VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 10, Number 3, 2010

DOI: 10.1089/vbz.2009.0014

Sterile-Insect Methods for Control of Mosquito-Borne Diseases: An Analysis

Luke Alphey,[1,2] Mark Benedict,[3] Romeo Bellini,[4] Gary G. Clark,[5] David A. Dame,[6] Mike W. Service,[7] and Stephen L. Dobson[8]

Abstract

Effective vector control, and more specifically mosquito control, is a complex and difficult problem, as illustrated by the continuing prevalence (and spread) of mosquito-transmitted diseases. The sterile insect technique and similar methods control certain agricultural insect pest populations in a species-specific, environmentally sound, and effective manner; there is increased interest in applying this approach to vector control. Such an approach, like all others in use and development, is not a one-size-fits-all solution, and will be more appropriate in some situations than others. In addition, the proposed release of pest insects, and more so genetically modified pest insects, is bound to raise questions in the general public and the scientific community as to such a method's efficacy, safety, and sustainability. This article attempts to address these concerns and indicate where sterile-insect methods are likely to be useful for vector control.

Key Words: SIT—RIDL—Vector control—Intervention—Mosquito(es).

The Sterile Insect Technique

SIT is a species-specific and environmentally nonpolluting method of insect control that relies on the release of large numbers of sterile insects (Knipling 1955, 1979, 1998, Krafsur 1998, Dyck et al., 2005a). Mating of released sterile males with native females leads to a decrease in the females' reproductive potential and ultimately, if males are released in sufficient numbers over a sufficient period of time, to the local elimination or suppression of the pest population.

Highly successful, area-wide SIT programs have eliminated the screwworm fly *Cochliomyia hominivorax* Coquerel from the United States, Mexico, and Central America, also from Libya, where SIT was used in the successful control of a serious outbreak in 1989 (Lindquist et al., 1992). Other targets of area-wide SIT programs include the Mediterranean fruit fly (Medfly) *Ceratitis capitata* Wiedemann and other tephritid fruit flies in the United States, Central and South America, South Africa, Europe, and Asia, and the pink bollworm *Pectinophora gossypiella* Saunders in the United States and codling moth *Cydia pomonella* L. in Canada. These programs can succeed on very large scales—the El Pino facility in Guatemala alone produces around 2 billion sterile male medflies per week (~20 tonnes/week), primarily for use in California and Guatemala. SIT is a proven, cost-effective strategy for eradication or suppression of target populations, or to protect areas against invasion or re-invasion.

[1]Oxitec Limited, Oxford, United Kingdom.
[2]Department of Zoology, University of Oxford, Oxford, United Kingdom.
[3]Entomology Unit, International Atomic Energy Agency, Vienna, Austria.
[4]Centro Agricoltura Ambiente "G.Nicoli," Crevalcore, Italy.
[5]Mosquito and Fly Research Unit, USDA-ARS-CMAVE, Gainesville, Florida.
[6]Entomological Services, Gainesville, Florida.
[7]Liverpool School of Tropical Medicine, Liverpool, United Kingdom.
[8]Department of Entomology, University of Kentucky, Lexington, Kentucky.

1. Frequently Asked Questions

We have found that several objections or questions are frequently raised in respect of the use of sterile-insect methods to control vector populations. Common ones are explicitly addressed in the course of this discussion of pros and cons of sterile-release methods. These are extracted below, listed in the order in which they appear in the text.

- **SIT is a top-down, centralized, command-and-control approach unsuitable to the new world of community-based practice.**
- **What makes a 'good' or 'bad' target for SIT?**
- **You could never get the sterile males in the right place at the right time to do the job.**
- **SIT programs are too slow to be useful, and can only be conducted over (impractically) huge areas.**
- **Density-dependent effects will reduce, eliminate, or even reverse the beneficial effect of an SIT program.**
- **Genetic control is too management- and operations-intensive to succeed in developing countries.**
- **How does SIT fit into a program of elimination or eradication of a vector-borne disease?**
- **You could not (should not, or would not be allowed to) release biting female mosquitoes.**
- **Sterile mosquitoes will not be able to compete equally/adequately for mates with wild mosquitoes.**
- **I can imagine blanketing Africa with bednets, drugs, and IRS. I cannot imagine blanketing Africa with sterile males or some other genetic control technology.**
- **You could never rear enough mosquitoes.**
- **How many do you need?**
- **Unlike screwworm and Medfly—the two most successful SIT programs—mosquito reproduction rates are too rapid for genetic control to be effective.**
- **The expense of genetic population control seems to make it prohibitive for many disease-endemic areas.**
- **Many areas have such high vector populations that it is inconceivable that genetic control could eliminate or even suppress them in such places.**
- **No one would ever let you do that (e.g., release GM mosquitoes).**
- **Why aren't you doing this in your own country first?/Because genetic methods might entail risks, they should be tested first in the countries in which they have been developed rather than developing countries.**
- **What does genetic control offer that we don't have already?**
- **Mosquito population suppression will result in loss of human population immunity, thus increasing transmission.**
- **Genetic control programs might work at first, but would not be sustainable.**
- **GM strains are unstable and will rapidly break down.**
- **Resistance may emerge and negate the strategy.**
- **What are the ecological consequences of suppressing or removing a pest?**
- **Eliminating a single vector may leave an empty niche that will be invaded by another, perhaps more harmful, vector.**
- **Looking ahead to a future of area-wide control of vectors by IVM with a strong SIT component against key vector species, how do we get there from here?**

2. Introduction

Vector control has long been seen as the only available tool against some major vector-borne diseases, for example, dengue. It is increasingly also seen as a major tool for malaria control.

The sterile insect technique (SIT[1]) is a method of pest insect control with a strong record of success against a range of agricultural pest insects (Dyck et al. 2005a). Field trials in the 1970s and 1980s demonstrated that the SIT could also be made to work against mosquitoes, even with the technology then available (Lofgren et al. 1974, Benedict and Robinson 2003). Interest in SIT for vector control has reemerged recently, driven by the availability of new technologies that have the potential to provide significant cost-effectiveness improvements for SIT, as well as by recognition of the limitations of current vector control strategies.

[1]Despite the name, the insects used in SIT are not strictly sterile, in the sense of agametic sterility. Rather, they are capable of mating, but some or all of the progeny of mating between the sterile insects and wild insects are nonviable. Several sterilizing methods are available. Here we use the terms "sterile," "sterility," and the like, for all of these methods, and the term "SIT" to encompass the use of any or all of them. These include:

- Radiation, which is used in all current agricultural programs. Radiation generates random dominant lethal mutations in the affected gametes.
- *Wolbachia*-induced cytoplasmic incompatibility, in which sperm from *Wolbachia*-infected males fail to function correctly after fertilizing eggs from uninfected females.
- Recombinant DNA methods, for example, the use of engineered repressible dominant lethal mutations (RIDL) that lead to the progeny of any cross involving an RIDL parent being nonviable unless provided with a suitable antidote (repressor) from the lethal genetic system. In one embodiment of this system, the lethal effect is female specific, so that only female progeny die.

Other methods have been used historically, including chemosterilants, or incompatible matings, through the use of either sibling species or else the use of artificially induced chromosome rearrangements.

These new technologies include:

- Genetic modification of mosquitoes, which has several potentially transformative applications to SIT (e.g., RIDL®, Oxitec Ltd., Oxford, United Kingdom) (Thomas et al. 2000, Alphey et al. 2007b, Phuc et al. 2007)
- Much better understanding of the biological basis and potential application to SIT of cytoplasmic incompatibility (CI) (e.g., Brelsfoard et al. 2008)
- A suite of technical improvements to SIT, developed over the past 20 years by the large agricultural programs. One example is the use of Global Positioning System and Geographic Information Systems, which represents a major breakthrough for area-wide control methods and has transformed the practice of SIT.

Although we believe that SIT has enormous potential for disease control in many contexts and settings, it is not a panacea. Conversely, that there are cases where SIT is clearly not a technically appropriate strategy does not obviate the potential value of this approach in other cases. In many instances the optimal use of SIT would be within an integrated vector management (IVM) program that used several approaches simultaneously (see Section 3.2.10 below). The applicability and attractiveness of SIT in a particular context depends in part on specific technical features of the method, which are highlighted and discussed below. Use of specific technologies (e.g., recombinant DNA technology, irradiation, aerial release, and *Wolbachia*) in the context of SIT may also be constrained by cultural, political, or regulatory issues; these are also discussed.

"SIT is a top-down, centralized, command-and-control approach unsuitable to the new world of community-based practice."

SIT and related techniques are typically thought of as large-scale, top-down programs. This is often correct, but it is not the only way in which these tools and strategies can be used, and most ongoing operational SIT programs now include community-based components. Further, new technology will allow much more distributed, community-based programs instead of, or in addition to, more centralized programs.

In the 20th century, large-scale vector control programs successfully brought pathogen transmission levels to zero, or near-zero levels, over huge areas. Examples include control of yellow fever and malaria in Cuba and Panama led by Gorgas and Ross (1901–1910), elimination of *Anopheles gambiae* Giles from Brazil around 1940, and the elimination of urban yellow fever from the Americas, the elimination, under the auspices of the Pan American Health Organization, of *Aedes aegypti* L. from all but 4 of 27 American countries by 1960 (Soper and Wilson 1943, Soper 1963). More recently, Cuba came close to eradicating *Ae. aegypti* in the 1980s (Kouri et al. 1986) and Singapore has kept that mosquito down to very low levels for over 30 years, though dengue incidence has recently increased (Ooi et al. 2006, Egger et al. 2008). Beyond mosquitoes, the Onchocerciasis Control Programme in West Africa (1974–2002) achieved its goals in 10 of 11 program countries (Amazigo and Boatin 2006). These programs relied on the large-scale, organized environmental use of broad-spectrum insecticides, notably Paris Green and then dichlorodiphenyltrichloroethane (DDT) but later a wide range of chemicals, supplemented in some cases with drug treatment, from quinine in the early days of malaria control to ivermectin for onchocerciasis today.

These programs were highly successful, and their conceptual descendants can be today, benefiting from the same organizational/cultural advantages of running large-scale public health programs but using more environmentally friendly chemicals, or application methods, and a better understanding of the mechanisms and spread of insecticide resistance. However, they have not generally produced sustainable results in terms of eliminating the vectors, so transmission is likely to persist or return when the intensity of program declines after its initial success. These methodologies, nevertheless, provide excellent tools for the population reductions likely to be a required before SIT releases.

SIT programs can provide many of the attractive features of such programs, without some of the disadvantages. Sterile insects are environmentally benign, with no toxic residues and minimal nontarget impact. Resistance, though theoretically possible, has very rarely been seen in the 50+ year history of large-scale SIT programs against agricultural pests; these programs have proven to be highly sustainable. Further, SIT programs, though needing the high degree of organization characteristic of any effective area-wide control program, especially with a goal of eradication or long-term suppression, are far less intrusive than most other such methods. In particular, SIT does not require access to households.

Some new technical developments should allow SIT programs to be operated in a much more decentralized or community-based manner, where this is desirable. In particular, genetic (transgenic) methods that allow for the release of eggs, rather than pupae or adults, have this characteristic. Potentially suitable strains for this strategy have recently been developed in *Ae. aegypti* and *Ae. albopictus* Skuse (both vectors of dengue and chikungunya viruses) (Alphey, unpublished).

3. Characteristics and Applicability of SIT

"What makes a 'good' or 'bad' target for SIT?"

Every control method has specific characteristics that tend to make it more suitable in some cases and less so in others. This cannot be considered in isolation, as the optimal strategy obviously depends on whether superior alternatives are available. In many cases the optimal strategy may involve a combination of several individual methods; our analysis leads us to conclude that in many instances the optimal strategy is likely to be an IVM program with a significant SIT component but also using other methods, especially insecticides.

3.1. Species specific

SIT is extremely species specific since released males mate only with females of the same species.

3.1.1. Pro: environmentally friendly. SIT will control the target species with minimal off-target effects on other species. The only nontarget effects are indirect, for example, the potential effect on other members of the ecosystem of removing or suppressing one species (the target vector species). No toxic chemicals or chemical residues are released into the environment.

3.1.2. Con: one species at a time. Although species specificity is highly attractive from an environmental perspective, it may be a limitation where several vector species

need to be controlled simultaneously. SIT is therefore best suited to areas with a single dominant vector for a particular disease; two would be manageable, but many more than that would point to a more broad-spectrum approach. There are many instances of such locations. For example, *Ae. aegypti* is the primary vector of dengue viruses worldwide; *Ae. albopictus* was the key vector for chikungunya virus in La Réunion in 2005–2006 and Italy in 2007, whereas *Ae. polynesiensis* Marks is an important vector of diurnally subperiodic *Wuchereria bancrofti* Cobbold filariasis in Polynesia. In contrast, some important areas of malaria transmission have several major vector species. In other situations, however, there may be a single major vector species, but also several secondary vectors, in which case the merit of controlling the main vector alone would need to be assessed.

3.1.2.1. Cryptic species and mating barriers

It is important for an SIT program to know which species to culture and with what it will mate. This might seem obvious and trivial, but in the context of species complexes and cryptic species it may not be. If the target species is in fact two species, or two (or more) sympatric populations with strong premating barriers between them, rearing and releasing only one type may be of limited benefit. This would apply to the *Anopheles gambiae* complex where *An. gambiae s. str.* and *An. arabiensis* can both be major vectors and in many areas both are present. Moreover, within these species there may be mating barriers between different chromosomal forms or types of these species, although the strength of such mating barriers does not seem entirely clear at present. This is an area that would need to be clarified before initiating an SIT program against this species. On the other hand, there are many major vector species and populations for which this seems not to be an issue.

In this context it should be noted that "significant mating barriers" is not synonymous with "detectable genetic differences" between populations. With forensic DNA methods it is possible to find genetic (molecular) differences between essentially any two humans on the planet. This does not mean that there are significant mating barriers. Similarly, that different insect populations can be distinguished by molecular methods does not by itself mean that there are mating barriers that would affect an SIT program. For example, SIT programs against agricultural pests have typically not found significant mating barriers between widely separated populations, despite detectable genetic differences (e.g., Cayol et al. 2002). Ongoing work with a transgenic strain of *Ae. aegypti* introgressed into a Latin American–derived genetic background and also an Asian-derived genetic background suggests that both strains are competitive when competing with males of the Asian strain for access to Asian-strain females. (Institute of Medical Research 2007, Lee et al. 2008). Similarly, Girod et al. (2001) found no mating incompatibility between genetically distinguishable populations of *Anopheles arabiensis* Patton.

3.1.3. Other methods. Other genetically modified (GM) vector methods tend to share the species specificity of SIT. Few other methods are so precisely targeted. Insecticides are much more broad spectrum, though a sufficiently good and specific attractant could potentially limit this.

3.2. Suitable target species and populations

The "single dominant pest" issue discussed above leads to a requirement that successful suppression or elimination (by SIT) of one or a small number of target species should provide sufficient benefit (e.g., in reduced human morbidity/mortality) to justify the cost of the program. There are several other criteria that would make a potential target species or population more or less attractive or suitable as a target for SIT:

3.2.1. Lack of cheap, effective alternatives.

- Other vector control methods, but also drugs and vaccines.
- On the other hand, within an IVM system, SIT may have a place even if not the cheapest option, for example, for resistance management (Alphey et al. 2007a), or "getting the last ones" in an elimination or eradication campaign (see below).

3.2.2. Ability to mass-rear the target species.

- Where not currently feasible for a particular species, this may be a research priority.
- Even where rearing is already well-known, investment in scale-up and improved rearing technology will improve cost effectiveness.
- Other genetic control strategies also require the ability to rear the target species, though possibly in somewhat lower numbers.

3.2.3. Dispersal range of the target insect.

- If very low, releases would have to be conducted on a very fine spatial scale. If very high, immigration from outside the control area might be a problem, unless the control area was very large.
- In practice, most agricultural SIT targets have adult dispersal distances that range from a few hundred meters to a few kilometers. Dispersal distance is probably not a limitation for most mosquito vectors.

3.2.4. Mating habits. **"You could never get the sterile males in the right place at the right time to do the job."**

- Males will tend to disperse themselves and seek out wild females without human intervention (unlike chemical insecticides, for example). This is particularly helpful where control program personnel lack ready access to some areas, for example, private property.
- Sterile males are typically released periodically. In some cases this is planned to match a wild target population that is synchronized by environmental forcing, for example, seasonal weather or rainfall. More commonly, releases are of sufficient frequency, for example, weekly, to maintain a permanent standing population of sterile males in the target area, so that females seeking mates always have a high chance of mating with a sterile male.
- However, a very complex mating system, such as the intense, limited-duration mating flights of some ants, would make it difficult to get the sterile males into the

target population at the right time and place to compete for mates.
- Parthenogenetic species, which can reproduce without mating, are also unlikely to be suitable.
- However, multiple mating behavior by target females will not affect the outcome, as long as sterile males are comparable with wild males in postcopulatory aspects such as induction of female refractoriness to remating and sperm competition (Knipling 1955, Whitten and Mahon 2005).
- These issues do not seem to rule out many vector species.

3.2.5. Generation time.

- Time to observable impact of the program will depend in part on the generation time of the target vector species. On the whole a shorter generation time is therefore preferable, though SIT has been used against insects with only one generation per year, at least experimentally (Bloem et al. 2005).
- Developing new strains, whether by classical genetics or recombinant DNA methods, will typically be faster for insects with a shorter generation time.
- Shorter generation time may also have a concomitant disadvantage in that the target population would recover more rapidly if there were a prolonged disruption to the program.

3.2.6. Which form causes the damage?

- SIT is more attractive where a harmless form can be released, for example, adult male mosquitoes that do not bite and cannot transmit diseases. Where the released sterile insects are themselves damaging, the program would be a trade-off between this damage and the benefit of the program from suppressing the target population. This trade-off would have to be evaluated on a case-by-case basis. Certainly, many SIT programs have been run on this basis (e.g., bisexual release of tephritid fruit flies [Enkerlin 2005, Klassen and Curtis 2005], some early mosquito SIT trials [Benedict and Robinson 2003, Klassen and Curtis 2005]).
- One SIT strategy that may be very attractive in some instances posits the release of eggs, rather than the pupae or adults generally released in current programs (Alphey et al. 2007b). This approach has some clear potential benefits in terms of community participation, lower distribution costs, and the like. However, it is unlikely to be appropriate for vectors where immature forms are damaging (e.g., triatomines) (as compared, for example, with mosquitoes, where the immature stages are harmless to humans and several key vector species are very cheap and easy to rear). Nonetheless, there is a precedent for releasing eggs: the F_1 sterility program against gypsy moth, *Lymantria dispar* L., where egg masses were released (reviewed by Bloem et al. 2005). This was seen as a potentially attractive option despite the fact that these eggs would hatch into larvae that would eat tree foliage just like wild gypsy moth larvae. The benefit of control (in this program, the released eggs emerge as sterile adults) was considered to outweigh the modest amount of damage inflicted by larvae from the released eggs.

3.2.7. Scale (time and space). **"SIT programs are too slow to be useful, and can only be conducted over [impractically] huge areas."**

- SIT programs do not have immediate effects on vector numbers. The sterile males impact the size of the wild population in the next generation, not the current one. Significant population reduction should be seen after a small number of generations, but this is likely to be weeks or months rather than hours or days.
- Similarly, there is a minimum effective scale for an SIT program. It is not easy to envisage protecting an individual, or even an individual household, by this method, but individual villages—or other isolated populations—up to or beyond the scale of large cities, are all feasible. Larger populations do not need to be treated homogeneously—a rolling program may well be more efficient. Agricultural SIT programs have operated successfully on scales ranging from a few hundred or few thousand hectares (e.g., painted apple moth in New Zealand; codling moth in Canada) up to continental scales (e.g., New World screwworm [NWS]).
- These characteristics mean that SIT methods are good for protecting significant areas for extended periods of time, but not suitable for individual protection for a traveler, transient, nomad, or soldier moving through a new area. We normally think of suitable areas in terms of cities, towns, villages (or, for agriculture, contiguous crop or livestock areas), but mining or oil camps, military bases, or other defined areas needing medium- or long-term protection are all potentially suitable.

3.2.8. Density-dependent effects. **"Density-dependent effects will reduce, eliminate, or even reverse the beneficial effect of an SIT program."**

- It has been shown that density dependence, for example, in terms of larval survival, could be a problem for sterile-release methods (Weidhaas et al. 1971, Rogers and Randolph 1984, Dobson et al. 2002), although this was not observed with *Anopheles albimanus* Wiedemann SIT in El Salvador (Weidhaas et al. 1974). Early death of some individuals (progeny of sterile×wild matings) would leave the remaining larvae with more resources (e.g., food) per larva, leading to less competition and a higher survival rate. This would immediately tend to counter the effect of the control program. The extent of this problem will clearly vary from species to species, and possibly from one locale and/or season to another.
- This problem can essentially be eliminated by manipulating the time of death of the affected individuals (Atkinson et al. 2007, Phuc et al. 2007, Yakob et al. 2008). If the affected individuals (i.e., progeny of sterile male× wild female) die later in development, after competing for food, rather than dying early, then the problem essentially disappears. Arranging death as pupae (rather than as embryos) would accomplish this. This has been demonstrated in the laboratory for RIDL strains of

Ae. aegypti and *Ae. albopictus* and could presumably be extended to other mosquito species without major difficulty (Alphey 2002, 2007, Alphey and Andreasen 2002, Alphey et al. 2007b, Phuc et al. 2007).

3.2.9. Infrastructure availability. **"Genetic control is too management- and operations-intensive to succeed in developing countries."**

- Many endemic countries do not have sufficient infrastructure and management experience to conduct a genetic control program. In the absence of extensive outside support, these countries will not be good candidates for genetic control programs (conversely, with such support they would be, and there would clearly be a significant capacity-building component and outcome to such support). Many developing and developed countries do have sufficient infrastructure to conduct genetic control programs. These include numerous densely populated urban areas in which both infrastructure and benefits are favorable.
- Issues about availability of resources, infrastructure, political will, trained personnel, and the like, apply to any organized program—vector control, vaccines, mass drug administration, and so on—and are not specific to genetic control. Ultimately, the availability of sufficient resources is critical for any successful program; key resources are money and political will, maintained over the long term. It is not necessarily the case that the management requirement of an *effective* SIT program would be higher than that for an *effective* control program using other methods.

3.2.10. Combining SIT methods with other tools (IVM). Though the SIT is often perceived or considered as a stand-alone method, in fact it is rarely used that way—current and historical practice is to use the SIT as a principal component of an integrated pest management system (Dyck et al. 2005a). SIT methods will combine well with any other method so long as it does not specifically target sterile males over wild males. Methods targeted at immature stages or adult females would be particularly compatible, indeed synergistic with mosquito SIT methods. The synergy comes from at least two directions—SIT methods work better (are less expensive) against target populations that have already been reduced to some extent by another method (see Sections 3.3.3.2 and 3.3.3.2.1). Also, separately, the use of SIT methods may help manage resistance against other tools; one version of RIDL is particularly powerful in this regard (Alphey et al. 2007a, 2008, 2009).

It should also be clear that an SIT program involves more than simple release of sterile insects. Entomological surveillance of the vector population is essential to monitor the impact of any vector control program, and for the SIT it is also desirable to assess the field performance of the released sterile males, for example, survival, dispersal, and mating success.

3.2.11. Elimination and eradication. Elimination is the local removal of a pest or pathogen (local eradication); we use the term "eradication" in the sense of global elimination.

How does SIT fit into a program of elimination or eradication of a vector-borne disease?

SIT fits very well. It is one of the few available vector control tools with a proven record of pest elimination over large areas, including up to continental scales. Clearly, elimination of an obligate vector will eliminate transmission. True elimination of a pest has some potential difficulties (e.g., immigration or dormant individuals; see below). However, elimination of a pathogen from an area (by vector control) does not strictly require elimination of the vector, merely reduction below a critical transmission threshold for a period of time. In many cases, this threshold may be far below the current population level.

3.2.11.1. Outbreaks/new introductions

SIT methods are exceptionally suitable for dealing with new introductions (or reinvasion of cleared areas) of vectors. This has been proven in multiple instances, for example, New World Screwworm in Libya (Lindquist et al. 1992, Vargas et al. 1994), and various moth and fruit fly species around the world (Koyama et al. 2004, Bloem et al. 2005, Enkerlin 2005, Kean and Suckling 2005). This is due to some fundamental properties of sterile-release methods:

- Effectiveness is related to the ratio of released sterile males to wild fertile males.
  - Density and/or geographic distribution of the newly introduced population is relatively small, so relatively easy to overwhelm with sterile insects.
- Sterile males will actively seek out wild females.
  - So you don't need to find these relatively rare insects.
  - Can target these few hard to find individuals and reduce the population from low to zero, that is, eliminate.
- Rapid response possible (c.f. Libya/screwworm).
  - But only if rearing infrastructure has already been established somewhere (not necessarily nearby—for screwworm sterile insects from Mexico program were flown to Libya).

If frequent reinvasion is expected, continuous prophylactic release of sterile insects, either uniformly across the at-risk area or at key entry points (barrier program) may be preferable (see Section 5.2.1).

3.2.11.2. Immigration

As long as reestablishment is prevented, at least for a suitable period of time, occasional immigration of the pest is extremely unlikely to sustain disease transmission, even though this might prevent true elimination of the pest.

3.2.11.3. Dormant individuals

Some species have a dormant stage, which can survive long periods of inclement conditions and then resume development or activity later. If these resume activity only after a variable and indeterminate time, lack of detectable adults, even for a period greatly in excess of one (normal) generation, cannot be taken to demonstrate elimination. However, it may well be enough to break the cycle of transmission. This in turn depends on the life cycle of the pathogen—if humans remain

infected/infectious for an extended period (e.g., *Plasmodium vivax*, lymphatic filariasis), then elimination of the vector will prevent transmission but not eliminate the pathogen. For other pathogens, such as dengue, where humans become noninfectious after a few days, elimination of the vector, even for a few weeks, would be sufficient to eliminate the pathogen from an area.

3.2.11.4. Endgame

The strategy and the endgame of an area-wide elimination strategy will require considerable thought and planning. For various reasons (immigration, persistent dormant stages, and inaccessible or cryptic populations), this endgame is likely to take quite a while, and requires vigilance and resource allocation for a considerable period after pathogen transmission has ceased, or been reduced to an undetectable level. SIT methods are well suited to this endgame; key properties include

- Nonintrusive, minimal action required by individual citizen.
  - Hard to maintain individual action/attention once transmission levels very low.
- Sterile males actively seek out wild females.
  - So, unlike most other methods, you don't have to find the last insects or breeding sites.
- Proven record of success in pest insect elimination.
  - Multiple examples across wide range of species, settings, and spatial scales

3.3. Mass rearing and mass release

Sterile-release methods are based on the distribution into the environment of large numbers of sterile insects. These insects compete for mates with their wild counterparts; a wild female that mates a sterile male will have no viable progeny, or fewer than she would otherwise have had, and so the next generation of the target population will tend to be smaller than it would otherwise have been. If a sufficient number of sterile insects can be released over a sufficient period of time, the target population will decline and collapse. This may lead to local elimination of the target species. Since the SIT principle was proposed more than 50 years ago (Knipling 1955, Klassen and Curtis 2005), it has been comprehensively validated in multiple, large-scale, long-term, successful, sustainable control programs (Dyck et al. 2005a).

The mode of action of SIT has some consequences. It is obviously necessary to be able to rear large numbers of the target insect. Some important vectors, for example, *Anopheles darlingi* Root, a major malaria vector in Brazil and elsewhere, cannot yet be reared in the laboratory at all, let alone on the scale required for SIT. However, this may represent more of a research need than a fundamental limitation.

3.3.1. Sex separation and genetic sexing. Genetic sexing is the genetics-based separation of males from females of the same species.

3.3.1.1. Rationale

"You could not [should not, or would not be allowed to] release biting female mosquitoes."

In most cases it is considered desirable to release only sterile males, rather than a mixed population of sterile males and sterile females. There are two principal reasons for this.

(i) For some species, for example, mosquitoes, adult males are harmless, but adult females are biting pests and potential disease vectors. Therefore, release of large numbers of sterile females, though perhaps acceptable for the long-term benefit of suppressing the wild vector population, might nonetheless cause some transient damage. This might also complicate public acceptance of the strategy.

(ii) Large-scale field experiments with the Mediterranean fruit fly (Medfly, *Ceratitis capitata* Wiedemann) demonstrated that sterile males are 3–5 times more effective (per male) when released alone than when released with similar numbers of sterile females (Rendón et al. 2004). An effect of this magnitude would justify considerable effort to remove the females.

3.3.1.2. Methods

Since most potential target species normally produce approximately equal numbers of males and females, releasing only males obviously requires the ability to separate large numbers of males from a similar number of females. For some species there is sufficient sexual dimorphism or other difference to allow adequate sex separation. This might be eclosion time (tsetse) or pupal size (*Ae. aegypti* [Ansari et al. 1977, Focks 1980]). However, for many vectors (e.g., *Anopheles* mosquitoes), no adequate method is available. For *An. albimanus* Wiedemann, giving adults access to citrated blood with 0.5% malathion killed 95% of the females, though also some males (Lowe et al. 1981); more efficient separation was considered to be important. In such cases, it may be possible to induce a suitable sexual dimorphism by genetics. This may be a visible difference between males and females or, preferably, a female-specific conditional lethal phenotype so that females can be removed by applying the condition. A lethal phenotype is preferable as it allows large numbers of females to be removed simultaneously; visible differences require that every insect be individually examined and sorted—even with automation this may be a costly process in the numbers required.

3.3.1.2.1. Classical genetics

Classical methods use an autosomal marker artificially linked by a translocation to the male-determining Y chromosome. Extremely effective sexing strains have been developed for Medfly (Franz 2005); these are based on a temperature-sensitive lethal mutation on an autosome with a covering translocation to the Y chromosome. Mosquito sexing strains have generally used an insecticide resistance gene, translocated to the Y chromosome so that males survive exposure to the toxin but females do not, for example, propoxur resistance for *An. albimanus* (Kaiser et al. 1978). This approach has some limitations:

- The strains can take a long time to develop (10–15 years for Medfly), though just 1–2 years for *An. albimanus* (Seawright et al. 1978).

- It may be undesirable to release mosquitoes carrying an insecticide resistance gene.
- The strains generally have significantly reduced fitness due to the mutations and chromosome rearrangements they carry (this may primarily affect productivity in rearing rather than field performance).
- Not all vectors have a usable Y chromosome. Tight linkage to a small "male determining locus" is then hard to achieve.

3.3.1.2.2. Recombinant DNA methods

Several new methods have been proposed, with at least proof of principle for genetic sexing. These include

- Sex-limited or sex-linked expression of a fluorescent protein, combined with fluorescence-based sorting (Catteruccia et al. 2005, Condon et al. 2007);
- Conditional female-specific lethality (Heinrich and Scott 2000, Thomas et al. 2000, Fu et al. 2007); in these examples the females die unless provided with tetracycline (or a suitable chemical analog) during larval development.

Such methods potentially provide significant efficiency improvements for sterile-release methods.

Female lethal strains of *Ae. aegypti* and *Ae. albopictus* have already been developed (Alphey, unpublished). There are no data giving reason to anticipate any major difficulties in applying this technology to other mosquito species.

3.3.1.3. Efficiency

Physical separation is unlikely to be 100% effective (e.g., 99–99.9% effective for *Ae. aegypti* [Ansari et al. 1977]). Classical sexing strains may also leave 0.1–1% females. Transgenic methods may be more effective, but this remains to be demonstrated in actual program use. However, it is noted that 100% sex separation has been achieved with RIDL Medfly strains ($n > 50{,}000$, Fu et al. 2007, Alphey, unpublished).

What separation rate is required? This depends on the circumstances.

- Program effectiveness. In terms of program effectiveness in suppressing the target wild population, removing females is not strictly necessary at all, though as noted above substantial efficiency gains may be achieved by doing so. However, this does not have to be 100% effective—98% would certainly be adequate. An SIT program against *An. albimanus* was successful despite releasing approximately 15% females (Lofgren et al. 1974, Weidhaas et al. 1974, Klassen and Curtis 2005).
- Impact on disease transmission. Though it needs to be assessed separately for each species, an SIT program might, for example, aim for a sterile:wild ratio in the field of around 10:1. For a male-only release that in fact contained 1% females, this would mean that sterile females would be present at about one-tenth of the wild female level. This is unlikely to have a significant negative impact, especially if prior population suppression methods have been applied, and particularly since the total number of females (including steriles) should rapidly decline if the program achieves any degree of success.
- There may be specific technical reasons where highly efficient sex separation is required. One example is the use of *Wolbachia*-induced CI as the method of sterilization. Release of even a small number of infected females may lead to *Wolbachia* invading the target population (i.e., population replacement [Alphey et al. 2002, Xi et al. 2005, Brelsfoard et al. 2008, Alphey 2009]); depending upon the strategy, females in the replaced population would be fertile with additional released males and so would be effectively resistant to the sterilizing method.

3.3.2. Insect quality. It is essential that the released males successfully mate a sufficient percentage of the wild females to reduce the wild population. Since there will also be wild males around, this implies that the sterile males must be reasonably competitive with wild males. However, various aspects of mass-rearing, handling, distribution, sterilization, genetics, and the like, may reduce the quality of the males. Several aspects of this issue are worth exploring.

3.3.2.1. Field and rearing quality

For SIT, insects are reared in captivity, sterilized as necessary, and released. After release, the key property is the ability of the sterile males to mate wild females. Male mating performance may have several components, including longevity and dispersal, as well as the ability to find and successfully court females, also postcopulatory traits such as ability to induce refractoriness to remating in a mated female, and adequate sperm competition if she does remate. However, other aspects of fitness in the field, for example, adult female host-seeking ability and fecundity, are irrelevant to the SIT (though they may be critical to other genetic control strategies).

Other aspects of strain performance, such as productivity in mass rearing, may have some impact on program cost, but they are unlikely to be critical to program success.

3.3.2.1.1. Measuring quality

One gap in the current state-of-the-art is in our ability to assess mosquito quality. Productivity in rearing is relatively easy to measure—one can easily count the number of males produced per female, per unit of diet, space, or other resource. However, it is much harder to know how good these males are.

Ideally, we would have a quick, inexpensive laboratory test that would accurately predict the performance of males in the field. At present we have only proxies of uncertain value. These include pupal or adult size/weight, biochemical composition (e.g., protein, lipid, and sugar reserves), and mating competitiveness in laboratory or field cages assays of varying degrees of realism.

However, once a large-scale program is implemented, it may well be possible to directly measure mating success in the field. The SIT program against the New World Screwworm measured the degree of sterility in egg masses laid on sentinel animals. Analogous monitoring for an SIT program against a mosquito would be to sample eggs laid in the field. For *Aedes*

and *Ochlerotatus* species, ovitrapping is already a very widely used technique for monitoring field populations (Service 1993, Silver 2008). Measurement of egg hatch rate would give an indication of induced sterility; where there is a detectable genetic difference between sterile and wild insects (e.g., where GM mosquitoes are used), precise assessment of parentage is possible (this assumes that females lay similar number of eggs whichever type of male they mate; if not, then suitable correction can be made). Where eggs can be obtained in traps and hatched reasonably easily, this method may be as simple as examining the resulting larvae for expression of a fluorescent marker (which would indicate a sterile father).

3.3.2.2. Threshold quality

"Sterile mosquitoes will not be able to compete equally/adequately for mates with wild mosquitoes."

Though the released sterile insects would ideally be equally competitive with wild males, this is neither likely nor necessary. In general, a deficiency in competitiveness can be overcome by releasing more sterile males. The quality-control manual for Medfly SIT programs recommends a minimum relative sterility index of 0.2, which means that the sterile males, mixed with wild males and wild females at 1:1:1 ratio, obtain only 20% of the mates (FAO/IAEA/USDA 2003). In laboratory and field cage studies, mosquitoes sterilized by radiation, CI, or genetics (RIDL) have all done far better than this, and several methods have been validated in open release experiments (Benedict and Robinson 2003, Klassen and Curtis 2005).

3.3.2.2.1. Genetic engineering and mosquito performance

Several articles have been widely misinterpreted as indicating that transgenic mosquitoes will inevitably have severely impaired performance. In the case of two high-profile articles, the observed effects were likely caused, in part or in whole, by inbreeding depression rather than a direct effect of the transgene insertion (Catteruccia et al. 2003, Irvin et al. 2004). The potential problem of inbreeding stems from the fact that the transgenic strain starts with a single founder individual. However, this issue also applies to classical genetic strains such as translocation strains; wild-type genetic material can be bred into the strain by out-crossing and genetic diversity and performance restored (e.g., Seawright et al. 1978). In any case, an observation that some strains have poor performance does not imply that all strains will be inadequate. In fact, several more recent studies have shown little or no adverse effect from transgenesis (Allen et al. 2004, Moreira et al. 2004, Marrelli et al. 2006, 2007).

There are no data at present to indicate any fundamental limitation to generating highly suitable strains of mosquitoes engineered with specific properties to augment SIT.

3.3.3. Cost/scale. Rearing insects may seem relatively expensive, at least when many millions may be required. However, this perception may not be correct. There is considerable accumulated experience of the costs and other issues around mass-rearing insects, primarily from agricultural programs (Dyck et al. 2005a, FAO/IAEA 2008), but also from previous trials with various mosquito species.

3.3.3.1. Scale

3.3.3.1.1. Area

"I can imagine blanketing Africa with bednets, drugs and indoor residual spraying (IRS). I cannot imagine blanketing Africa with sterile males or some other genetic control technology."

Clearly, genetic control programs will not operate on such a wide scale in the first instance, whether in Africa or elsewhere. We do believe that developing the technology and implementing it in a stepwise manner will create methods and efficiencies that will increase the breadth of application. In fact, few other approaches can match the SIT's proven record of pest elimination on scales up to and including continental. Unlike the continuous investment in IRS, insecticide-treated bed nets, and drugs, it may offer the potential for vector elimination—a truly sustainable outcome.

3.3.3.1.2. Numbers

"You could never rear enough mosquitoes."

The largest single sterile-insect production facility in the world produces around 2 billion sterile male Medflies per week—around 20 tonnes per week of Medflies (USDA 2006). There are around 20 Medfly production facilities around the world, and several other species are reared for SIT in numbers around 100 million per week (Dyck et al. 2005a).

Mosquitoes have not been reared at quite this scale, but particular *Anopheles*, *Aedes*, and *Culex* species have been reared at 10^5 to $>10^6$ per week, and *An. albimanus* at $>10^6$ males daily, this production level being maintained continuously for over a year (Bailey et al. 1979). Only a few mosquito species in these three genera have been reared. Obviously, some species are harder to rear than others, but there seems no reason to think that there are any insuperable obstacles to rearing sufficient numbers for many vector species, given sufficient resources.

Of course the other side of the question, "Can you rear enough?" is, "How many do you need?" This may vary considerably from one vector to another. Some may feel that unlike screwworm and Medfly—the two most successful SIT programs—mosquito reproduction rates are too rapid for genetic control to be effective, but this is simply incorrect. For *Ae. aegypti*, Dye (1984) estimated the net reproductive rate in the field to be between 3 and 11. Weidhaas et al. (1974) estimated this value for *An. albimanus* in the field to range between 0.4 and 4.8 depending on season. The value for Medfly is very similar, probably at the upper end of this range. Thus, these mosquitoes at least do not have a reproduction rate that is significantly different from agricultural pests that have been successfully controlled or eliminated by the SIT.

Seasonal variation in population size and growth rate can also be helpful—targeting the population at its most vulnerable time may considerably reduce the number of sterile mosquitoes required.

The flip side of high reproductive rate in the field is high reproductive rate in the rearing facility, which would tend to lead to the ability to rear large numbers quickly and inexpensively. In fact one current SIT program where cost (relative to alternatives) does seem to be a controversial or negative issue is for tsetse, an insect with a famously low reproductive rate (Rogers and Randolph 2002, Vale and Torr 2005,

Enserink 2007, but see also Feldmann et al. 2005, Vreysen et al. 2007).

Numbers from a specific example: RIDL strains against *Ae. aegypti* and dengue. Models suggest that the minimum effective release ratio for successful control of the target population (to an effectively zero level) is approximately 1.7:1 (Atkinson et al. 2007, Phuc et al. 2007). Allowing a considerable margin for error, we assume an initial release ratio of 10:1. In many settings (Focks et al. 2000, Focks and Alexander 2006), this would correspond to releasing around 20 males per human to be protected (i.e., per inhabitant of the control zone) per week. For a city of 5 million people, this would correspond to releasing 100 million males per week. This number is well within the range of current agricultural SIT programs. It could be reduced substantially by prior suppression of the target population by alternative methods, an approach strongly advocated by Knipling and others (Knipling 1979, Klassen 2005, and see below). After initial suppression, ongoing maintenance—if based on use of sterile males—would likely require fewer, thus freeing up spare capacity for use in other locations.

3.3.3.2. Cost

"The expense of genetic population control seems to make it prohibitive for many disease endemic areas."

The SIT may be effective, but is it cost effective? A study led by Stanford Business School suggested that an SIT program using RIDL against dengue was likely to be superior in terms of both cost and efficacy to other currently available methods (Atkinson et al. 2007).

Acceptable cost depends in part on an assessment of the alternatives. Data on some of these were summarized by the Bill and Melinda Gates Foundation and Boston Consulting Group (2007) and can be used for comparison. The costs and benefits suggest that the use of a RIDL strain against *Ae. aegypti* and dengue would be attractive relative to alternatives. (Alphey et al; unpublished).

These calculations are based on the use of SIT as the sole control measure. As noted above, this is almost certainly not the optimal strategy, but provides a conservative upper cost estimate.

3.3.3.2.1. Alternatives

There are no specific drugs or licensed vaccine for dengue, so vector control is the only option. Bednets are ineffective against this day-biting mosquito, though other applications of insecticide treated materials (ITMs) may be useful for dengue control (Kroeger et al. 2006). Most dengue control programs depend on source reduction and some application of adulticides, but only in very few cases have current methods been able to reduce vector densities to a low enough level to prevent significant transmission (Halstead 2000, Heintze et al. 2006, Egger et al. 2008).

3.3.3.2.2. Cost of SIT

Production costs for sterile males of the *An. albimanus* MACHO strain in El Salvador in 1979 were estimated at $156 per million (Dame et al. 1981) and for *Ae. aegypti* and *Culex pipiens fatigans* pupae at $58 and $50, respectively, per million in India in 1975 (Ansari et al. 1975, 1977, Singh and Razdan 1975) (converting to 2008 values using GDP chained price index [U.S. Office of Management and Budget 2008], these figures would correspond to ca. $389, $228, and $196).

A significant fraction of the program cost is the cost of finance—the raising of money to build the facility as a loan and paying interest on this until the facility is fully operational. Costs would be considerably lower, perhaps by a factor of two in terms of ongoing costs if funds from philanthropic or beneficiary sources were available upfront.

SIT has historically been seen as a premium method; the key virtues have been effectiveness and, perhaps secondarily, attractive environmental profile, though still at an acceptable cost. In the context of pest insect elimination (and sustainability thereof), SIT clearly has a strong record of success, perhaps unmatched since the days of DDT, and of course with a more attractive environmental profile. We believe that the application of new technologies to sterile-release methods, particularly genetic methods, will significantly improve the cost profile without losing any of the attractive features of such methods, indeed enhancing some of them.

3.3.3.2.3. Market

The delivery is a little different, in that bednets are provided (whether sold or given) to individuals, who may or may not then use them. For bednets, there is a significant additional community benefit when coverage (use) approaches 100%, but this is difficult to arrange; certainly, it is not directly part of the product or service. SIT methods may be able to do this more efficiently and economically. SIT tools would probably not, in most instances, be sold/provided directly to individual consumers (though this is conceivable for some variants, including RIDL). Thus, the customer (the entity actually paying for the product or service) is more likely to be local or national government agencies, or donor agencies, than individual citizens.

3.3.3.2.4. Cost-effectiveness profile

One unusual or unique feature of SIT is its cost-effectiveness profile. Most vector control interventions show a diminishing return. For example, a given amount of pesticide must be used regardless of whether the target insect population in a given area is large or small; this amounts to decreased efficiency (fewer insects killed) with smaller target populations. In contrast, releasing a given number of sterile insects becomes more efficient as the target population becomes smaller because a higher ratio of sterile to indigenous males is achieved (Knipling 1955, Dyck et al. 2005a).

Or, for example, a given investment (e.g., public education, or vector control teams) may lead to the elimination of a certain proportion (say 50%) of the *Ae. aegypti* larval habitats in an area. Doubling this investment will give only a modest additional reduction, certainly not another 50% (to 100% total). This profile (continuously diminishing return) is likely to be true for larvicides, adulticides, bednet coverage, vaccines, mass drug administration, and so on.

In contrast, SIT has a different profile. Imagine spending a certain amount of money to build and operate a rearing facility and control program with a given capacity (in terms of sterile males per week). If this is enough above a certain threshold, it will eliminate the target population. At a capacity somewhat below that threshold, the target population will

typically decline at a slower rate to a new, lower equilibrium. At a capacity significantly below that threshold the effect on the target population will be negligible. This implies a very different cost-effectiveness or cost-benefit relationship. With decreasing population density as control takes effect the sterile:fertile ratio increases rapidly without increasing the numbers of released insects. Another way of phrasing this is that SIT is better at getting the last vectors than it is at getting the first ones, which is quite unlike other strategies. Further, release numbers could be reduced after the wild population has dwindled sufficiently, allowing the unused sterile males to be used to extend the area of control.

This means that the optimal strategy for vector elimination (for a species reasonably amenable to SIT control, see above) is almost inevitably first to suppress by all available conventional techniques and/or time the initial releases to periods of predictably low population density, then to use SIT to deal with the now-reduced target population. Since SIT strategies are compatible with most other control methods, in practice this means using several methods simultaneously—in other words IVM with a significant SIT component.

3.3.3.2.5. Size of vector population

"Many areas have such high vector populations that it is inconceivable that genetic control could eliminate or even suppress them in such places."

This is true for some vectors in some settings. Even in these settings, SIT is likely to be a highly desirable component of an IVM system. Even in the best-known successes of SIT, it was not used as the only control method, though often the major one. As noted above, the optimal strategy is likely to involve initial reduction of the target population by other methods, leading into or combined with an SIT program.

4. Safety, Regulatory, and Public Perception Issues

"No one would ever let you do that (e.g., release GM mosquitoes)."

This is often seen as an issue primarily for SIT strategies that employ recombinant DNA methods, that is, transgenic mosquitoes. However, the use of isotope or X-ray irradiators, or microbial (*Wolbachia*)–based sterilization methods, or simply the release of mosquitoes, all have associated regulatory and public perception issues, as indeed does the use of insecticides. More broadly, any public health program needs community acceptance, even if the methods used do not require direct individual participation or acceptance of invasive treatments. This is even more the case for programs that do need substantial community participation to succeed, and for programs using new technologies that may be viewed with suspicion, or at least not be readily understood and accepted, by those in affected communities. However, methods for achieving this are not well understood; even defining the affected community can be nontrivial (Lavery et al. 2008). Though SIT programs have generally been well accepted, a proposed large trial in India was derailed before it started by severe negative publicity (Nature 1975, WHO 1976, Dyck et al. 2005b). However, consideration of the recent problems with polio vaccination in Nigeria shows that even a well-established program with a strong record of success and using tried-and-tested technology is not immune to accusations and negative publicity (e.g., Jegede 2007). Nonetheless, we focus here on issues related to genetic modification.

At present, the key precedent for the field use of GM insects is the pink bollworm SIT program in the United States. Pink bollworm (*Pectinophora gossypiella* Saunders) is a major lepidopteran pest of cotton and for many years has been successfully controlled by a conventional SIT program in the Southwestern United States. The rearing facility for this U.S. Department of Agriculture (USDA)–led program is based in Phoenix, Arizona, and can produce ~200 million sterile moths per week. The first genetic transformation of pink bollworm was described in 2000 (Peloquin et al 2000). A number of transgenic strains have been developed subsequently. After a series of laboratory and field cage trials, open releases were initiated in 2006. In 2007 approximately 1.2 million transgenic moths were released into three cotton fields in Arizona (ca. 1/3 ground releases, 2/3 from aircraft), along with an equal number of standard strain. This experiment was a detailed comparison of the performance of the transgenic moths relative to the standard SIT strain; the transgenic strain was observed to have field performance equal or superior to that of the standard strain for the measured parameters (Simmons 2007, Simmons et al. 2007, USDA/APHIS 2007). In 2008, the program used this transgenic strain, rather than the standard strain, to control pink bollworm in an area of ~2500 acres. This involved the release of ~1 million GM moths per week in this area, from aircraft. The project ran for approximately 4 months (to the end of the cotton season) and involved the release of a total of ~15 million GM moths.

Each aspect of this program was performed under the regulatory oversight of the U.S. system, primarily USDA–Biotechnology Regulatory Services for the GM aspects. A series of Environmental Assessments (EAs), triggered by each significant new step (e.g., importation of transgenic moths into the United States, field cage experiments, and open release experiments), all reached a finding of no significant impact. In addition, USDA has developed an Environmental Impact Statement (EIS), the gold-standard environmental analysis in the U.S. system, for its potential program use of autocidal methods (e.g., RIDL) in its fruit fly and pink bollworm control programs (EIS available at www.aphis.usda.gov/plant_health/ea/geneng.shtml). Each of these EAs and the EIS have had public comment periods; in the case of the EIS this included a series of public meetings as well as the solicitation of written comments from the public during scoping and again after publication of a complete draft EIS. The technology covered by this EIS is similar to that being developed for mosquitoes (e.g., Phuc et al. 2007).

This extensive precedent suggests that this technology can pass rigorous independent scrutiny from the perspective of safety and environmental impact, leading to the issue of all necessary permits for the activities so far; it is also significant that the many public comment periods and consultations have passed with little negative comment from members of the public or special interest groups.

4.1. Safety

This is not the place for a comprehensive discussion of the safety of sterilized mosquitoes, whether irradiated, engineered (RIDL), *Wolbachia*-infected, or the like. Comprehensive analyses, such as the EAs and EIS above, and similar

studies for conventional SIT, have found not only that the benefits outweigh potential concerns, but also that there are few or no realistic areas of concern at all. Briefly, conventional SIT has a long history of safe use, including against mammal-targeting insects like the New World Screwworm, as well as crop pests. RIDL strains have a short additional segment of DNA; this does not encode any toxin or toxic gene product, or, importantly, any component that would confer a selective advantage to any insect or microbe that might somehow take up all or part of this DNA. Microbes sample DNA in their environment at some (low) frequency, but for insects the phylogenetic record shows that horizontal gene transfer (transfer other than by interbreeding) is extremely rare even on a multi-million year timescale between closely related species. For all of these methods, to a predator or a scavenger eating the mosquito, or a mammal being bitten by one, the consequences would be exactly the same as if this were a normal wild mosquito.

4.1.1. Public perception. Historically, sterile-release methods for control of agricultural pest insects have been well accepted relative to alternatives (e.g., insecticides) because of (i) low environmental impact and (ii) relatively unobtrusive deployment. This has been particularly noticeable in urban areas.

There are a number of complex issues relating to the deployment of any new technology. In our experience, when lay members of the public are given the opportunity to discuss and ask questions about the potential use of GM vectors for disease control, they tend to have a specific set of questions or concerns irrespective of the educational background of the person concerned. It also does not vary much between disease-endemic countries (DECs) and nonendemic countries, though individuals from DECs generally see the potential benefit of disease control more clearly and give more weight to this in any (informal) cost-benefit analysis that they may perform. The initial level of understanding of vectors, vector control methods, disease transmission, and current and future control methods is understandably rather low (typically), but the response to new ideas or methods appears to be sceptical but interested; certainly, one does not find the deeply entrenched positions that one might find regarding GM food, for example. One would likely find a similar response to proposals for IRS, or to put predators in stored drinking water, or other proposed or novel control methods.

Many of the frequent or typical questions are addressed elsewhere in this article (e.g., the first one is generally, "What happens if someone is bitten by a GM mosquito?" then the largest set are about ecosystem consequences: "What happens if a predator or scavenger eats a GM mosquito?" "What are the ecological consequences of success, that is, if you successfully suppress or eliminate the target species?"). Some other concerns relate more to the process of introducing a new technology, rather than specifically to recombinant DNA technology, or even vector control, and are addressed here:

"Why aren't you doing this in your own country first?" which might alternatively be phrased as

"Because genetic methods might entail risks, they should be tested first in the countries in which they have been developed rather than developing countries."

Insect strain construction and laboratory testing have indeed been initially performed in developed countries. We do not see this as essential, or even necessarily desirable, but it is a simple consequence of the geographic distribution of research groups and laboratories capable of developing new methods in sterile-release, especially higher-tech methods such as the use of recombinant DNA methods. However, this is not necessarily the case for field testing and deployment. It would seem generally desirable that this should be conducted in locations that have the vector, and probably also the disease, that is, DECs. No simulation outside of the potential control area will equal experiments performed in the target site and conducting them in an unrealistic location will delay the benefits to the endemic setting.

"What does SIT offer that we don't have already?"

Technical aspects are addressed in many other parts of this article. A key aspect is, "What do you have already?" In a few instances, for example, yellow fever vaccine, perhaps ivermectin for onchocerciasis, current methods can give good control. For many major vector-borne diseases, current methods appear ineffective, or insufficiently effective as applied (almost by definition, given that these are major diseases despite current control methods and programs). Currently effective methods, if any, are under constant threat from potential resistance in pathogen or vector. There is very wide consensus in the scientific community and beyond that new control methods, strategies, and ideas are desperately needed.

5. Consequences of Success

The sections above relate to various issues around, "Will it work?" There are also some issues that effectively come down to, "What if it does?"

5.1. Human consequences—herd immunity

"Mosquito population suppression will result in loss of human population immunity, thus increasing transmission in some settings."

One consequence of any successful disease control measure will be that fewer people will be infected. With the arguable exception of a vaccination program, this means that the level of immunity in the human population will decrease, leading to the presence of more susceptible individuals. Under some circumstances this could increase the risk of a disease epidemic, at least if the successful control is abandoned. It is also likely that decreased herd immunity will, over time, result in a decreased entomological threshold for transmission. If this threshold is thereby lowered to a level below the level of control actually achieved, disease transmission may resume, although at a lesser level than previously. It has been suggested that this phenomenon has occurred in Singapore, where rigorous vector control gave good protection against dengue for some years, but a significant number of cases have been recorded in the past several years (Reiter 1993, Ooi et al. 2006, Burattini et al. 2008).

SIT is, in fact, very well placed to reduce the vector population below a transmission threshold even with zero herd immunity. More precisely, situations in which reduction of transmission will result in a loss of immunity in the local human population that may result in increased transmission should consider a control method leading to vector elimination, such as SIT, which is very effective against low vector populations. If a vaccine were to become available, even

modest coverage of the population, or an incompletely effective vaccine, would eliminate this issue of herd immunity. Conversely, for vector-borne diseases such as dengue, effective vector control is likely to be required in conjunction with any vaccine, in all but the most of optimistic of scenarios.

5.2. Sustainability

"Genetic control programs might work at first, but would not be sustainable."

None of these issues relate specifically to genetic control programs such as SIT approaches. Any successful control that is then abandoned runs the risk of allowing resurgence of the disease. For example, *Ae. aegypti* was effectively eliminated from large regions of South America, primarily by use of DDT (Soper 1963, Gubler 1998). This control effort lapsed in the early 1970s due to a conjunction of several circumstances. The mosquito gradually reinvaded and by 1995 its distribution was similar to that of the 1940s, before the elimination program began. Nevertheless, it was a decade or so before the mosquito was widely reestablished, and longer before dengue virus was reintroduced. Though the situation now is that the mosquito and all four dengue serotypes are widely distributed in the region, the original program gave decades-long protection, even without maintenance.

There are also abundant precedents from agricultural SIT demonstrating how to maintain protection after a successful program. The ideal method will vary depending on the characteristics of the vector species, whether local elimination was achieved, the reintroduction (invasion) pressure, and so on. Three examples follow, with two very different pest insects.

5.2.1. New World Screwworm (NWS)-release in a barrier zone. The original NWS SIT program eradicated this pest from the United States by 1966 (Bushland 1985), though there were outbreaks in the 1970s and 1980s. The program then moved south in several phases, through Mexico and Central America as far as Panama. South America remains infested. The North and Central America are maintained free of screwworm by ongoing weekly releases of 40–50 million sterile NWS flies in a barrier zone in the Darien Gap (southern Panama and northern Colombia) to prevent reinvasion from South America. Production has been carried out since the 1970s at a mass-rearing facility in Tuxtla Gutierrez in Mexico, which has a maximum capacity of 500 million sterile flies per week, though fewer are needed to maintain the barrier. This facility now lies in an NWS-free zone, and production is being moved to a new facility in Panama.

Over the whole ca. 45-year period, the NWS eradication programs in the United States, Mexico, and Central America cost an estimated U.S. $1200 million (in 2005 dollars) (Meyer 1994, FAO 2005, Vargas-Teran et al. 2005). In contrast, the *annual* estimated benefit to these regions is estimated at U.S. $1300 million (Wyss 2000, Vargas-Teran et al. 2005). Reinvasion from South America is prevented by release of 50 million sterile flies per week in a barrier zone in eastern Panama and northern Colombia. At a cost of $\sim$$10 million per year to protect Central and North America, this continues to represent extremely good value for money.

This barrier strategy is likely to be the most efficient when the reintroduction/reinvasion route is known or predictable and so can be blocked by such a barrier.

5.2.2. Medfly–fire-fighting and preventative release program. Medfly was eliminated from California by a combination of methods including SIT as a substantial component. After elimination, monitoring (entomological surveillance) was conducted in at-risk areas; detection of Medfly infestation led to an elimination response comprising delimiting trapping, release of sterile insects, and perhaps use of insecticides and other methods, as appropriate. This might be characterized as a fire-fighting approach. This is likely to be an efficient strategy when the introduction rate is relatively low, but the routes of introduction unclear or varied, so that a barrier program is not feasible.

Over time, it became clear that the invasion or outbreak rate was relatively high (introduction routes are somewhat uncertain, but are likely to be importation of infested host material, either by private citizens or commercial). A new, alternative maintenance strategy was therefore introduced in 1994. This was continuous, prophylactic release of sterile Medflies over the at-risk area, the so-called Preventative Release Program. Sterile males are always present, so any newly introduced females—typically low in number—are likely to mate the much larger number of sterile males, so the new infestation is rapidly and automatically eliminated. The switch from a reactive approach to preventative release program resulted in a 96% drop in the number of wild Medfly captures (Enkerlin 2005), a halving of the overall cost of the Medfly control program, and only two Medfly outbreaks in the treated area between 1994 and 1998 (Enkerlin 2005), compared with an annual average of seven or eight before its implementation (USDA/APHIS 1992). This method has been clearly demonstrated to be effective, sustainable, and significantly cheaper than the fire-fighting strategy. This is likely to be the most efficient strategy when the reintroduction rate is relatively high.

5.2.3. Strain stability. **"GM strains are unstable and will rapidly break down."**

This statement is incorrect in the context of SIT strategies. Modeling suggests that instability rates of 1% or so would not compromise program effectiveness given appropriate quality control to prevent the accumulation of breakdown products in the rearing facility (Phuc et al. 2007). In principle, spontaneous mutation should lead to a breakdown rate of 10^{-6} to 10^{-7} per generation, but this is too low to be readily detectable in laboratory-scale experiments. Recombination may lead to breakdown of translocation-based strains at a rather higher rate than this. However, there is substantial experience with the use of unstable translocation-based strains in Medfly SIT, for which breakdown is certainly detectable at a significant rate, but, despite this, rearing systems have been developed to maintain high-quality, consistent output from the rearing facility (clean filter rearing system [Franz 2005]). Such systems have been widely implemented and are currently used to produce $>$2 billion sterile males per week. Therefore, we do not anticipate any insuperable difficulties for mosquito sterile-release programs due to strain (in)stability.

5.2.4. Resistance. **"Resistance may emerge and negate the strategy."**

Resistance is a potential issue for any vector control strategy, or indeed for drugs or vaccines. However, in practice the SIT has an excellent record for (lack of) resistance. Resistance

through assortative mating has been reported in several cases, but was generally found to be associated with a loss of quality in the mass-reared insects, probably due to inbreeding; this was rapidly reversed by improved genetics. We know of only one instance of reasonably well-documented resistance through assortative mating in the absence of clear decline of sterile insect production quality in the entire 50+ years of SIT (Hibino and Iwahashi 1991, Koyama et al. 2004). One might argue that we have less experience with new sterilization methods (genetic, *Wolbachia*, etc.) than with radiation in this regard, and there may be some potential for biochemical resistance to the effector molecules used in these systems. This is not a new issue, and would apply also to any other chemical, insecticidal, or genetic strategy. Large-scale field experience will determine the rate at which such resistance may emerge—it has not been detected in laboratory studies. Fortunately, there are many available effector molecules, so if this turns out to be a problem it should not be difficult to develop replacement strains, perhaps with multiple (stacked) effectors.

Some SIT strategies have additional resistance management properties that would further slow or reverse the spread of any incipient resistance in the target population (Alphey et al. 2007a, 2009).

5.3. Ecosystem consequences

In our experience, most questions from people outside of insect control and allied disciplines, including lay members of the public in potential release areas, relate much more to ecosystem consequences than to human health. Thus, what are the ecological consequences of suppressing or removing a pest? Again this is clearly a "what if the program succeeds" question.

This is something that needs to be considered on a case-by-case basis. *Ae. aegypti* is native to some parts of Africa but has been inadvertently spread around the world by humans in the relatively recent past. One would not therefore expect any native species in the Americas, for example, to be dependent on this vector species. Indeed, removal or suppression of an invasive exotic species might be considered somewhat desirable. In general, though many species (birds, fish, arthropods, scavengers, and microbes) will feed on live or dead mosquitoes (adult or immature stages) if they find them, there seem to be rather few specialists that depend on mosquitoes. Even then, the species-specific nature of SIT means that only a single species would be removed or suppressed; this approach is perhaps as precisely targeted and environmentally benign as any vector control method can be. Further, should unacceptable consequences be observed, SIT suppression is reversible before global eradication of the target species.

5.3.1. Empty ecological niche. **"Eliminating a single vector may leave an empty niche that will be invaded by another, perhaps more harmful, vector."**

This clearly needs to be considered in advance, but is not likely to be a significant problem in many settings. SIT will be used to target the major vector in a given setting. Are other vectors, especially more serious vectors, excluded from this setting or ecological niche by the presence of the principal vector? In the case of dengue viruses, *Ae. aegypti* is clearly the major vector around the world. In some areas its range overlaps with *Ae. albopictus*. It is possible that controlling *Ae. aegypti* would allow some increase in the numbers or range of *Ae. albopictus*, though there seems to be little actual evidence for this. Since the result would be to replace the most severe vector with a less anthropophilic, less effective vector, such an outcome would likely represent only a marginal reduction in the net beneficial effect of controlling *Ae. aegypti*. In some cases an SIT program against *Ae. albopictus* may well be desirable in any case, and this could certainly be combined with, or follow on from, a successful program against *Ae. aegypti*.

6. Priorities for Investment

"Looking ahead to a future of area-wide control of vectors by IVM with a strong SIT component against key vector species, how do we get there from here?"

What are the key steps and critical path? The single step that would transform the field is a successful contemporary demonstration of existing or novel SIT methodologies against a mosquito vector. No series of laboratory or cage experiments (e.g., measuring components or predictors of success, such as male mating competitiveness) can substitute for this. A pilot program would scale up from laboratory to cage to field, eventually validating (or otherwise) the strategy in town/city size pilot programs. As a side benefit, a successful field trial would yield much practical and basic information (e.g., about mosquito ecology and genetics).

The basic technology (rearing, sterilization procedure, and strain development) is in place for only a few vector species. There is considerable opportunity to improve currently available technologies by further strain development. This would logically involve (i) improving today's best technologies, working on those few lead species in which they are most developed and (ii) translating these technologies to other key vector species for which the necessary strains and methods are not yet available. In particular, some investment could usefully be directed to developing strains of key *Anopheles* species that match the best *Aedes* strains.

An additional priority for investment would be the development of improved rearing and distribution systems for relevant species. Technology exists or can be adapted from prior mosquito trials (1970s) and ongoing agricultural programs, but needs to be built and tested. A modest investment in methods development would reap considerable returns in cost effectiveness in the longer term. A third investment priority is development of methods for producing and recognizing high-quality males that are competitive and capable of impacting the targeted population at relatively low release ratios. The fourth investment priority is related to communication and capacity building—specifically, better defining the regulatory approval process and assessing needs related to community acceptance/participation.

7. Concluding Remarks

SIT may be considered an emerging viable approach to vector control. In contrast to other genetic control methods, the necessary strains and technology for SIT have already been developed for several vector species and are on the verge of field assessment. Several technical aspects warrant further investment, related to efficiency, cost-effectiveness improvements, and field validation of the new methodologies. However, there are no fundamental technical barriers

to large-scale field use of SIT for many important mosquito species.

Acknowledgments

The authors were members of a Working Group SIT including RIDL at the Vector Control Consultation meeting organized by the Bill and Melinda Gates Foundation in Seattle, July 28–30, 2008. This article arose from those discussions. We thank Nina Alphey for contributions to the development of this paper and Jorge Hendrichs and Marc Vreysen for comments on a near-final version.

Funded in part by a grant to the Regents of the University of California from the Foundation for the National Institutes of Health through the Grand Challenges in Global Health initiative (L.A.).

Disclosure Statement

L.A. has employment and equity interest in Oxitec Ltd, a company developing methods (e.g. RIDL) discussed in this article. R.B. is currently employed by CAA "G. Nicoli," a public society developing SIT methods against mosquitoes. The other authors declare no competing interests.

References

Allen, M, Berkebile, D, Skoda, S. Postlarval fitness of transgenic strains of *Cochliomyia hominivorax* (Diptera: Calliphoridae). J Econ Entomol 2004; 97:1181–1185.

Alphey, L. Re-engineering the sterile insect technique. Insect Biochem Mol Biol 2002; 32:1243–1247.

Alphey, L. Engineering insects for the sterile insect technique. In: Vreysen, M, Robinson, A, Hendrichs, J, eds. *Area-Wide Control of Insect Pests: From Research to Field Implementation.* Dordrecht, The Netherlands: Springer, 2007:51–60.

Alphey, L. Natural and engineered mosquito immunity. J Biol 2009; 8:40.

Alphey, L, Andreasen, MH. Dominant lethality and insect population control. Mol Biochem Parasitol 2002; 121:173–178.

Alphey, L, Beard, B, Billingsley, P, Coetzee, M, et al. Malaria control with genetically modified vectors. Science 2002; 298:119–121.

Alphey, N, Bonsall, M, Alphey, L. Combining pest control and resistance management: synergy of engineered insects with Bt crops. J Econ Entomol 2009; 102:717–732.

Alphey, N, Coleman, PG, Bonsall, M, Alphey, L. Proportions of different habitat types are critical to the fate of a resistance allele. Theor Ecol 2008; 1:103–115.

Alphey, N, Coleman, PG, Donnelly, CA, Alphey, L. Managing insecticide resistance by mass release of engineeered insects. J Econ Entomol 2007a; 100:1642–1649.

Alphey, L, Nimmo, D, O'Connell, S, Alphey, N. Insect population suppression using engineered insects. In: Aksoy, S, ed. *Transgenesis and the Management of Vector-Borne Disease*. Austin, TX: Landes Bioscience, 2007b:93–103.

Amazigo, U, Boatin, B. The future of onchocerciasis control in Africa. Lancet 2006; 368:1946–1947.

Ansari, M, Singh, K, Brooks, G, Malhotra, P, Vaidyanathan, V. The development of procedures and techniques for mass rearing of *Aedes aegypti*. Ind J Med Res 1977; 65(Suppl):91–99.

Ansari, M, Singh, KRP, Brooks, G, Malhotra, P, Vaidyanathan, V. *The Development of Procedures for Mass Rearing of* Aedes aegypti. Geneva: World Health Organization, 1975:9.

Atkinson, MP, Su, Z, Alphey, N, Alphey, LS, et al. Analyzing the control of mosquito-borne diseases by a dominant lethal genetic system. Proc Natl Acad Sci USA 2007; 104:9540–9545.

Bailey, D, Fowler, J, Lowe, R. Production efficiency and rate of increase of a mass-reared laboratory colony of *Anopheles albimanus* Wiedemann. Mosq News 1979; 39:640–644.

Benedict, M, Robinson, A. The first releases of transgenic mosquitoes: an argument for the sterile insect technique. Trends Parasitol 2003; 19:349–355.

Bill and Melinda Gates Foundation and Boston Consulting Group. *Market Assessment for Public Health Pesticide Products;* Seattle, WA. 2007:51.

Bloem, KA, Bloem, S, Carpenter, JE. Impact of moth suppression/eradication programmes using the sterile insect technique or inherited sterility. In: Dyck, VA, Hendrichs, J, Robinson, AS, eds. *Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management*. Dordrecht, The Netherlands: Springer, 2005:677–700.

Brelsfoard, C, Sechan, Y, Dobson, S. Interspecific hybridization yields strategy for South Pacific filariasis vector elimination. PLoS Negl Trop Dis 2008; 2:e129.

Burattini, MN, Chen, M, Chow, A, Coutinho, FAB, et al. Modelling the control strategies against dengue in Singapore. Epidemiol Infect 2008; 136:309–319.

Bushland, RC. Eradication program in the southwestern United States. *Symposium on the eradication of the Screwworm from the United States and Mexico. Misc. Pub.* Entomol Soc Am Misc Publ 1985; 62:12–15.

Catteruccia, F, Benton, J, Crisanti, A. An *Anopheles* transgenic sexing strain for vector control. Nat Biotechnol 2005; 23:1414–1417.

Catteruccia, F, Godfray, H, Crisanti, A. Impact of genetic manipulation on the fitness of *Anopheles stephensi* mosquitoes. Science 2003; 299:1225–1227.

Cayol, JP, Coronado, P, Taher, M. Sexual compatibility in the Medfly (Diptera: Tephritidae) from different origins. Fla Entomol 2002; 85:51–57.

Condon, K, Condon, G, Dafa'alla, T, Fu, G, et al. Genetic sexing through the use of Y-linked transgenes. Insect Biochem Mol Biol 2007; 37:1168–1176.

Dame, D, Lowe, R, Williamson, D. Assessment of released sterile *Anopheles albimanus* and *Glossina morsitans morsitans.* In: Kyoto, RP, Kitzmiller, J, Kanda, T, eds. *Cytogenetics and Genetics of Vectors. Proc XVI Internat. Cong. Entomol.* New York: Elsevier Science, 1981:231–248.

Dobson, S, Fox, C, Jiggins, F. The effect of *Wolbachia*-induced cytoplasmic incompatibility on host population size in natural and manipulated systems. Proc Roy Soc Lond B 2002; 269:437–445.

Dyck, VA, Hendrichs, J, Robinson, AS, eds. *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management.* Dordrecht, The Netherlands: Springer, 2005a.

Dyck, VA, Regidor Fernandez, EE, Reyes Flores, J, Teruya, T, et al. Public relations and political support in area-wide integrated pest management programmes that integrate the sterile insect technique. In: Dyck, VA, Hendrichs, J, Robinson, AS, eds. *Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management.* Dordrecht, The Netherlands: Springer, 2005b:547–559.

Dye, C. Models for the population dynamics of the yellow fever mosquito, *Aedes aegypti.* J Anim Ecol 1984; 53:247–268.

Egger, J, Ooi, E, Kelly, D, Woolhouse, M, et al. Reconstructing historical changes in the force of infection of dengue fever in

Singapore: implications for surveillance and control. Bull WHO 2008; 86:187–196.

Enkerlin, WR. Impact of fruit fly control programmes using the sterile insect technique. In: Dyck, VA, Hendrichs, J, Robinson, AS, eds. *Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management.* Dordrecht, The Netherlands: Springer, 2005:651–676.

Enserink, M. Welcome to Ethiopia's fly factory. Science 2007; 317:310–313.

FAO. *La cooperación internacional en al control, erradicación y prevención del gusano barrenador del ganado.* Rome, Italy: FAO, 2005.

FAO/IAEA. *Model Business Plan for a Sterile Insect Production Facility.* Vienna, Austria: IAEA, 2008.

FAO/IAEA/USDA. *Manual for Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies V5.0.* IAEA: Vienna, Austria. 2003.

Feldmann, U, Dyck, VA, Mattioli, RC, Jannin, J. Potential impact of tsetse fly control involving the sterile insect technique. In: Dyck, VA, Hendrichs, J, Robinson, AS, eds. *Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management.* Dordrecht, The Netherlands: Springer, 2005:701–723.

Focks, DA. An improved separator for separating the developmental stages, sexes and species of mosquitoes. Mosq News 1980; 19:144–147.

Focks, DA, Alexander, N. *Multicountry Study of* Aedes aegypti *Pupal Productivity Survey Methodology: Findings and Recommendations.* Geneva, Switzerland: WHO-TDR, 2006:48.

Focks, DA, Brenner, RJ, Hayes, J, Daniels, E. Transmission thresholds for dengue in terms of *Aedes aegypti* pupae per person with discussion of their utility in source reduction efforts. Am J Trop Med Hyg 2000; 62:11–18.

Franz, G. Genetic sexing strains in mediterranean fruit fly, an example for other species amenable to large-scale rearing for the sterile insect technique. In: Dyck, VA, Hendrichs, J, Robinson, AS, eds. *Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management.* Dordrecht, The Netherlands: Springer, 2005:427–451.

Fu, G, Condon, KC, Epton, MJ, Gong, P, et al. Female-specific insect lethality engineered using alternative splicing. Nat Biotechnol 2007; 25:353–357.

Girod, R, Coetzee, M, Salvan, M, Hunt, R. Polymorphisme chromosomique des populations d'*Anopheles arabiensis* (Diptera: Culicidae) de l'ile de la Reunion et inter-fertilite avec des populations d'Afrique continentale. Parasitologia 2001; 43:99–103.

Gubler, D. Dengue and dengue hemorrhagic fever. Clin Microbiol Rev 1998; 11:480–496.

Halstead, S. Successes and failures in dengue control—global experience. Dengue Bull 2000; 24:60–70.

Heinrich, J, Scott, M. A repressible female-specific lethal genetic system for making transgenic insect strains suitable for a sterile-release program. Proc Natl Acad Sci USA 2000; 97: 8229–8232.

Heintze, C, Garrido, M, Kroeger, A. What do community-based dengue control programmes achieve? A systematic review of published evaluations. Trans Roy Soc Trop Med Hyg 2006; 101:317–325.

Hibino, Y, Iwahashi, O. Appearance of wild females unreceptive to sterilized males on Okinawa island in the eradication programme of the melon fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae). Appl Entomol Zool 1991; 26:265–270.

Institute of Medical Research. Annual Report of the Director-General of Health Malaysia (Laporan-laporan Teknikal Tahunan Ketua Pengarah Kesihatan Malaysia), Kuala Lumpur, Malaysia, 2007.

Irvin, N, Hoddle, M, O'Brochta, D, Carey, B, Atkinson, P. Assessing fitness costs for transgenic *Aedes aegypti* expressing the GFP marker and transposase genes. Proc Natl Acad Sci USA 2004; 101:891–896.

Jegede, A. What led to the Nigerian boycott of the polio vaccination campaign? PLoS Med 2007; 4:e73.

Kaiser, PE, Seawright, JA, Dame, DA, Joslyn, DJ. Development of a genetic sexing for *Anopheles albimanus*. J Econ Entomol 1978; 71:766–771.

Kean, J, Suckling, D. Estimating the probability of eradication of painted apple moth from Auckland. NZ Plant Prot 2005; 58:7–11.

Klassen, W. Area-wide integrated pest management and the sterile insect technique. In: Dyck, VA, Hendrichs, J, Robinson, AS, eds. *Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management.* Dordrecht, The Netherlands: Springer, 2005:39–68.

Klassen, W, Curtis, CF. History of the sterile insect technique. In: Dyck, VA, Hendrichs, J, Robinson, AS, eds. *Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management.* Dordrecht, The Netherlands: Springer, 2005:3–36.

Knipling, E. Possibilities of insect control or eradication through use of sexually sterile males. J Econ Entomol 1955; 48:459–462.

Knipling, E. *Agriculture Handbook No. 512: The Basic Principles of Insect Population Suppression and Management*. Washington, DC: USDA, 1979.

Knipling, E. Role of parasitoid augmentation and sterile insect techniques for area-wide management of agricultural insect pests. J Agric Entomol 1998; 15:273–301.

Kouri, G, Guzman, M, Bravo, J. Hemorrhagic dengue in Cuba: history of an epidemic. Bull Pan Am Health Org 1986; 20:24–30.

Koyama, J, Kakinohana, H, Miyatake, T. Eradication of the Melon Fly *Bactrocera cucurbitae* in Japan: importance of behaviour, ecology, genetics and evolution. Ann Rev Entomol 2004; 49:331–349.

Krafsur, E. Sterile insect technique for suppressing and eradicating insect populations: 55 years and counting. J Agric Entomol 1998; 15:303–317.

Kroeger, A, Lenhart, A, Ochoa, M, Villegas, E, et al. Effective control of dengue vectors with curtains and water container covers treated with insecticide in Mexico and Venezuela: cluster randomised trials. Br Med J 2006; 332:1247–1250.

Lavery, JV, Tinadana, PO, Scott, TW, Harrington, LC, et al. Towards a framework for community engagement in global health research. 2008 (in review).

Lee, H, Vasan, S, Nazni, W, Shanaz, M. *Scientific Report on the Innovative Application of* Aedes aegypti *RIDL-Sterile Insect Technique to Combat Dengue and Chikungunya in Malaysia*. Kuala Lumpur, Malaysia: Institute of Medical Research, 2008.

Lindquist, DA, Abusowa, M, Hall, MJ. The New World screwworm fly in Libya: a review of its introduction and eradication. Med Vet Entomol 1992; 6:2–8.

Lofgren, CS, Dame, DA, Breeland, SG, Weidhaas, DE, et al. Release of chemosterilized males for the control of *Anopheles albimanus* in El Salvador. III. Field methods and population control. Am J Trop Med Hyg 1974; 23:288–297.

Lowe, R, Fowler, E, Bailey, D, Dame, D, Savage, K. Separation of sexes of adult *An. albimanus* by feeding of insecticide-laden blood. Mosq News 1981; 41:634–638.

Marrelli, M, Li, C, Rasgon, J, Jacobs-Lorena, M. Transgenic malaria-resistant mosquitoes have a fitness advantage when feeding on *Plasmodium*-infected blood. Proc Natl Acad Sci USA 2007; 104:5580–5583.

Marrelli, MT, Moreira, CK, Kelly, D, Alphey, L, Jacobs-Lorena, M. Mosquito transgenesis: what is the fitness cost? Trends Parasitol 2006; 22:197–202.

Meyer, NL. *History of the Mexican-United States Screwworm Eradication Program.* New York: Vantage Press, 1994.

Moreira, L, Wang, J, Collins, F, Jacobs-Lorena, M. Fitness of anopheline mosquitoes expressing transgenes that inhibit plasmodium development. Genetics 2004; 166:1337–1341.

Nature. Editorial—Oh, New Delhi; Oh, Geneva. Nature 1975; 256:355–357.

Ooi, E, Goh, K, Gubler, D. Dengue prevention and 35 years of vector control in Singapore. Emerg Infect Dis 2006; 12:887–893.

Peloquin, JJ, Thibault, ST, Staten, R, Miller, TA. Germ-line transformation of pink bollworm (Lepidoptera: Gelechiidae) mediated by the *piggyBac* transposable element. Insect Mol Biol 2000; 9:323–333.

Phuc, HK, Andreasen, MH, Burton, RS, Vass, C, et al. Late-acting dominant lethal genetic systems and mosquito control. BMC Biol 2007; 5:11.

Reiter, P. Dengue control in Singapore. In: Goh, KT, ed. *Dengue in Singapore*. Singapore: Institute of Environmental Epidemiology, Ministry of the Environment, 1993:213–242.

Rendón, P, McInnis, D, Lance, D, Stewart, J. Medfly (Diptera: Tephritidae) genetic sexing: large-scale field comparison of males-only and bisexual sterile fly releases in Guatemala. J Econ Entomol 2004; 97:1547–1553.

Rogers, D, Randolph, S. From a case study to a theoretical basis for tsetse control. Insect Sci Appl 1984; 5:419–423.

Rogers, D, Randolph, S. A response to the aim of eradicating tsetse from Africa. Trends Parasitol 2002; 18:534–536.

Seawright, J, Kaiser, P, Dame, D, Lofgren, C. Genetic method for the preferential elimination of females of *Anopheles albimanus*. Science 1978; 200:1303–1304.

Service, M. *Mosquito Ecology. Field Sampling Methods, 2nd Edition.* London: Chapman and Hall, 1993.

Silver, J. *Mosquito Ecology: Field Sampling Methods, 3rd Edition.* Dordrecht, The Netherlands: Springer, 2008.

Simmons, G. Genetically modified pink bollworm. CPHST News 2007; 4:7.

Simmons, G, Alphey, L, Vasquez, T, Morrison, NI, et al. Pink bollworm *Pectinophora gossypiella* in area-wide eradication or suppression programmes. In: Vreysen, MB, Robinson, AS, Hendrichs, J, eds. *Area-Wide Control of Insect Pests.* Dordrecht, The Netherlands: Springer, 2007:119–123.

Singh, KRP, Razdan, RK. *Mass Rearing of* Culex pipiens fatigans *Wied. Under Ambient Conditions.* Geneva: World Health Organization, 1975:6.

Soper, F. The elimination of urban yellow fever in the Americas through the eradication of *Aedes aegypti*. Am J Public Health 1963; 53:7–16.

Soper, F, Wilson, D. Anopheles gambiae *in Brazil, 1930 to 1940.* New York: The Rockefeller Foundation, 1943.

Thomas, DD, Donnelly, CA, Wood, RJ, Alphey, LS. Insect population control using a dominant, repressible, lethal genetic system. Science 2000; 287:2474–2476.

USDA. *Exotic Fruit Fly Strategic Plan FY 2006–2010.* USDA/APHIS, ed. Riverdale, MD. 2006:28.

USDA/APHIS. *Center for Plant Health Science and Technology Accomplishments, 2007*. Raleigh, NC, 2007:48.

USDA/APHIS. *Risk Assessment: Mediterranean Fruit Fly.* Riverdale, MD. 1992.

U.S. Office of Management and Budget. *Historical Tables, Budget of the United States Government, Fiscal Year 2009*. Washington, DC: U.S. Government Printing Office, 2008.

Vale, G, Torr, S. User-friendly models of the costs and efficacy of tsetse control: application to sterilizing and insecticidal techniques. Med Vet Entomol 2005; 19:293–305.

Vargas, T, Hursey, B, Cunningham, E. Eradication of the screwworm from Libya using the sterile insect technique. Parasitol Today 1994; 10:199–122.

Vargas-Teran, M, Hofmann, HC, Tweddle, NE. Impact of screwworm eradication programmes using the sterile insect technique. In: Dyck, VA, Hendrichs, J, Robinson, AS, eds. *Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management.* Dordrecht, The Netherlands: Springer, 2005:629–650.

Vreysen, M, Robinson, AS, Hendrichs, J. *Area-Wide Control of Insect Pests: From Research to Field Implementation.* Dordrecht, The Netherlands: Springer, 2007.

Weidhaas, DE, Breeland, SG, Lofgren, CS, Dame, DA, Kaiser, R. Release of chemosterilized males for the control of *Anopheles albimanus* in El Salvador. IV. Dynamics of the test population. Am J Trop Med Hyg 1974; 23:298–308.

Weidhaas, D, Patterson, R, Lofgren, C, Ford, H. Bionomics of a population of *Culex pipiens quinquefasciatus* Say. Mosq News 1971; 31:177–182.

Whitten, M, Mahon, R. Misconceptions and constraints. In: Dyck, VA, Hendrichs, J, Robinson, AS, eds. *Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management.* Dordrecht, The Netherlands: Springer, 2005:601–626.

WHO. WHO-supported collaborative research projects in India: the facts. WHO Chronicle 1976; 30:131–139.

Wyss, JH. Screw-worm eradication in the Americas—overview. In: Tan, KH, ed. *Area-Wide Control of Fruit Flies and Other Insect Pests.* Penang: Penerbit Universiti Sains Malaysia, 2000:79–86.

Xi, Z, Khoo, C, Dobson, S. Wolbachia establishment and invasion in an *Aedes aegypti* laboratory population. Science 2005; 310:326–328.

Yakob, L, Alphey, L, Bonsall, M. *Aedes aegypti* control: the concomitant role of competition, space and transgenic technologies. J Appl Ecol 2008; 45:1258–1265.

Address correspondence to:
Luke Alphey
Oxitec Limited
71 Milton Park
Oxford OX14 4RX
United Kingdom

E-mail: luke.alphey@oxitec.com