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Preventing the establishment of invasive exotic mosquitoes

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The boosted sterile insect technique, which involves releasing sterile males treated with a biocide such as pyriproxyfen to contaminate breeding sites, has been shown to be effective against *Aedes* vectors. This approach could prevent the establishment of invasive mosquitoes sharing the same larval habitats, such as *Anopheles stephensi*.

Threats from invasive mosquito species

Vector-borne diseases represent a major global health challenge, accounting for 17% of all infectious diseases and resulting in more than 1 million deaths annually, as reported by the World Health Organization (WHO) [1]. Of these diseases, malaria, transmitted by *Anopheles* mosquitoes and arboviral diseases such as dengue, chikungunya, yellow fever, and Zika, transmitted by *Aedes* mosquitoes, are of particular concern.

Malaria remains a public health priority worldwide, with 263 million cases reported by the WHO in 2023, mainly in tropical Africa. *Anopheles stephensi*, which was originally an urban vector of malaria in India, is invading the entire African continent, raising fears of a resurgence of this disease in this already particularly affected region. Its presence was confirmed in eight African countries since 2012 [2]. Islands of the Indian Ocean region are also at a high risk of invasion and include Mayotte, Comoros, Madagascar (still endemic for malaria) and Réunion, Mauritius, and Seychelles (free of the disease). The risk of invasion is even more worrying for the region because its recent establishment in Kenya raises fears of a probable extension toward Tanzania, Mozambique, and neighboring countries, which have active, extensive commercial and human international links with those islands, notably with Comoros. Although several methods could effectively reduce the density of *An. stephensi* [3], none would allow its elimination after its introduction into a new territory.

The incidence of arboviruses is also increasing every year. Europe has largely been invaded by *Aedes albopictus* during the 2000s, whereas *Aedes aegypti* remained limited to Madeira and the areas bordering the Black Sea. The invasion of Europe by *Aedes aegypti* would be catastrophic in terms of public health because it is the most competent vector for most arboviruses (dengue, chikungunya, Zika). However, its invasion of Europe is more and more likely because of globalization and climate change, especially because it is regularly introduced, as, for example, in Marseille in 2018 [4]. It has also successfully established itself in Cyprus since 2021 [5].

These challenges and threats are prompting health managers, supported by research teams, to strengthen surveillance systems of these vector-borne diseases and response capacities to their risk of expansion and resurgence. It is generally established that preventing a potential invasion far outweighs the curative cost associated with disease casualties as well as economic impacts of invasive mosquito vectors [6,7]. Here, we propose such a prevention strategy based on the heterospecific impact of the boosted sterile insect technique (SIT) (Figure 1A).

Lessons from boosted SIT trials

During a case-control field trial carried out in Valencia, Spain, sterile male *Ae. albopictus* mosquitoes treated with pyriproxyfen and released at a density of 1700–2700 sterile males/ha/wk led to a 89% and 98% suppression of *Ae. albopictus* in two villages. In Reunion Island, sterile males of *Ae. aegypti* treated with pyriproxyfen and released at a density of ~600 males/ha every 2 weeks led to a >90% suppression of adult *Ae. aegypti* in 3 months. This trial also led to a 60% suppression of *Ae. albopictus*, which shares some of the larval habitats with *Ae. aegypti* in the release area, demonstrating the heterospecific impact of this method [8]. Similarly, in China, releasing 15 000 *Wolbachia*-carrying male *Ae. albopictus* treated with pyriproxyfen per ha per week resulted in a 72.1% suppression of adult *Ae. albopictus* [9]. These releases also significantly reduced the density of *Culex quinquefasciatus*, although this species only occasionally overlaps the breeding sites of *Ae. albopictus* and has different dominant habitats and peak seasons.

During a semifield experiment carried out in Reunion [10], we found that pyriproxyfen-treated sterile males of *Ae. albopictus* are capable of contaminating larval breeding sites even in the absence of females! Actually, the concentration of pyriproxyfen in the breeding sites, represented by ovitraps in this experiment, was similar to the presence or absence of females (Figure 1B), showing direct contamination of the larval habitats by boosted males.

Lessons learned from surveillance of entry points in Mauritius

Because of its connections with Asia and Africa, Mauritius is particularly exposed to the risk of introduction of *An. stephensi*. A network of 38 artificial breeding sites imitating the larval habitats of this species is therefore regularly monitored in the seaport of Mauritius [11]. During 25 successive surveys carried out from July 2022

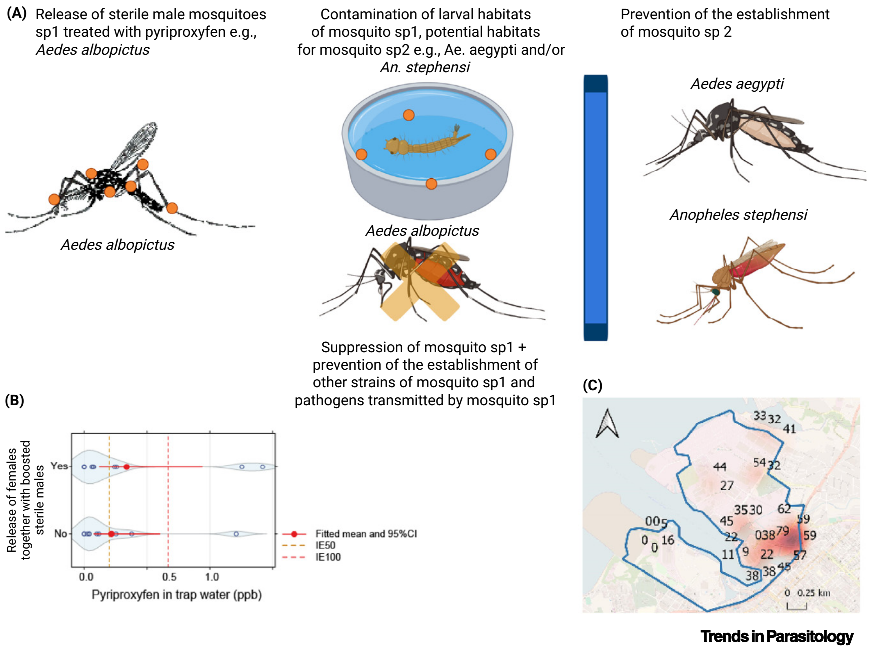

Figure 1. Application of the boosted sterile insect technique to suppress a local mosquito species while preventing the establishment of exotic species. (A) Principle of the strategy. (B) Amount of pyriproxyfen transferred by boosted sterile male *Aedes albopictus* in ovitrap water from large field cages, conditionally on the presence of untreated females; vertical red doted lines represent the concentration leading to 10% and 95% inhibition of adult emergence (source: modified from Figure 2 of [10]). (C) Heat map showing the frequency of artificial mosquito breeding sites positive for larvae of *Ae. albopictus* in the seaport of Mauritius; the numbers represent the density of larvae (source: modified from Figure 3 of [11]). Created with BioRender. Abbreviations: CI, confidence interval; IE50, inhibition emergence 50%; IE100, inhibition emergence 100%; Sp, species.

to April 2023, no specimens of *An. stephensi* were detected. However, 85% of these breeding sites (n = 32) were positive for *Ae. albopictus* (Figure 1C), with about one-third of potential breeding sites positive for *Ae. albopictus* during each survey. This ability of this species to occupy the same breeding sites as *An. stephensi* in tropical areas has also been highlighted [12].

Proposition of a new strategy to prevent mosquito invasions

On the basis of these two results, we propose to use boosted SIT against a local species of major public health interest (e.g., *Ae. albopictus*, the vector of dengue and chikungunya in Reunion and Mauritius) to contaminate the larval habitats that may be shared by other species and that are threatening to invade a given territory, with pyriproxyfen or other biopesticides active at low concentration (Figure 1A). In this particular case, this method, applied to points of entry, can prevent the introduction of notable species such as *An. stephensi* and *Ae. aegypti* on these two islands. It will also suppress homospecific populations at points of entry, preventing the transmission of pathogens by this species, as well as the introduction of other populations of the same species, which may be more competent or resistant to insecticides than the already established populations. In Europe, this strategy can be applied to prevent the introduction of *Ae. aegypti* using *Ae. albopictus* and the reverse in African, Asian, or American countries not yet colonized by *Ae. albopictus*. It is actually much more difficult to apply genetic control against these species when they are both present because of the risk of competitive release, where the specific suppression of a target species can favor another one sharing the same ecological niche [13]. A major advantage of the strategy we are proposing is that besides its cost-effectiveness and rapidity of deployment, it circumvents the risk of importing an invasive species into a country it has not yet colonized for its mass production and sterilization. Instead, sterile males of a local vector species of major interest sharing similar breeding sites could be mass produced in existing SIT facilities [14] to counter a potential invasion. This strategy has many advantages over other methods: it is efficient on a low-density target species, it can reach and treat cryptic or inaccessible breeding sites, and it presents no risk of resistance. Research is needed to evaluate in the field the density and frequency of sterile males to be released to achieve a treatment coverage and a concentration of biocide in the larval habitats that will completely inhibit potential invaders at points of entry. Also, it will be important to improve the pyriproxyfen formulation in order to reduce the large variations of the concentration observed during the semifield trial [10], because exposure to sublethal doses may lead to the development of resistance to this molecule or even to cross-resistance to other insecticides [15] in the species used to contaminate the breeding sites. Given that the response to a potential invasion must be rapid and effective, this strategy will require investment in appropriate infrastructure and the strengthening of local capacity to mass produce local species of public health interest as a preventive measure, which is never easy to finance. Also, it will always be necessary to improve surveillance systems in order to be able to detect the presence of an invasive mosquito at an early stage and to monitor the efficiency of this strategy to prevent its establishment and spread.

Finally, some large breeding sites suitable to *An. stephensi* may not be shared with *Aedes* species, and it will be necessary to destroy or treat them with other insecticides (e.g., *Bacillus thuringiensis*) to avoid the selection of different oviposition preference in this species. Fortunately, these breeding sites are much easier to locate and control around entry points that the cryptic ones.

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Declaration of interests

J.B. is cofounder and CEO of MoSITouch, a company offering vector control services, particularly the boosted sterile insect technique.

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