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# 1    **Yes, Irradiated Sterile Male Mosquitoes Can Be Sexually Competitive!**

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8    **Keywords:** fried competitiveness index, sterile insect technique, *Aedes*, *Anopheles*, dengue,  
9    malaria

10

## 11    **Abstract**

12    Adequate sexual competitiveness of sterile males is a prerequisite for genetic control methods,  
13    including the sterile insect technique. During the last decade, several semi-field and open field  
14    trials demonstrated that irradiated male mosquitoes can be competitive.

15

Although the biological quality or competitiveness (see Box 1) of released sterile male insects is essential to ensure success in genetic insect pest control programmes, in most cases this is rarely assessed [1]. Entomological effectiveness in all male release programmes- including irradiated, *Wolbachia*-infected, and transgenic males - that use colonized insects can only be proven by calculating the competitiveness of the released males. Here, we argue that a reduction in quality of the produced sterile male insects is mostly related to the mass-rearing, handling, marking and release processes, rather than radiation *per se*. As an example, in SIT programmes against tsetse flies, it was demonstrated that chilling and transport of sterile male pupae were the main factors impacting their quality [2].

The competitiveness  $C$  of a sterile male is the odds of a wild female being mated with a sterile male compared to a wild male when exposed to both in equal numbers. A  $C$  value of 1 indicates that sterile and wild males are equally competitive. A  $C$  value of 0.5 indicates that females are two times more likely to be mated with wild males. The sterile insect technique (SIT) is a genetic control tactic used for the management of selective insect pests and relies on the release of mass-produced male insects that are sterilized by ionizing radiation. A mating of a wild female insect with a sterile male will result in no offspring. Insemination of the oocyte with sperm that contains numerous dominant lethal mutations will cause embryonic arrest and death. In the case of SIT, an appropriate irradiation dose must be selected that ensures adequate sterility without impairing  $C$ .

### **Insights from semi-field trials**

Eleven studies with four mosquito species (*Aedes aegypti*, *Ae. albopictus*, *Anopheles arabiensis* and *An. coluzzii*) were analysed, and in most cases the irradiation treatment was given during the pupal stage (see supplemental Table S1). The studies used different doses,

type of irradiators, male age when irradiated and when offered the females for mating, which all can affect the amount of dominant lethal mutations in the sperm [3] and, hence, the  $C$  value. Also, the ratio  $R$  of sterile to wild males was variable which can impact the observed  $C$  (Box 1). In general, *Anopheles* species required a higher dose to obtain the same level of sterility as compared with *Aedes* species and the irradiation dose was negatively correlated with the  $C$  values (Figure 1A). However, this was not consistent, and  $C$  was sometimes very variable at the same dose, e.g. a  $C$  value between 0.4 and 1 for *Ae. albopictus* that were irradiated with a dose of 35 Gy. However, the  $C$  value of *An. arabiensis* treated as adults with 75 to 120 Gy was similar and overall better than irradiating them as pupae [4]. In addition to improved  $C$  values, irradiating adults resulted in better insemination rates as compared with irradiating pupae. These data are indicative that irradiating pupae induced higher levels of somatic cell damage than irradiating adults probably because more stem cells were negatively affected in pupae. Similar observations were made with other insect species [1]. Moreover, contrary to adult irradiation, irradiation of pupae can result in partial recovery of fertility with age [5].

In all studies, the  $C$  value was above 0.2, which is considered the lower threshold for cost-effective projects. A lower value would require an asymptomatic increase in the amount of sterile males to be released as was indicated by two independent modelling studies [6, 7] (Figure 1C&D).

### **Insights from recent field trials**

Although there are world-wide 34 SIT mosquito trials presently implemented [8], only four reported an estimation of the  $C$  value; all on *Aedes* species (Figure 1B). These projects reported a  $C$  value above the 0.2 threshold, resulting in the successful suppression of *Ae.*

*albopictus* in China [9], Italy [10] and Mauritius [11]. A combined SIT-Incompatible Insect Technique (IIT) approach was used in the China trial, releasing triple *Wolbachia*-infected male *Ae. albopictus* that were irradiated with only 40 Gy. The low radiation dose was enough to sterilize accidentally released female mosquitoes and the males conferred the conditional sterility created by the *Wolbachia* symbiont: this resulted in a higher *C* value. The main objective of the trial in Brazil was to test a release system mounted on an unmanned aerial vehicle (drone) for the aerial release of sterile male *Ae. aegypti*. During three successive releases, ~2500 sterile males were dispersed per ha and this resulted in a maximum sterile to wild male ratio of 0.8:1 in the release area [12]. The proportion of unviable eggs collected in the release area increased by >50% as compared with that of a neighboring control area where no sterile mosquitoes were released; this corresponded to a *C* value of 0.26.

## **Conclusions and perspectives**

All these studies confirm that irradiated male mosquitoes can be competitive with the native mosquito population after release. Recent advances in the R&D of mosquito SIT has made all the components of the SIT package more effective, which has contributed to increased and adequate competitiveness of the released male mosquitoes that made these trials a success [13]. Male quality reducing processes (mass-rearing, handling, marking and release) are not specific to the SIT and also affect programmes using *Wolbachia*-induced sterility or transgenic males. It is off course obvious that treating insects with excessive high radiation doses will impair their competitiveness, but in most programmes it has been possible to select a trade-off dose obtaining >99% male sterility without significantly reducing their biological quality<sup>i</sup>.

87 To increase the probability of success, a phased conditional approach (PCA) was proposed for  
88 the management of mosquito populations using the SIT. In this approach, support to the next  
89 phase is conditional on the completion of activities in the previous phase and the scope,  
90 expense and commitment increase along the process [8]. An assessment of the  $C$  value is part  
91 of the baseline data collection phase (I), and should be conducted first under laboratory  
92 conditions, followed by studies under semi-field and field conditions before commencing  
93 pilot trials (phase II). Having knowledge of  $C$  values is considered a crucial and mandatory  
94 milestone, and a necessity that allows estimating the required sterile to wild male ratios to  
95 suppress the target population. As many factors other than irradiation can cause a loss of  
96 quality of sterile males, this estimate of the  $C$  value in the field must be obtained using the  
97 same protocols of production, handling, irradiation and release as those that will be used in  
98 the operational programme. This should be done through a specific mark-release-recapture  
99 protocol where the ratio of sterile to wild males is measured using adult traps in the same sites  
100 simultaneously with assessing induced sterility in the native female population using ovitraps.  
101 An IAEA guideline is available in this respect <sup>ii</sup> and can be used with technologies based on  
102 *Wolbachia*-infected or transgenic mosquitoes, but such estimates are rare in the literature. As  
103 an example, the Verily team carried out an impressive suppression trial in California using  
104 *Wolbachia*-infected *Ae. aegypti* male mosquitoes, but no competitiveness metrics were  
105 measured, which significantly reduces the value of this study. The ground release trial with  
106 engineered RIDL<sup>®</sup> (Release of Insects carrying a Dominant Lethal) mosquitoes was a notable  
107 exception, but the  $C$  value obtained was less than 0.06 (cited in [12]). This value is much  
108 lower than any estimate obtained for irradiated mosquitoes, and to date, no other genetic  
109 control method has provided higher estimates of  $C$  than those of irradiated males, despite the  
110 general inappropriate dogma in the literature that irradiated male mosquitoes cannot be  
111 competitive.

Ground releases are very ineffective (giving point releases), labour and transport intensive and require prolonged detrimental chilling of the sterile males to cover the targeted areas [2]. Therefore, it will be important to improve aerial distribution of sterile male mosquitoes in the future to enhance their quality [12]. Improved aerial dispersal systems would also make the technology more cost effective, as it would reduce the time and staff necessary to treat a given area [9].

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## Resources

<sup>i</sup> [http://www-naweb.iaea.org/nafa/ipc/public/Guidelines-for-MRR-Aedes\\_v1.0.pdf](http://www-naweb.iaea.org/nafa/ipc/public/Guidelines-for-MRR-Aedes_v1.0.pdf)

<sup>ii</sup> <https://www.iaea.org/sites/default/files/aedes-who-iaea-2020.pdf>

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## Box 1. What is competitiveness?

The competitiveness  $C$  is often estimated using Fried's index following the formulae:  $C = \frac{(Ha-E)/(E-Hs)}{R}$ , where  $Ha$  is the natural fertility of females,  $E$  the observed fertility rate under a given ratio  $R$  of sterile over wild males and  $Hs$  the residual fertility of males [14].  $Hs$  is often neglected when the residual fertility of males is below 1% [10].  $Ha$  is estimated as the percentage of fertile eggs in a cage with non-irradiated males (or a control site),  $E$  the percentage of fertile eggs from a cage with a given ratio  $R$  (or a release site), and  $Hs$  the hatch rate of eggs from females mated with sterile males. To estimate a reference value of  $C$ , it is suggested to use a sterile male - wild male - wild female ratio of 1:1:1. Increased sterile to wild male ratios will increase competition between sterile males themselves, and this may reduce their quality. Under field conditions, the release site must be isolated or  $R$  below 4 to reduce the impact of fertile females immigration [15] on the underestimation of  $C$  (Figure I.).

**Figure I. Impact of the ratio ( $R$ ) of sterile to wild males on the observed competitiveness ( $C$ ) of sterile males in field conditions assuming immigration of fertile females in the release site.** Red line = real  $C$ ; blue points = observed  $C$ . See supplemental Table S2 for details.

## Figure legends

**Figure 1 Competitiveness of irradiated male mosquitoes and impact on the necessary release rates in operational programmes.** Competitiveness ( $C$ ) of irradiated sterile male mosquitoes were measured in semi-field trials (**A**) and field trials (**B**), as a function of the irradiation dose (see Supplemental Table S1 for details). Values marked with a ‘\*’ correspond to sterile males irradiated as adults whereas all others are irradiated as pupae. The value marked with a ‘#’ corresponds to a combination between the incompatible and the sterile insect technique [9]. (**C**) Relationship between the competitiveness and the daily release rate of sterile males to obtain eradication of a local population of *Aedes albopictus* in one year. The release rate ( $RR$ ), expressed as a proportion of the initial male population ( $M$ ), is increasing asymptotically for a competitiveness below a threshold of 0.2. Source: modified from [6]. (**D**) Relationship between the competitiveness and the daily release rate (sterile males per ha per week) to obtain a given suppression rate of female mosquitoes. The suppression rate of adult females is indicated along the isoclines from 0.1 to 0.8. Source: modified from [7]. In all panels, the red dashed lines represent a competitiveness of 0.2.

$R$

