INMN Draft Conference Proceedings Submission

Title

Does the source matter for SIT? Negligible effects of irradiator source, X-ray vs. gamma, for sterilization and sterile male mating competitiveness in the mosquito *Aedes aegypti.*

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Keywords

SIT, insect pests, disease vectoring mosquito, alternative technologies for radioactive sources

Abstract

Sterile insect technique (SIT) has proven to be very effective as a key component in area-wide integrative pest management for multiple agricultural pests, and in recent years interest has been growing in applying SIT to mosquito vectors of importance to human health. SIT may be particularly useful for peridomestic disease vectors like Aedes aegypti that live in close proximity to humans, occupy cryptic larval breeding sites with adults that rest in places difficult to reach with sprays, and that have some populations showing resistance to common chemicals used in mosquito control. For SIT, males are typically sterilized by exposing them to gamma radiation. However, these irradiators typically use Co-60 or Cs-137; high activity sources that are vulnerable to theft or misuse and thus require substantial security and monitoring. In support of the National Nuclear Security Administration (NNSA) Office of Radiological Security (ORS) mission to reduce the risk of these sources by encouraging use of non-radioisotopic technologies, Sandia National Laboratories has funded a study to compare the efficacy of gamma versus X-ray-based irradiation for SIT. Specifically, this study seeks to compare an established Cs-137 gamma irradiator used for an operational *Ae. aegypti* SIT pilot program with a new X-ray irradiator for sterilizing male pupae and adults while maintaining male performance after irradiation. We show negligible differences in efficacy of sterilization or post-irradiation male performance between the X-ray and gamma sources. Our results show that insect SIT programs can successfully transition from gamma to X-ray sources and suggests that new programs can begin their programs using X-ray irradiation sources to avoid regulatory and cost hurdles associated with installation of new gamma-radiation sources, overall showing that X-ray irradiators provide a viable alternative technology to radioactive gamma sources for insect pest control.

Paper Body

The sterile insect technique (SIT) is a biologically based method to control insect pests without using chemical pesticides (Bakri et al. 2005). The basic methodology for SIT starts with raising hundreds of thousands to millions of males of a particular pest insect species in a laboratory facility/factory. Factory-grown male insects are then sterilized with a dose of ionizing radiation sufficient to generate many double-strand breaks in the insects genomic DNA, known as dominant lethal mutations (Robinson 2005). These sterile males are then released in the field *en masse* to overwhelm the number of wild males and mate with wild females. Due to the DNA double-strand breaks carried by males irradiated at sterilizing doses, proper embryogenesis cannot occur and offspring die early in development (Robinson 2005), thus suppressing the pest insect population. SIT has been successfully used in a wide range of insects, from agricultural pests that may affect food security to blood sucking insects that may vector important human and animal diseases (Bakri et al. 2005). Due to a combination of public distaste for chemical pesticides and a regulatory landscape wherein the products available for use have consistently been reduced over the last two decades (Deming et al. 2016; Moyes et al. 2017), there is need for further development and use of non-chemical approaches like SIT in the control of pest insects.

Most active SIT programs irradiate males with gamma sources, usually Cs-137 or Co-60. Yet, these gamma sources have a number of drawbacks, including the necessity for expensive and restrictive security measures to prevent misuse or theft, such as continuous monitoring and extra security against inappropriate access. Furthermore, access to these sources often requires passing a series of trainings and security checks that can reduce the ease of use and introduce lags in how quickly a new user can expect to wait until they are cleared to use the irradiator for their work. X-ray irradiators provide a viable alternative to gamma irradiators for mass sterilization of male insects for SIT programs because they have been shown to be effective in sterilizing male insects (Mastrangelo et al 2010), and X-ray irradiators are in use by a few small to moderate sized operational SIT programs (Du et al. 2019). To support the goals of the National Nuclear Security Administration (NNSA) Office of Radiological Security (ORS) mission with regard to reducing the risk of gamma sources by promoting use of non-radioisotopic sources, the objective of our study was to compare the efficacy of an X-ray irradiator with a gamma irradiator for sterilization of males of the mosquito *Aedes aegypti*, a disease-vectoring mosquito for which standardized operational SIT programs are in the early phases of development.

Specifically, we compared sterilization dose-repone relationships of cohorts of *Ae. aegypti* irradiated in either a RadSource RS-2400 low energy X-ray irradiator and a Gammacell 1000 137Cs-GC45 gamma irradiator. Dosimetry across both systems was performed with Gafchromic HD-V2 film chips in each run read by a RadGen DoseReader 4 spectrophotometer. In X-ray, the Gafchromic film was calibrated to a NIST-tracible Radcal 10X6-0.18 ion chamber interfaced to an Accu-Gold digitizer. For gamma, Gafchromic film was calibrated to alanine pellet dosimeters provided by NIST and read by their dosimetry service. The X-ray chamber delivered 20.94 Gy/min and the gamma 8.8 Gy/min, thus time in the irradiator determined the radiation dose.

Aedes aegypti were cultured in the lab using standard methods, including separation of males from females by size using a glass wedge as described in Moreno et al. (2021). To generate a dose-response relationship in each life stage we either used male pupae that were ~30 h old or adult males that were ~24h old. Methods for assessing sterility followed Chen et al. (2023).

As expected, we found that the proportion of eggs hatching decreased with increasing radiation dose pupae were exposed to (GLM, estimate=0.13, z=57.18), but with no statistically detectable effect of radiation source (gamma vs X-ray) on sterilization in male pupae (GLM, estimate=0.05, z=0.89, p=0.38). No eggs hatched at our 60 Gy dose from either source, and only 3 eggs hatched from 1415 eggs laid in one 50 Gy gamma treatment. The estimated 99% sterilization dose for male pupae was 47.03 Gy (95% CI: 45.70-48.36) for gamma and 44.77 Gy (95% CI: 43.59-45.95) for X-ray. Similarly to our results for pupae, proportion of eggs hatching significantly decreased with increasing radiation delivered to 24-48h old adult males (GLM, estimate=0.08, z=55.42, $p < 0.001$), but with no statistically detectable effect of radiation source (gamma vs X-ray) on sterilization (GLM, estimate=0.05, z=0.67, p=0.42). Only 6 eggs hatched from 1029 eggs in one of the replicates in our 75 Gy x-ray treatment, and 7 eggs hatched from total 2232 eggs in two of the replicates in our 75 Gy gamma treatment. The estimated 99% sterilization dose for male adults was 68.91 Gy (95% CI: 67.13-70.69) for gamma and 65.87 Gy (95% CI: 64.13-67.61) for X-ray. Taken together, it took a much higher radiation dose to induce 99% sterility in adult males than male pupae, but there was no significant effect of radiation source on the degree of sterility induced by a particular dose.

While we are still in the process of finalizing data collection to more thoroughly test for differences in sterility induction by X-rays vs gamma rays in our mosquito SIT system, our observations thus far suggest a negligible effect of source on the efficacy of sterility induction in *Aedes aegypti* male adults or pupae. Our results demonstrate that SIT programs can move from gamma to X-ray sources effectively. Further, we suggest that new programs simply begin with X-ray irradiators rather than gamma irradiators because of the prohibitive costs and regulatory difficulties involved with new gamma irradiator installation. Taken together, we believe that X-ray irradiators provide a viable alternative to radioactive gamma sources to control insect pests.

Acknowledgements
This study was funded by Sandia National Laboratories. We thank Hunter Martin and Jared Walters for assistance with mosquito rearing and laboratory maintenance.

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