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DISSERTATION

Advancing the Sterile Insect Technique for tsetse (Diptera: Glossinidae): Exploring alternative radiation sources and protocols

ausgeführt zum Zwecke der Erlangung des akademischen Grades eines Doktors der Naturwissenschaften unter der Leitung von

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Dedication

To my dearest wife, Placide, and my cherished children, Aaron Chris-Ryan and Adina Chris-Hérine, I want to express my profound gratitude for the enduring patience and unwavering support you have graciously offered throughout this extended period of separation. Adina, your arrival in the midst of this extraordinary journey has not only brought immeasurable joy to our lives but has seamlessly woven into the fabric of this thesis, making it all the more special.

May the love and resilience we share as a family serve as a wellspring of inspiration propelling each of you toward greater heights. Every one of you holds a unique place in my heart, and I eagerly anticipate the joyous moment when our family is reunited.

To the Kaboré, Sané and linked families, may this endeavour serve as a source of inspiration and motivation for generations to come !

Acknowledgment

"Bless the LORD, O my soul, and forget not all his benefits, *Psalms 103:2.*"

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Finally, I am grateful to the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture for fully funding this research, with special appreciation to the Insect Pest Control Laboratory for hosting me and providing all the necessary support services throughout my journey. I extend my sincere thanks to all my colleagues at the IPCL, as well as the technicians in the tsetse group, for their invaluable support in conducting our experiments.

Table of contents

List of abbreviations

Abbreviations	Definitions
137Cs	Cesium-137
^{60}Co	Cobalt-60
AAT	African Animal Trypanosomosis
AW-IPM	Area-wide integrate Pest Management
CIRDES	Centre International de Recherche-Développement sur l'Elevage en zone Subhumide
DNA	Deoxyribonucleic Acid
FAO	Food and Agricultural Organization of the United Nations
Ga	*Glossina austeni*
GLM	General Linear Model
Gb	*Glossina brevipalpis*
Gff	*Glossina fuscipes fuscipes*
Gpg	*Glossina palpalis gambiensis*
Gpp	*Glossina palpalis palpalis*
HAT	Human African Trypanosomiasis
IAEA	International Atomic Energy Agency
IBD-CETT	Insectarium de Bobo-Dioulasso-Campagne d'Eradication de la mouche Tsétsé et de la Trypanosomose
IPCL	Insect Pest Control Laboratory
L1	First instar larvae
L2	Second instar larvae
L3	Third instar larvae
NAFA	Nuclear Applications in Food and Agriculture
PATTEC	Pan-African Tsetse Fly and Trypanosomiasis Eradication Campaign
R&D	Research and Development
SIT	Sterile Insect Technique
US$	United States Dollar
WHO	World Health Organization

Abstract

Insect pests are responsible of public health concerns as well as economical losses throughout the world. Their control has been developed and regularly improved with targeting their containment, suppression, or eradication. Tsetse flies are responsible of the transmission of African Animal Trypanosomosis or nagana in animals and Human African Trypanosomosis or sleeping sickness in humans and constitute an obstacle of the agriculture development in around 10 million km^2 of land in Africa. In face of the constraints of the resistance to the trypanocides used to treat the cases and the persisting impact of the scourge, several methods of control of the disease and the vectors have been developed. These methods encompassing the preventive treatment, the use of trypanotolerant breeds and the vectors control through the spraying of insecticide and the use of bait technology have been deployed. Lastly, the sterile insect technique (SIT) which was successfully used against the new world screw worm has been successfully tested in an integrated pest management against tsetse. Regarding the principle of the SIT based of the release of male sterilized with ionizing radiation that compete with wild counterparts to mate wild females with no offspring expectation, the sterilization is a key point for an SIT program establishment. Gamma rays have been traditionally used in SIT against various insect pests. However, gamma-ray sources are facing a growing restriction about the purchase, the shipment, and the transport of isotope for reloading the irradiators since regulations about these aspects are becoming stringent. Therefore, alternative radiation sources are needed for the ongoing and future SIT programmes. X-rays, initially tested since the discovery of Knippling are shown as an alternative radiation source that could circumvent the aforementioned constraints. Thus, this thesis aimed to advances insect pest management through the alternatives and safe ionizing radiation sources and protocols for the SIT. For this purpose, the suitability of some available X-rays sources has been assessed on tsetse, fruit flies and mosquitoes. In addition, and in relation to the refinement of the existing protocols, an assessment of radiation under cold and low oxygen environment or fractionating radiation dose were evaluated. Key parameters such the adult emergence rate, the induced sterility, the flies flight propensity, their survival, and mating competitiveness have been measured. The results indicates that the Blood irradiator Raycell Mk2, the Rad Source 2400 and the X-Rad 320 Precision are suitable to induce 95-99% of sterility, required for the use for the SIT in tsetse as compared to gamma-rays. To induce 95% of sterility in *Glossina palpalis gambiensis*, X-rays irradiators requires less radiation dose compared to the optimal dose of 110 Gy for gamma-rays in air. In addition, there was no significant difference in the flight quality parameters and

the mating competitiveness of flies irradiated with X-rays as compared to those irradiated with gamma-rays. Assessing the impact of the dose fractionation using gamma-rays, there was no significant effect on induced sterility and flight quality parameters when the optimal dose was split into 10+100 Gy or 50+60 Gy separated by 1-, 2-, or 3-day interval. The same results have been obtained when splitting the optimal dose in equal dose of 55 Gy separated by 4-, 8-, or 24-hour interval. The nitrogen treatment exhibited a radioprotective effect, necessitating increased doses of 115 Gy and 135 Gy to achieve at least 95% sterility in male *G. p. gambiensis* when exposed to X- and gamma-rays, respectively, as compared to radiation in ambient air. In addition, the survival time under feeding regime was significantly increased. In the context of irradiation temperature, the results indicate that the X-RAD 320 effectively induced at least 95% sterility. Approximately 75 Gy and 90 Gy were required for pupae at 7 °C and ambient temperature, respectively, while adults needed 90 Gy in both treatments. Whether the flies were irradiated under chilled or non-chilled conditions, those irradiated as adult survived longer than those irradiated as pupae revealing the importance of the choice of the life stage to be sterilized. These findings significantly contribute to the discussion on the importance of implementing dose-response assessments for both new and ongoing programs, emphasizing the necessity of employing a reliable dosimetry system. The dual considerations of cost-effectiveness and the complexity of the SIT pose challenges, requiring the commitment of policymakers and governments. Additionally, there is a need for addressing concerns related to mass production and quality control from the perspective of researchers. The engagement of stakeholders and capacity building are emphasized as crucial elements. In conclusion, recommendations and perspectives are offered, addressing the selection of irradiation sources, and advocating for a realistic and beneficial integrated pest management approach.

Keywords: *Glossina palpalis gambiensis*, Dose-response, Insect pests, Ionizing radiation, SIT, AW-IPM

Zusammenfassung

Insekten als Schädlinge sind weltweit für Gesundheitsprobleme und finanzielle Einbußen verantwortlich. Verschiedene Ansätze der Schädlingskontrolle wurden bereits entwickelt und werden laufend verbessert indem gezielt auf ihre Eindämmung, Unterdrückung oder Ausrottung gesetzt wird. Tsetsefliegen sind verantwortlich für die Übertragung von Trypanosoma. Trypanosoma ist ein Protozoenparasit, der die afrikanische Tier-Trypanosomosis, auch genannt Nagana, in Tieren auslöst. Desweiteren löst der Parasit auch bei Menschen Trypanosomosis, auch Schlafkrankheit genannt, aus. Dies ist ein Problem für die landwirtschaftliche Entwicklung in mehr als 10 Millionen Quadratkilometer Landfläche in Afrika. Trypanozide werden verwendet um die Krankheit zu bekämpfen, aber in Anbetracht der entwickelten Tryipanozidresistenz und den anhaltenden negativen Folgen dessen wurden verschiedene Methoden zur Bekämpfung der Krankheit und der Vektoren entwickelt. Diese Methoden umfassen die vorbeugende Behandlung, den Einsatz trypanotoleranter Rassen (Kühen) und der Vektorenbekämpfung durch das Versprühen von Insektiziden und dem Einsatz von der Ködertechnologie. Schließlich wurde die Sterile-Insekten-Technik (SIT), die schon erfolgreich gegen die Neuwelt-Schraubenwurmfliege eingesetzt wurde, auch erfolgreich in einem integrierten Schädlingsmanagement gegen Tsetsefliegen getestet. Das Prinzip der Sterilen-Insekten-Technik basiert auf der Freilassung von mit ionisierender Strahlung sterilisierten Männchen, die mit wilden männlichen Artgenossen um die Begattung wilder Weibchen konkurrieren. Kommt es zur Fortpflanzung mit einem sterilisierten Männchen, gibt es keine Nachkommen. Daher ist eine erfolgreiche Sterilisation ein zentraler Punkt für die Einrichtung eines SIT-Programms. Gammastrahlen werden traditionell bei SIT gegen verschiedene Insektenschädlinge eingesetzt. Allerdings unterliegen Gammastrahlen zunehmenden Einschränkungen hinsichtlich des Kaufs, des Versands und des Transports von Isotopen zum Nachladen der Bestrahlungsgeräte, da die Vorschriften zu diesen Aspekten immer strenger werden. Daher werden für die laufenden und zukünftigen SIT-Programme alternative Strahlungsquellen benötigt. Röntgenstrahlen, die schon seit Beginn der Entdeckung der SIT getestet worden sind, erweisen sich als alternative Strahlungsquelle, die die oben genannten Einschränkungen umgehen könnte. Daher ist es das Ziel dieser Doktorarbeit, die Insektenschädlingsbekämpfung durch alternative und sichere ionisierende Strahlungsquellen und Protokolle für die SIT voranzutreiben. Zu diesem Zweck wurde die Eignung der verfügbaren Röntgenstrahlung an Tsetsefliegen, Fruchtfliegen und Mücken beurteilt.

Zusätzlich, und im Zusammenhang mit der Optimierung der bestehenden Protokolle, wurde die Auswirkung der Strahlung in einer kalten und sauerstoffarmen Umgebung oder mit einer fraktionierten Strahlungsdosis bewertet. Schlüsselparameter wie die induzierte Sterilität, die Flug-Fähigkeit der Fliegen, ihr Überleben und ihre Paarungskonkurrenzfähigkeit wurden gemessen. Die Ergebnisse zeigen, dass das Blutbestrahlungsgerät Raycell Mk2, die Rad Source 2400 und das X-Rad 320 geeignet sind, mindestens 95–99% Sterilität herbeizuführen, die für die Verwendung in der SIT bei Tsetsefliegen erforderlich ist. Um eine Sterilität von 95% zu erreichen, benötigen Röntgenbestrahlungsgeräte eine geringere Strahlendosis im Vergleich zur optimalen Dosis von 110 Gy bei Gammastrahlen. Darüber hinaus gab es keinen signifikanten Unterschied in den Flugqualitätsparametern und der Paarungskonkurrenzfähigkeit von Fliegen, die mit Röntgenstrahlen bestrahlt wurden, im Vergleich zu denen, die mit Gammastrahlen bestrahlt wurden. Bei der Beurteilung der Auswirkung der Dosisfraktionierung mithilfe von Gammastrahlen ergab sich kein signifikanter Effekt auf die induzierten Sterilitäts- und Flugqualitätsparameter, wenn die optimale Dosis in 10+100 Gy oder 50+60 Gy aufgeteilt und durch 1-, 2- oder 3-Tages-Intervalle geteilt wurde. Die gleichen Resultate wurden erzielt wenn die optimale Dosierung auf zu gleichen Teilen von 55 Gy aufgeteilt wurde und in einem 4,-8 oder 24 Stunden Intervall angewendet wurde. Die Stickstoffbehandlung zeigte eine strahlenschützende Wirkung und erforderte erhöhte Dosen von 115 Gy bei Röntgenstrahlen und 135 Gy bei Gammastrahlen, um bei männlichen *Glossina palpalis gambiensis* eine Sterilität von 95 % zu erreichen. Diese Ergebnisse stehen im Vergleich zur Verwendung der Strahlen in Raumluft. Darüber hinaus verlängerte sich die Überlebenszeit der Fliegen die regelmäßig gefüttert wurden. Im Zusammenhang mit der Bestrahlungstemperatur deuten die Ergebnisse darauf hin, dass das X-RAD 320 eine Sterilität von mindestens 95 % herbeiführte. Für Puppen waren bei 7 °C etwa 75 Gy erforderlich und bei Raumtemperatur 90 Gy. Unterdessen benötigten erwachsene Fliegen bei beiden getesteten Temperaturen 90 Gy. Unabhängig davon, ob die Fliegen unter gekühlten oder nicht gekühlten Bedingungen bestrahlt wurden, überlebten diejenigen, die als Adulttiere bestrahlt wurden, länger als diejenigen, die als Puppen bestrahlt wurden, was zeigt, wie wichtig die Wahl des zu sterilisierenden Lebensstadiums ist. Die Ergebnisse dieser Arbeit tragen erheblich zur Diskussion über die Wichtigkeit der Implementierung von Dosis-Wirkungs-Bewertungen sowohl für neue als auch für laufende Programme bei und unterstreichen die Notwendigkeit des Einsatzes eines zuverlässigen Dosimetriesystems. Der Aspekt der Kostenwirksamkeit und die Komplexität des SIT stellt Herausforderungen dar, die das Engagement von politischen Entscheidungsträgern und Regierungen erfordern. Darüber hinaus müssen sich Forscher weitere Gedanken über die

Optimierung von Massenproduktion und Qualitätskontrolle machen. Als entscheidende Elemente werden die Einbindung von Stakeholdern und der Kapazitätsaufbau hervorgehoben. Abschließend werden Empfehlungen und Perspektiven gegeben, die sich mit der Auswahl von Strahlungsquellen befassen und sich für einen realistischen und vorteilhaften integrierten Schädlingsbekämpfungsansatz einsetzen.

Schlüsselwörter: *Glossina palpalis gambiensis*, Dosis-Wirkung, Schadinsekten, Ionisierende Strahlung, SIT, AW-IPM

Declaration

I hereby declare that this doctoral thesis entitled "*Advancing the Sterile Insect Technique for tsetse (Diptera: Glossinidae): Exploring alternative radiation sources and protocols*" was carried out and written by me for the degree of Doctor of Philosophy in English under the Doctoral programme of the Natural Sciences Diploma programme: Technical Chemistry under. This thesis was conducted under the guidance and supervision of Prof. Robert L. Mach from the Institute of Chemical, Environmental and Biological Engineering, and Dr Chantel J. de Beer from the Insect Pest Control Laboratory, Joint FAO/IAEA Divisions of Nuclear Techniques in Food and Agriculture. I also confirm that this thesis is the product of original research work obtained at the Insect Pest Control Laboratory, Joint FAO/IAEA Divisions of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria. Additionally, I have exercised due diligence to guarantee the originality of the work, avoiding any infringement of copyright laws from external sources. Proper citation and acknowledgment have been meticulously employed where applicable. I have not submitted the doctoral thesis for any other degree or professional qualification. The experimental work is almost entirely my work and all the collaborative contributions have been indicated clearly and acknowledged. The data presented in this thesis were acquired through experiments conducted in close collaboration with colleagues from the Insect Pest Control Laboratory from tsetse, fruit flies and Mosquito group, mainly Hanano Yamada, Inna, Arooj Nawaj and Yeudieul Gomez from Programa Operativo Moscas, Mexico, Gisel Marie Sophie Sanon and Soumaila Pagabeleguem both from the Ministry of Agriculture and Livestock Resources in Burkina Faso.

This thesis incorporates contributions from both my colleagues and me. The content presented herein corresponds to the publications listed in the next section, ***"List of Publications".***

List of publications originating from this study

1. Yamada H, **Kaboré BA**, Bimbilé Somda NS, Ntoyi NL, de Beer CJ, Bouyer J, et al. Suitability of Raycell MK2 Blood X-ray Irradiator for the Use in the Sterile Insect Technique: Dose Response in Fruit Flies, Tsetse Flies and Mosquitoes. Insects. 2023;14: 92. doi:10.3390/insects14010092
2. **Kaboré BA**, Nawaj A, Maiga H, Soukia O, Pagabeleguem S, Ouédraogo/Sanon MSG, et al. X-rays are as effective as gamma-rays for the sterilization of *Glossina palpalis gambiensis* Vanderplank, 1911 (Diptera: Glossinidae) for use in the sterile insect technique. Sci Rep. 2023;13: 17633. doi:10.1038/s41598-023-44479-8
3. **Kaboré BA**, Taqi SD, Mkinga A, Morales Zambrana AE, Mach RL, Vreysen MJ, et al. Radiation dose fractionation and its potential hormetic effects on male *Glossina palpalis gambiensis* (Diptera: Glossinidae): a comparative study of reproductive and flight quality parameters. Parasite. 2024;31: 4. doi:10.1051/parasite/2024001
4. **Kaboré BA**, Vreysen MJB, Mach RL, de Beer CJ. Effects of low temperature and nitrogen-enriched environment on the sterility and survival of male *Glossina palpalis gambiensis* (Glossinidae: Diptera) using gamma and x-ray sources. Journal of Pest Science.
5. Dera KM, Barro TD, **Kaboré BA**, Gstöttenmayer F, Pagabeleguem S, Weiss BL, et al. *Spiroplasma* infection in colonized *Glossina fuscipes fuscipes*: Impact on mass rearing and the Sterile Insect Technique. Insect Science.

List of communications originating from this study

1. **Kaboré BA**, de Beer CJ, 2024. Regional Training Course on SIT components: "Dosimetry and Irradiation procedures for Supporting SIT Programmes to Management Tsetse and mosquitoes", 18-22 March 2024, Vienna, Austria.
2. **Kaboré BA**, de Beer CJ, 2024. Advancing the Sterile Insect Technique for tsetse (Diptera: Glossinidae): Exploring alternative radiation sources and protocols, Joint FAO/IAEA Insect Pest Control Laboratory Seminar, 29 February 2024, Vienna, Austria.

Chapter 1:

General introduction

1. Introduction

The purpose of this introduction is to offer an overview of the thesis subject. Commencing with insights into African trypanosomiasis and its vectors, the diverse control strategies developed are summarized, placing specific emphasis on the sterile insect technique (SIT). Beyond delving into the history and successes of SIT, a crucial facet of this technique - specifically, the sterilization of male insects - is examined, encompassing the various sources of radiation, the dominant lethal mutations pathways, and their biological effects. Additionally, the necessity for exploring alternative sources of radiation is underscored. Concluding this section, the study's context and objectives are presented.

2. African trypanosomosis

Human African Trypanosomosis (HAT) and African Animal Trypanosomosis (AAT) caused by trypanosomes transmitted by tsetse limit the development of sustainable agriculture in the humid and sub-humid zones of sub-Saharan Africa, by affecting approximately 10 million km^2 south of the Sahara [1,2].

2.1 Epidemiology of Human African Trypanosomosis

In sub-Saharan Africa, Human trypanosomosis are caused by two uniflagellar parasites in the subspecies of *Trypanosoma brucei* i.e., *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesience,* discovered more than a century ago [3] (Figure 1). The chronic form of sleeping sickness, is caused by *T. b. gambiense*, which occurs in West, Central Africa and is manly transmitted by tsetse of the *palpalis* group[4–6]. Concerning *T. b. rhodesience,* responsible for the acute form, it is distributed in Eastern and Southern Africa and transmitted by tsetse of the *morsitans* group[4,5].

Regarding their epidemiological aspect, the *gambiense* form is endemic in 24 countries and causes more than 90% of reported cases of sleeping sickness while the *rhodesiense* form is endemic in 13 countries, representing less than 10% of reported cases globally [6,7]. However, both types can be involved in an endemic or epidemic way [3,8]. Without timely intervention and appropriate treatment, HAT can be fatal, however this is not always the case, as untreated infections with *T. b. gambiense* does not lead to a 100% fatality rate [9]. Therefore, the importance of prompt and suitable measures as well as the epidemiological surveillance cannot be overstated [10], as they are crucial for averting adverse consequences and mitigating the severity of the disease. For example, since 2004, HAT is a part of the Resolutions of the World

Health Assembly (WHA) on elimination and eradication of selected neglected tropical diseases [7].

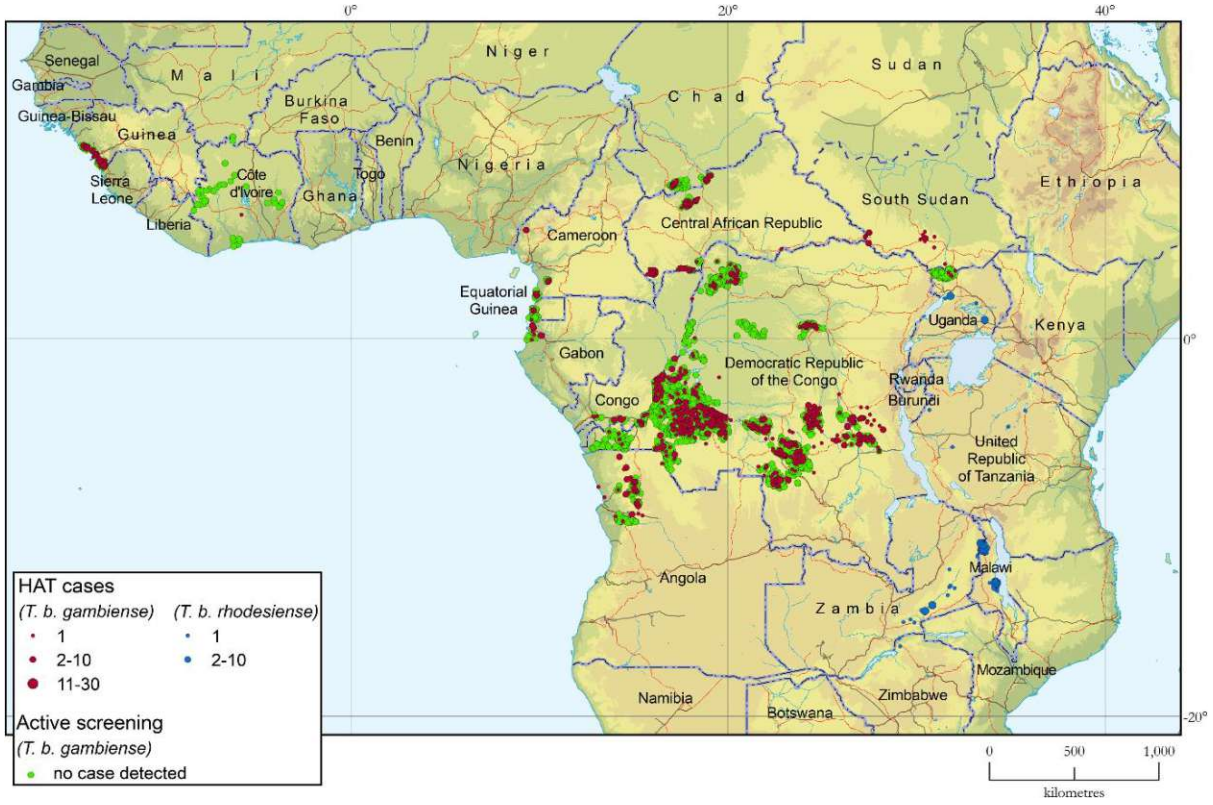

Figure 1: Geographical distribution of reported infections of human African trypanosomiasis in relation to World Health Organization road map targets for 2020 [11]

2.2 Epidemiology and consequences of African Animal Trypanosomosis

African Animal Trypanosomosis known as nagana is also caused by Trypanosoma parasites and is also affecting livestock comprising affects species of wild and domestic mammals across approximately 10 million of km^2 in Sub-Saharan Africa [12]. The parasites are mainly transmitted cyclically by the genus *Glossina*, but also mechanically by biting flies belonging to the families of Tabanidae and Muscidae, e.g., *Stomoxys sp.* [13].

Nagana is acknowledged as a multifaceted disease, marked by the occurrence of either a mono-infection or co-infection with trypanosomes, affecting a broad spectrum of animal species [14]. However, it is mainly associated with the infestations of cattle by the primary pathogens *Trypanosoma congolense, Trypanosoma vivax* and *Trypanosoma brucei brucei*, considered as the most pathogenic, the most prevalent, and the least extensive, respectively [14–16]. Although these trypanosomes are particularly important, there is a wide range of trypanosome

species that can infect a wide range of domestic and wild animals such as sheep, goats, pigs, horses, donkeys, mules, camels, dogs, as well as a number of wild animals including reptiles [14,17,18]. For example, infestations in Camelidae and Equidae by *Trypanosoma evansi and Trypanosoma equiperdum* are specifically termed surra and dourine, respectively, and is mostly mechanically transmitted by tabanids and stomoxes [19]. The presence of mechanically transmitted trypanosome species has facilitated their spread to previously unaffected areas outside Africa. This is evident in the case of *T. vivax*, which reached South and Central America and, more recently, Asia [20–22] (Figure 2).

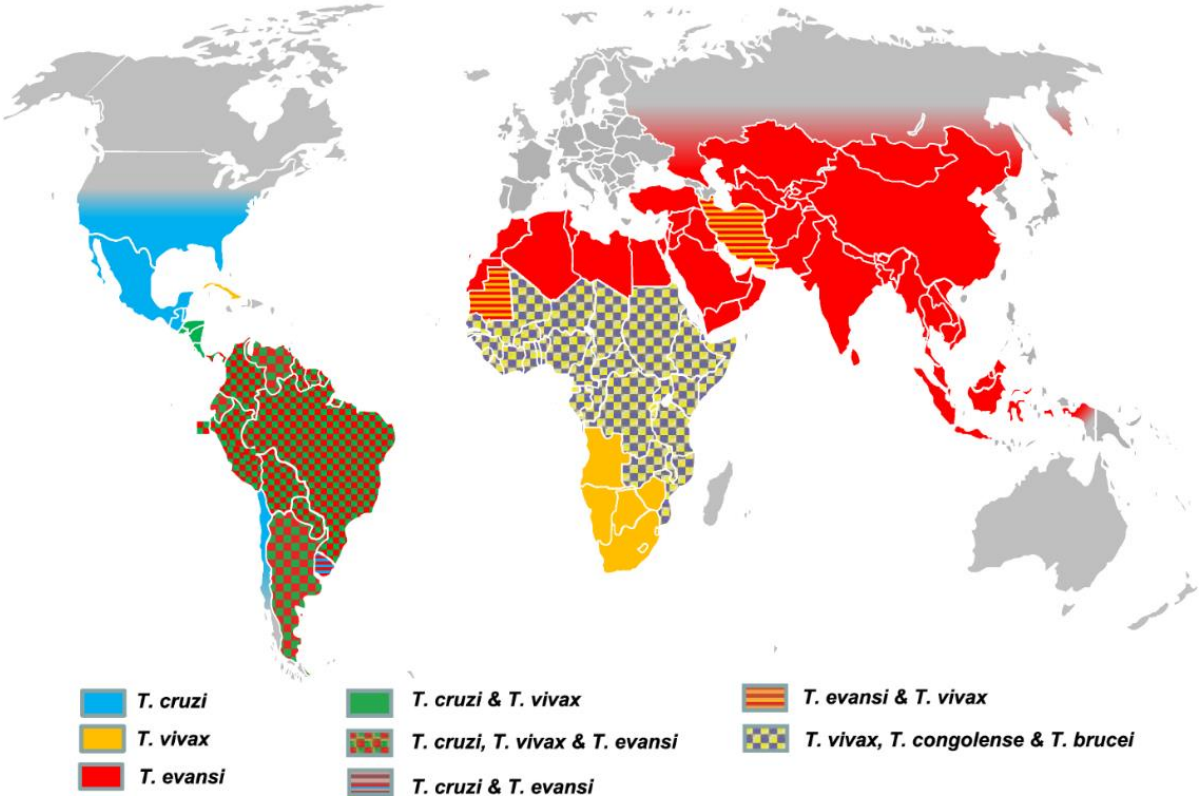

Figure 2: Geographical distribution of pathogenic mammal trypanosomes (*T. congolense, T. vivax and T. brucei*) [14].

Economically, AAT is a major obstacle to the expansion of livestock farming and livestock-based industries in Africa [23]. AAT is considered to have socio-economic implication to due to its negative impact on animal production leading to rural poverty and food insecurity [1,24]. The direct consequences of the disease are reduced milk and meat production, mortality, and the costs of control programs, which annual costs are estimated to be more than US$500 million [25]. Thus, it makes constraints to all initiatives for sustainable development of agriculture [26,27]. In view of the economic losses and the threat to animal and human health, it is

imperative that the vectors and disease is managed with the commitment of governments which has a crucial role to play [28]. Thus in 2000, during the African Union (AU) summit held in Lomé (Togo), the heads of state and government, realizing the important role of government against this disease, adopted a resolution calling for the total eradication of the tsetse in Africa. This resolution led to the creation of the Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) to eradicate this scourge [1].

3. Vectors of African trypanosomosis

African trypanosomosis vectors primarily consist of tsetse belonging to the genus *Glossina*, serving as the biological vectors for *Trypanosoma* species [29]. These flies have been categorized into three extant subgenera: *Austenina* Townsend (*Fusca* species group), *Nemorhina* Robineau-Desvoidy (*Palpalis* species group), and *Glossina* Wiedemann (*Morsitans* species group). Each subgenus is associated with specific habitats, with *Austenina* inhabiting forested areas, *Nemorhina* found in riverine habitats and areas with dense vegetation, and *Glossina* thriving in savannah regions [29].

The life cycle of these vectors involves distinct stages, including larval (instar larvae 1, 2 and 3), pupal, and adult phases. Female tsetse are viviparous, giving birth to fully developed larvae. They have a relatively low reproductive rates, producing only 8 - 10 progeny during her lifetime, which render them suitable for genetic control [29,30]. Both males and females are characterized by their unique ability to transmit trypanosomes during blood-feeding [29,31]. Research has illustrated that different species possess unique feeding habits. Consequently, the likelihood of a tsetse taking a blood meal is more reliant on its preference rather than solely on the availability of a host [32].

The distribution of tsetse is closely linked to environmental factors such as vegetation, temperature, and humidity. Tsetse are predominantly found in sub-Saharan Africa (Figure 3), particularly in regions with suitable environmental conditions, corresponding to the most fertile areas for the development of the agriculture [26]. However, anthropogenic factors, climate change, and ecological shifts can influence the distribution of these vectors, leading to changes in the epidemiology of African trypanosomosis [33,34].

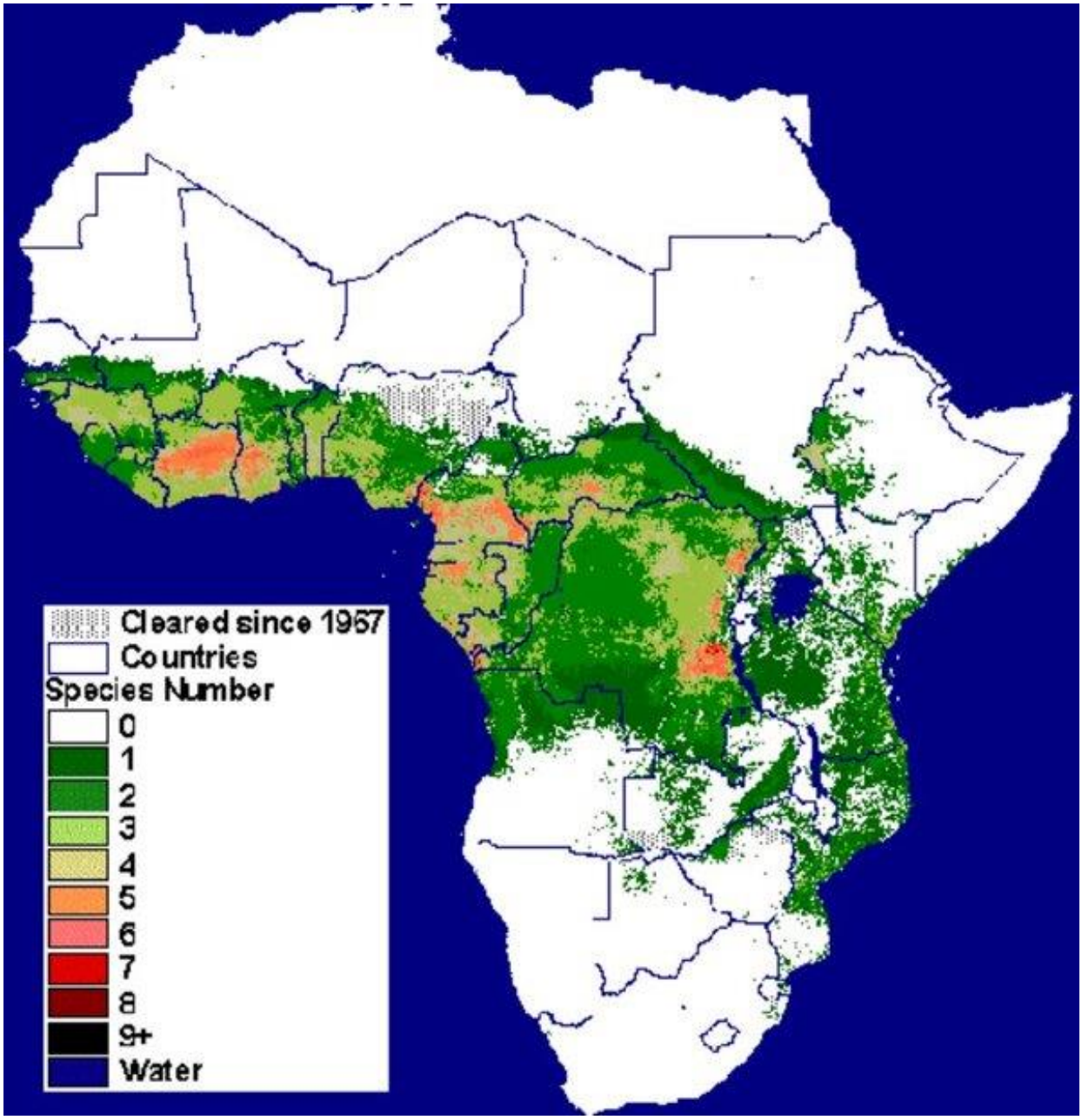

Figure 3 : Distribution map of tsetse across Sub-Saharan Africa [35]

4. Disease and vector control strategies and constraints

Since the discovery of trypanosomosis and the role of tsetse in its transmission [36], various control methods have been developed, essentially based on controlling the disease through diagnosis and curative/preventive treatment, but also through vector control (Figure 4). In addition, exploitation of trypanotolerant breeds has been developed to reduce the socio-economic impact of the disease and allow a sustainable livestock production [37–39].

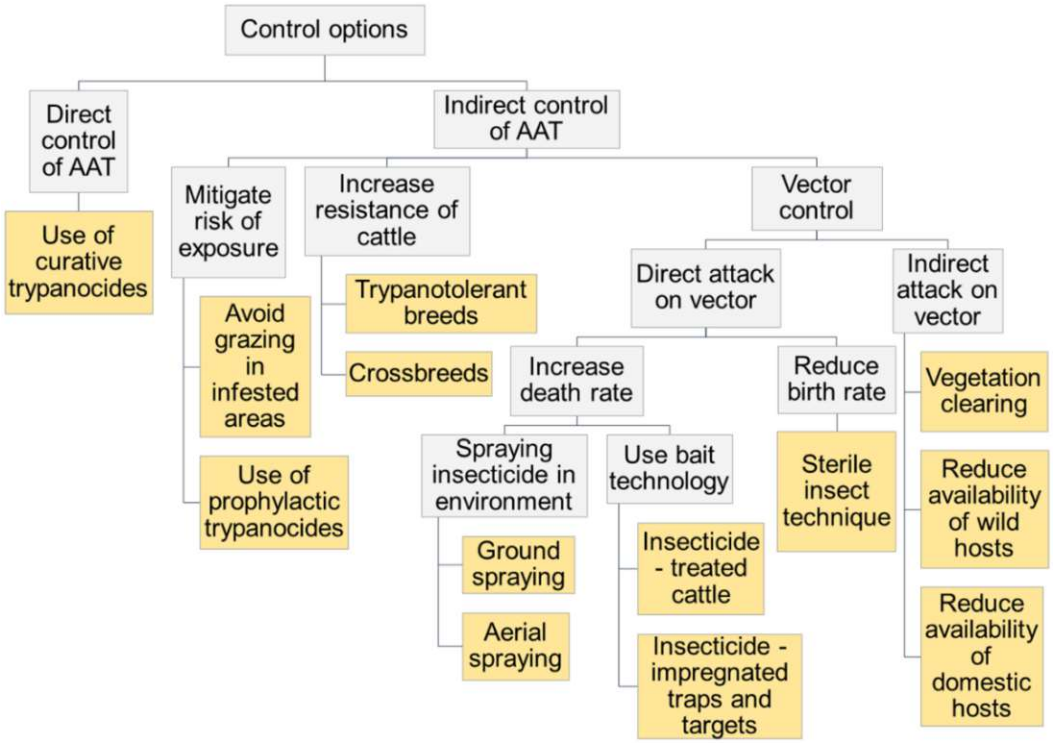

Figure 4: Summary of available approaches to control tsetse and AAT. Grey blocks indicate global strategies and yellow blocks, the practical applicable techniques [40]

The disease control in livestock has been depended primarily on the use of trypanocides drugs, mainly, isometamidium, diminazine and homidium salts [27,41]. These molecules have been in use for over 40 years, and with little interest shown by pharmaceutical companies to develop new trypanocides, drug resistance has begun to emerge [42,43]. Despite this situation, the chemotherapy stands out as the predominant approach for managing AAT in numerous countries, challenging the drug resistance phenomena and the presence of drug metabolites in animal products [44] and motivating the development of sustainable methods for the vector control [45].

Therefore, vector control plays a crucial role in mitigating the incidence of AAT. Approaches involving the elimination of game animals and the clearance of tsetse habitat were initially

successful to deprive tsetse of food and shelter [46–49]. However, these methods were ultimately abandoned due to their adverse environmental effect [48]. During the 20th century, insecticides have been employed through techniques such as ground spraying [47,50,51], the bait technology (odor-baited targets, insecticide treatment of cattle) [52–54], aerial spraying , have been successfully and successively developed in response to their acceptability and cost-effectiveness [55].

A separated use or a combination of aerial spraying, odor-baited targets and insecticide-treated cattle, in an Area-wide Integrated Pest Management (AW-IPM) program has been implemented successfully in several regions/countries to reduce desirably the tsetse population [54,56,57]. While these methods can reduce densities of vector and AAT prevalence, they are not always sustainable and re-invasion can occur after their removal [50,57].

The constraints in tsetse control through these methods may include their environmental impact [48,58,59], the logistical challenges, the community engagement [60], and necessary financial resources [61]. Some control methods, such as the use of insecticides, may have adverse effects on the environment, affecting non-target species [62,63]. Successful control strategies often require collaboration with local communities since the adoption of locally based tools such as bait technologies may depend on the engagement of communities [64]. In addition, tsetse control often involves working in remote and challenging environments, making it difficult to implement and sustain control measures. Finally, lack of funding, manpower, and resources can hinder the implementation of effective tsetse control in the Sub-Saharan Africa [40].

In the light of these challenges, a comprehensive initiative, the PATTEC, was launched in July 2000 during the African Union summit in Lomé, Togo. The collective goal of this initiative was to eliminate the threat of tsetse and trypanosomosis [1].

After two decades of implementation, the integration of the above various methods has successfully led to a significant reduction in tsetse density and disease incidence [57]. This success is exemplified by the effective application of an AW-IPM approach [65,66], specifically incorporating the SIT that allowed the eradication of *G. p. gambiensis* in the Niayes region of Senegal [67]. Indeed, the SIT is considered as an biologically based and environmentally acceptable method [68,69] which combined with the previous methods at the later stage of the control program can lead to the eradication (Figure 5).

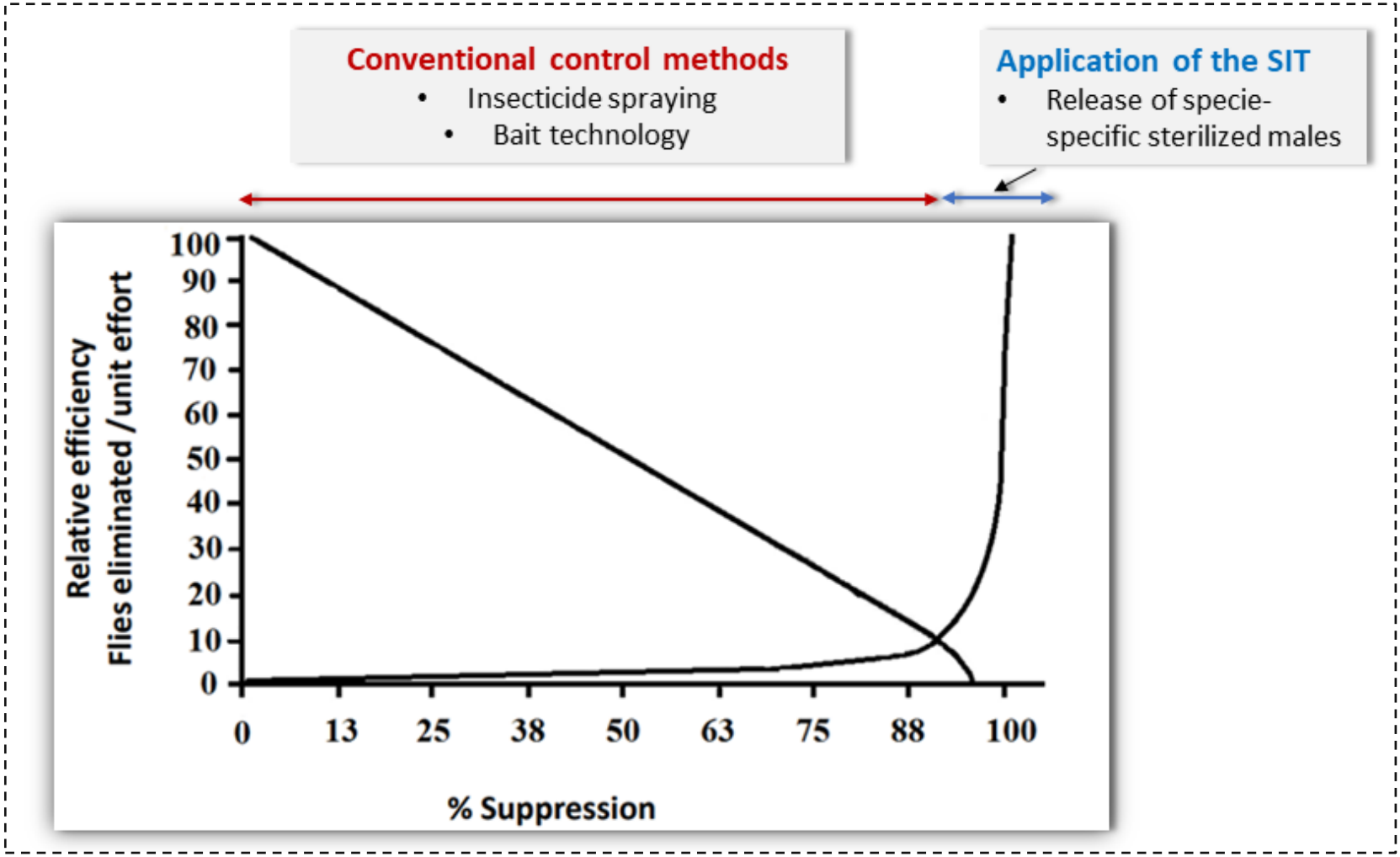

Figure 5: Strategies for integrating conventional control and SIT in an integrated and progressive approach to pest management. The conventional control methods (density dependent) cannot lead to 100% of suppression. They become less efficient as the tsetse density is low. The SIT will be effective only when the suppression reaches at least 95%. Updated from Sow [70].

5. Sterile insect technique (SIT)

5.1 Principles and requirements

The SIT as a genetic control method entails utilizing factors that induce reproductive failure, functioning essentially as a form of birth control [71]. It is a highly species-specific, effective, and environmentally acceptable method mostly used in combination with other control tactics within an AW-IPM strategy [68,71]. The fundamental principle of SIT centers on manipulating the fertility of insects to regulate population dynamics. This process includes mass production, sterilization, and the subsequent release of sterile male insects into the target population habitat [72] (Figure 6). The sterile males then mate with wild females in competition with wild counterparts, resulting in the production of no offspring [69]. The application of this advanced technique holds the potential to either eradicate or significantly suppress the targeted insect population [73].

Given that the implementation of SIT involves releasing sterile males, the mass production and sterilization of males through irradiation are essential prerequisites. When facing constraints in mass rearing, a critical aspect is radiation, which must achieve high sterilization while preserving the maximum quality of males, including their flight ability, competitiveness, and mating capacities [74]. Therefore, determining the lowest possible radiation dose for optimal sterilization with high-quality outcomes becomes crucial [75].

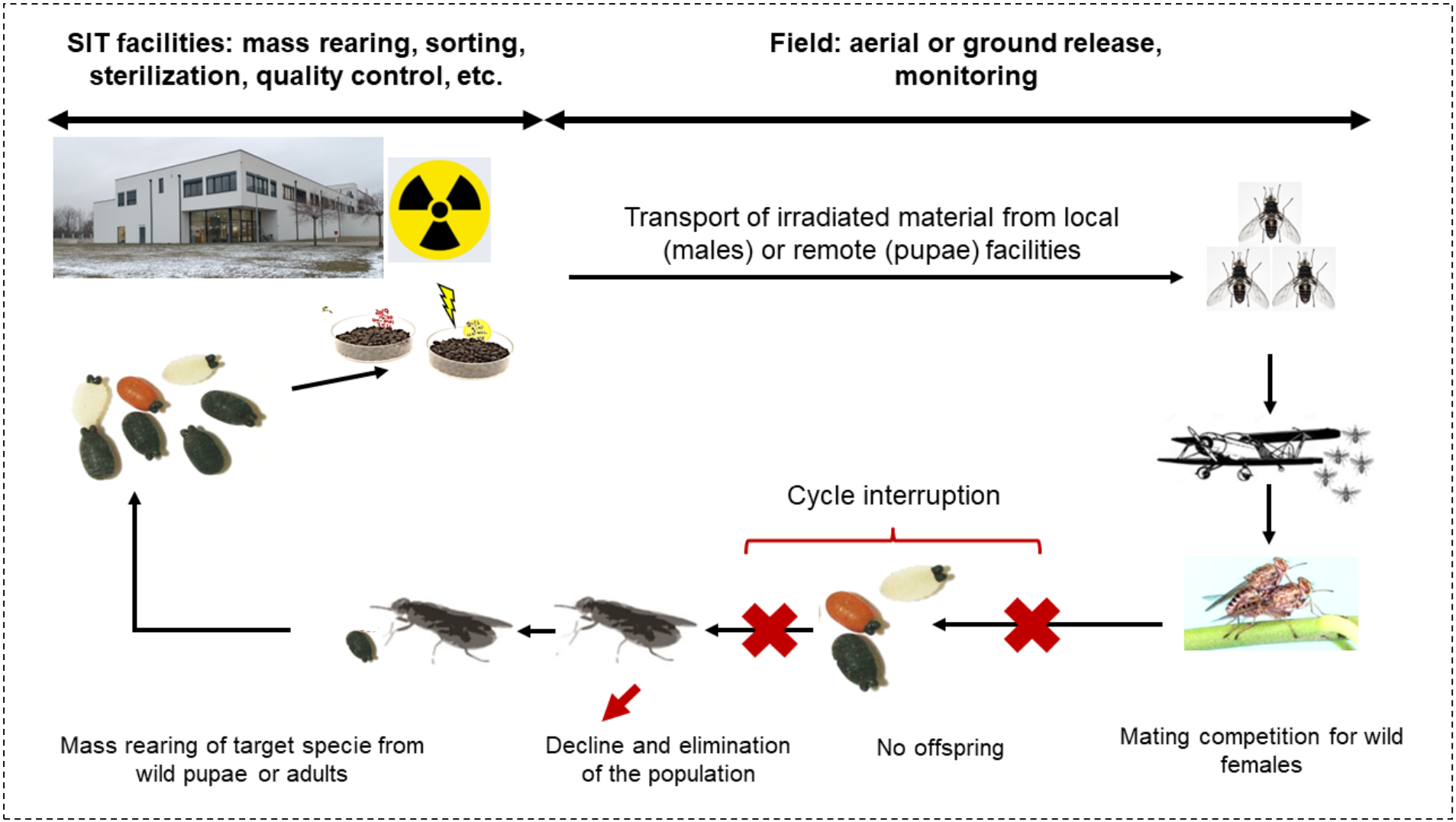

Figure 6: From the laboratory to the field: the principle of the sterile insect technique and the dynamics of pest population suppression and/or eradication.

5.2 History and success

Over the past sixty years, the technique of insect sterilization driven par Knipling [76,77] has been developed to target insect pests that attack crops and livestock or that are transmitting human diseases. In total, over 300 species of arthropods with mostly of economic importance, have been subjected to irradiation studies for purposes of research, disinfestation for quarantine or phytosanitary or different pest control applications, including the SIT [78]. The first success story of SIT is its use to eradicate the New World Screwworm *Cochliomyia hominivorax* (Coquerel) in the USA, Mexico, and Central America for the last 40 years [79]. It has been then applied to various insect pests such as *fruit flies*, tsetse, *moths* and mosquitoes for their prevention, containment, suppression and/or eradication across the world [69,80,81] (Table I).

Table I : Successful use of SIT for tsetse eradication in Africa using AW-IPM. Some eradicated areas have been reinvaded due to the absence of protective barriers or the cessation of activities.

Species	Country/zone	Associated methods	realease	Area (Km^2)	Sustainability	References
Ga	Tanzania (Island of Zanzibar)	attractive devices, treating livestock with insecticide	Aerial release	1,650	Sustained, no tsetse captured more 18 years after eradication	[82,83]
Gpg	Burkina Faso (Sideradougou)	insecticide application and trapping	Ground release	1,500	Unsustained, barriers not maintained, and tsetse reinvaded	[41,84,85]
	Senegal (Niayes)	insecticide-impregnated traps/targets and the use of "pour-on" for cattle	Ground and aerial release	1,000	Sustainable, barriers in place, control ongoing	[67]
Gpp	Nigeria	traps and insecticide-impregnated targets	Ground release	1,500	Unsustained, barriers not maintained, reinvasion occurred	[86,87]

Ga: Glossina austeni; Gpg: Glossina palpalis gambiensis; Gpp: Glossinna palpalis palpalis

6. Insect sterilization through irradiation

6.1 Primary radiation sources for sterilizing insects and their constraints

Radiation can originate from various sources, and it is categorized into two main types: ionizing and non-ionizing radiation [88]. Ionizing radiation has enough energy to remove tightly bound electrons from atoms, creating ions whereas non-ionizing radiation, on the other hand, lacks the energy to ionize atoms [88,89].

Gamma- and X-rays are waves in the electromagnetic spectrum, and gamma-rays have the shortest wavelength that are typically, but not always, shorter than those of X-rays (range from 10 pico- to 10 nanometers). These rays can be differentiated by their origin, i.e., gamma-rays are produced during nuclear decay of the nuclei of atoms, whereas X-rays are produced by electrons [90]. Gamma-rays have a stronger ionizing ability and X-rays have less penetrating power compared to gamma-rays (https://pediaa.com/difference-between-x-rays-and-gamma-rays/) and are widely used (Table II). In the 1920s, H.J. Muller made a groundbreaking discovery that X- or gamma-ray radiation could inflict significant genetic damage on insect reproductive systems, leading to the induction of sterility [91]. Thus, the suitability of a radiation type for SIT programs rely on its relative biological effect, penetrability, availability and safety [74]. The ability of radiation to induce chromosome aberrations depends on its linear energy transfer (LET) and is supposed to lead to higher effective sterility when LET is higher [92]. In this line, alpha and beta rays possess high LET but can penetrate only a fraction of a millimeter into a container of insects, making them unsuitable for SIT programs.

Traditional extensive methods involve the use of ionizing gamma radiation from radioactive isotopes, such as Cobalt-60 or Cesium-137 [93]. Demonstrated to be highly dependable, this method has proven itself to be extremely reliable in achieving the objective of sterilizing specific insect pests. However, initial attempts of using ionizing radiation on insect were based on the use of X-rays. At the end of the 19th century, several experiments aimed at inducing sterility in insects/mites were implemented, yielding negative results, which were later attributed to the rudimentary nature of the equipment used [94]. The first conclusive results were obtained by H.J. Muller almost a century ago when exposing *Drosophila melanogaster* sperm to heavy X-ray doses [95].

In addition to X-rays, electron beam irradiation has been tested not only for SIT but also for quarantine treatment of food [96]. Electron beam technology is widely used for sterilization in medical and food industries and has potential applications for insect sterilization [97]. Electron beam irradiation involves the use of high-energy electrons produced by accelerators and it is a non-radioactive method that does not leave residual radioactivity in treated items.

Table II displays the characteristics, advantages, and disadvantages of the available irradiators suitable or acceptable for sterilizing insects. It is crucial to emphasize that the selection of a radiation source is contingent upon various factors, including the size and characteristics of the insects, the intended degree of sterilization, and environmental considerations [98]. Concerns about the relative biological effectiveness (RBE) of X- versus gamma-rays for insect sterilization, along with regulatory approval and cost-effectiveness, are crucial in evaluating alternative radiation sources [98]. Regarding the constraints faced, due to growing apprehensions related to safety, procurement, and transportation of gamma rays sources, along with challenges in transboundary shipment of radioisotopes [98–102], regulations are becoming increasingly stringent. As a response, scientists are actively investigating alternative radiation sources and one promising avenue is the use of self-contained, low-energy X-ray irradiators for insect sterilization [103–110]. As a recent innovation, low-energy self-contained X-ray sources offer a unique perspective, warranting a comparative analysis with their self-contained gamma irradiators. While certain aspects, such as radiation safety requirements, exhibit notable similarities, distinctions emerge in areas such as reliability of operation, cooling requirements, mode of operation, and procedures for delivering a desired dose [102].

Table II: Typical characteristics of five radiation sources along with their advantages and disadvantages [98]

	Gamma ray (panoramic)	Gamma ray (self-shielded)	Electron Beam (accelerator)	X-ray (high energy)	X-ray (low energy)
Characteristics					
Photon/ electron energy range (MeV)	^{60}Co 1.17, 1.33	^{60}Co 1.17, 1.33/137Cs 0.66	1 – 20	1 – 7.5	0.15 – 0.225
Emission pattern	Isotropic	Isotropic	Narrow beam (~1 cm dia.) scanned across conveyor belt	Narrow beam (~1 cm dia.) scanned across conveyor belt	Isotropic
Penetration (cm in water to half dose rate)	High (20/23)	High (20/23)	Low (2-7)	Very high (15 – 40)	Low (5 – 10)
Typical dose rate (Gy.min^{-1})	Low-medium (5 – 20)	High (20 – 300)	Very high (100 – 10000)	Medium (5 – 500)	Low – medium (3 – 15)
Process method	Continuous/batch	Batch	Continuous	Continuous	Batch
Process time	Long	Short	Very short	Short – medium	Medium – long
Throughput (million fruit fly pupae/hr at 100 Gy)	6 – 12	0.2 – 0.8	0.05 – 19	0.08 – 54	0.025 – 0.37
Advantages and disadvantages					
Advantages	High throughput; Low running costs; Good dose uniformity; Very reliable; Capital costs lower than accelerator; Can be incorporated into a conveyor/automated system	Low running costs; Fairly good dose uniformity; Very reliable; Lower capital costs; No external shielding required	Can be integrated into automated system (conveyor belt feed); Licensing easier than cobalt	Can be integrated into automated system (conveyor belt feed); Licensing easier than cobalt; Good penetration allows load to be irradiated in transport containers	Cost effective options available; No shielding required; Redundancy by procuring two; Simple licensing and regulation
Disadvantages	Source replacement costs high; Radiological safety issues; Strict regulations on procurement and transport; Shielding required; Security costs (human resources and equipment)	Source replacement costs high; Radiological safety issues; Strict regulations on procurement and transport; Security costs (human resources); Difficult to integrate into automated system; Increased labour and handling	High capital and running costs; Requires shielding; No redundancy; Requires qualified service technicians; Very low penetration requires insects to be in shallow trays; Load must be flipped or two opposing accelerators to get adequate dose uniformity; Very high dose rate requires high conveyor speed	High capital and running costs; Requires shielding; No redundancy; Requires qualified service technicians; Load may need to be flipped or two opposing accelerators to get adequate dose uniformity; High dose rate requires high conveyor speed	Difficult to integrate into automated system; Increased labour and handling; Limited reliability record; Time and cost to replace X-ray tube; Requires qualified service technicians

The potential for the widespread use of such irradiators is gaining momentum due to their distinct advantages (Table II). Not only do they address safety concerns, but they also eliminate the need for intricate and expensive purchase and transportation of irradiators and radioactive sources across international borders. In addition, regulations involving safety and shielding requirements are limited. Continual research in this field is focused on developing efficient and sustainable methods to tackle agricultural and public health challenges associated with insect pests. Studies involving mosquitoes and fruit flies have revealed potential benefits and effects, indicating that the X-ray generator holds promise for SIT programs, making it not only applicable to other insect species but also a more cost-effective and practical alternative [105,107,109].

6.2 Pathways of the dominant lethal mutations

In the context of the SIT, radiation serves as a crucial tool for inducing sterility in insects. The targeted radiation dose causes damage to the reproductive cells of the insects, effectively compromising their ability to produce viable offspring [111,112]. The radiation induced dominant lethal mutations have been attributed to those chromosome aberrations that result in bridge formation during cleavage as depicted in the Figure 7 [113].

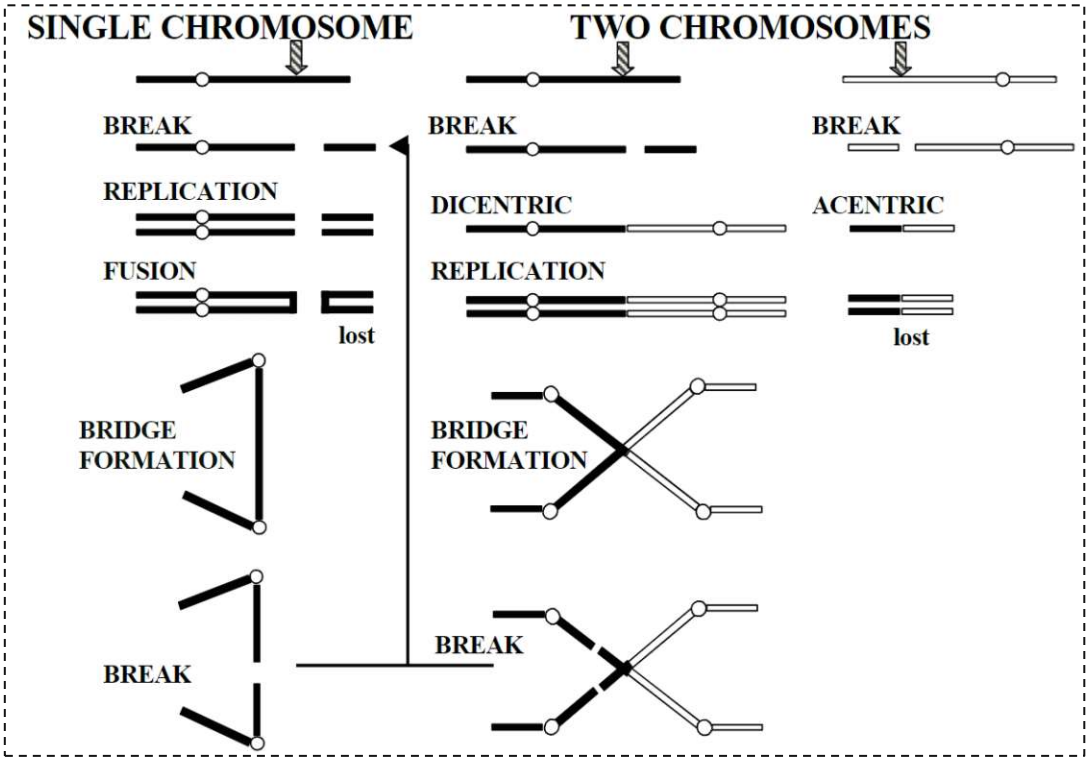

Figure 7: Schematic representation of radiation-dominant lethal mutations pathways during cell division that result in the accumulation of imbalances in the genetic information of daughter

cells. The primary lesion leading to a dominant lethal mutation is a break in the chromosome [113].

These mutations have been made possible through the oxygen radicals that are produced when the radiation penetrate the insect cells [114,115]. Radiation-induced ions and radicals engage with molecules, leading to the formation of secondary DNA radicals or initiating chain reactions. The consequential mutations in the DNA of cells are responsible for most notable biological effects. The occurrence of these mutations in germ cells does not interrupt the maturation of the cell into a gamete or the participation of the gamete in the formation of the zygote but it does result in the cell death or that of the developing embryo [113,116,117]. However, several factors may play a role in the amount of dominant lethal mutations induced [113] resulting in different radiosensitivity.

6.3 Factors of variation of the radiosensitivity

The required dose for the sterilization of arthropods varies widely, ranging from less than 5 Gy for the acridid *Schistocerca gregaria* to 400 Gy or even more for certain lyonetiid, noctuid, and pyralid moths [74]. There are several endogenous factors as well as extrinsic factors that influence the radiation sensitivity. In addition to the classic difference between germ cells, there exists an overall sensitivity difference between male and female insects to radiation. Generally, females exhibit higher sensitivity than males to sterilization through irradiation. This sensitivity has been shown for *Glossina sp.* [118,119], mosquitoes [120] and fruit flies [121]. This discrepancy implies the necessity of identifying the sterilization dose for the SIT based on the acceptable dose for males.

Concerning the life stage, in most insects, spermatogenesis occurs during the larval/pupal stage [93,122]. Therefore, the early stages of spermatogenesis exhibit higher sensitivity to radiation-induced irreversible damage compared to later stages but accompanied with higher somatic damage [113]. However, the selection of the life stage relies heavily on the operational requirements of the specific SIT program [78]. In tsetse control programs with an SIT component, both pupae and adult stages have already been utilized [67,83,123]. Beyond these factors, insect weight and size, nutritional status, and genetic differences may play a role in radiosensitivity, impacting variables like the correlation between dietary protein intake and both cuticular melanization and immune function [74,124].

In addition to inter and intra-specific variations in radiosensitivity, there are numerous other factors that can influence an organism's sensitivity to radiation. Some of these factors include

radiation atmosphere, temperature, dose fractionation as well as handling prior and after irradiation, however other factors may also have an impact on the sterilized insects quality [125–128]. Finally, radiosensitivity can be associated with characteristics such as the dose rate or energy range of photons/electrons from various radiation sources, independently of their specific constraints [74,129].

7. Tsetse irradiation for SIT: measurable biological effects of radiation on insects

When establishing irradiation protocols, a crucial objective is to determine the optimum irradiation dose that preserves the high competitiveness of irradiated males compared to their wild counterparts [130,131]. It is imperative to induce a level of sterility that effectively limits progeny while ensuring the continued viability of the released population and the rate of sterility induced in the wild population of insect pests [132]. The cost-effectiveness of a program with an SIT component depends on the balance between the sterility level and competitiveness of the sterilized males [125]. Achieving this balance allows effective control of insect populations and successful implementation of SIT as part of AW-IPM strategies.

7.1 Determining the optimum sterilization dose

The selection of an optimal radiation dose is a crucial aspect of feasibility studies aimed at launching SIT programs. Achieving the necessary sterility level, estimated to fall between 95% and 99%, necessitates accepting the lowest dose ensuring complete female sterility. Given the challenges in sex separation during early stages (larvae, pupae) and transportation constraints, current methods fall short of obtaining 100% male samples. In addition, in many SIT campaigns, mass rearing facilities are often situated far from release fields, prompting the dispatch of sterilized males as pupae or adults [80,123,126,133,134]. It is, therefore, crucial to prevent the simultaneous introduction of reared females and sterilized males. Furthermore, despite the pivotal role of irradiation in SIT, the procedures, the dosimetry, and the selection of an appropriate dose for maximizing sterility induction in wild females exhibit substantial variation. Establishing precise dose-response curves for the target insect, with accurate dosimetry, is imperative for both the feasibility study of SIT and the continuous improvement of operational programs.

Table III furnishes details regarding the optimal radiation dose required to induce a minimum of 95% sterility in females mated with irradiated males. Additionally, it outlines the life stage

of the insect and essential radiation treatments designed to minimize somatic damage. Above these established optimal radiation doses, it is imperative to conduct quality control tests to ensure the competitiveness of the sterilized males.

Table III : Summary of the radiation optimal doses for several stage of tsetse species of interest.

Species	Life stage	Age (days)	Irradiation treatments	Optimal radiation dose (Gy) (≥95% of sterility)	Induced sterility (%)	References
Glossina austeni	Late stage pupae	36	Air	80 and 100	99.0-100.0	[119]
	Adult males	4	Air	80 and 100	97.0–99.0	[119]
Glossina brevipalpis	Pupae	37-41	Air	40	>97.0	[135]
	Adult males	4-6	NA	50	95.0	[136]
	Adult males	4	NA	80	99.0	[135]
Glossina fuscipes fuscipes	Pupae	NA	Air	120	> 95.0	[137]
	Adult males	4-6	NA	80-100	95.0	[136]
Glossina morsitans centralis	Adult males	NA	NA	100	98.0	[138]
Glossina morsitans morsitans	Adult males	3-4	NA	190	100.0	[122]
	Male pupae	27	Nitrogen	120	99.0	[139]
	Male pupae	Emergence day	Nitrogen	120 and 170	99.9	[139]
Glossina morsitans submorsitans	Adult male	NA	NA	120	NA	[84]
Glossina pallidipes	Adult males	13	Air	120	96.0	[140]
Glossina palpalis gambiensis	Adult males	12	NA	110	97.7	[141]
	Male pupae	NA	Chilled at 8 °C	120	97.0-99.0	[142]
	Adult males	4-5	Air	120	100.0	[136]
Glossina palpalis palpalis	Male pupae	25-28	Air	120	100.0	[136]
	Adult males	NA	NA	118-120	NA	[87]
	Pupae	NA	Air	120	98.0	[143]
	Pupae	NA	Nitrogen	150	97.0	
Glossina tachinoides	Adult males	4-6	Air	120	95.0	[136]
	Adult males	1-9	NA	155	100.0	[122]
	Male pupae mid-satge	15	Nitrogen + dose fractionation	60-80	95.0	[136]
	Male pupae	20	Chilled at 15 °C for 9 days	10	95.0	[136]

NA: Not Available

7.2 Measurable biological effects of radiation on tsetse key quality parameters

Standardized tests are instituted to ensure that the optimal required dose for sterilization does not significantly impact the key quality parameters. These parameters encompass the adult emergence of irradiated pupae, flight propensity, survival rate, and mating competitiveness [119,134,135,144]. Table IV presents selected results illustrating the radiation impact on key quality parameters in some tsetse species.

Assessing adult emergence rate showed that radiation dose could have a negative effect as emergence rate is decreasing with increasing radiation dose [135]. However, most of the studies showed that the optimal radiation dose did not significantly decrease the emergence rate as compared to the non-irradiated pupae [126,127,144] (Table IV). Nevertheless, additional stress factors such as chilling and long transport as well as handling processes reduced significantly the adult emergence rate in *G. p. gambiensis* [126]. The younger age of the irradiated pupae in relation with their sensitivity could also impact negatively the emergence rate [119].

The flight propensity as well as the survival time test are implemented to follow-up the quality of biological products within the SIT facility or provided by partners or distant facilities to the operational SIT program [123,134,143]. The flight propensity is often impacted even with the optimal radiation dose (Table IV). As an example, the flight propensity was significantly affected by the radiation dose in *G. p. gambiensis* as compared to the control [144]. As for the survival rate, the available research results have shown that the survival time of irradiated flies under starvation was significantly reduced as compared to the control [141,144] contrasting with the findings on flies emerged from pupae chilled, irradiated and transported [126]. This indicates that many additional factors might be involving in the male survival rate (Table IV).

Concerning the mating competitiveness, some studies using the optimal radiation dose showed that the irradiation does not affect the ability of irradiated males to compete with the fertile males for non-irradiated females namely on *G. brevipalpis* [135], *G. pallidipes* [145], *G. p. gambiensis* [142,144] (Table IV). Moreover, the irradiation does not impact the sterile male insemination potential [135,144,145]. In male *G. p. gambiensis* irradiated with 120 Gy, the mating latency, the mating duration as well as the insemination rate did not differ between the irradiated and the control group [142]. The same results have been obtained in *G. pallidipes* using a range doses for the mating duration and the insemination rate [140]. This confirms that the irradiated male remain competitive and kept their ability to transfer sperm even the

spermathecae fill could be affected or not by the irradiation [140,142,144]. To accelerate the pest elimination and eradication, the release of large amount of sterile male in the field in a certain ratio is advised to compensate the lower relative mating index caused by the radiation [146].

Finally, handling procedures, characteristics of radiation sources, dosimetry implementation, operator influence, and geographical differences are among the various factors that may influence the radiation's effects. This literature review aimed to summarize the socio-economic impact of tsetse, the diseases they transmit, and the strategies employed for their control. Therefore, the crucial role of sterilization within the SIT as a component of an AW-IPM approach has been emphasized. This is achieved through the description of radiation sources, dose-response relationships, and their effects on flight quality and mating competitiveness. Considering the costs and complexities associated with the SIT, it is essential that feasibility studies, improvements to current protocols, and research into alternative radiation sources be grounded in relevant parameters.

Table IV: Examples of irradiation impact on emergence rate, flight propensity, survival time and mating competitiveness parameters of some tsetse species. Comparison have been made with the non-irradiated groups.

Species	Life stage	Irradiation treatments	Traget dose	Dosimetry	Emergence rate	Flight propensity	Survival	Mating competitiveness (PM/RMI)	References
Ga	4-day-old adult	Air	80, 100	No	Age effect	NA	NA	0.63/ 0.35 and 0.32	[119]
	Pupae of 32-36-day-old	Air	100	No	Age effect	NA	NA	0.41/0.36 and 0.24	
Gb	Pupae of 37-, 39- and 41-day old	Air	40, 60, 80, 100, 120 or 140	No	Negative effect with increasing radiation dose	NA	NA	NA	[135]
	Pupae of 37-, 39- and 41-day old	Air	40, 60, 80, 100, 120 or 140	No	Negative effect	NA.	Under feeing: reduction in average longevity before 56 days	NA	
	Adult of 4-day-old	Air	40 and 80	No	NA	NA	NA	0.57/0.33 and 0.41, no significant difference in the RMI	
Gp	Pupae of 25-28 day-old, 13-day-old adults	15 °C (24-72 h)	120	NA	NA	NA	NA	0.77-0.97/0.28, 0.29, 0.36, 0.36,0.31, 0.30 significant difference related to cold treatment	[127]
Gpg	Pupae after females energence	Chilling (8±2 °C), long transport, handling	110	No	Effect of the chilling, No effect of radiation	Reduction from 82 ± 13% to51 ±21%	No effect of the irradiation	NA	[126]
		Male pupae chilled at 8 °C	120	No	NA	NA	NA	0.460.33, no significant difference in RMI	[142]
			140	No	NA	NA	NA	0.46/0.15, significant lower RMI	
	Pupae after females energence	Chilling at 8–10 °C for 24 h	110	Yes	No significant effect	Reduction from 89.58% to 79.87%	Reduction from 5 to 4 days, on average	0.57/.44, no significant difference in RMI	[144]

NA: Not Available

8. Objectives of the research

8.1 Aim and general objective

This research was conducted at the Insect Pest Control Laboratory (IPCL) of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture and in collaboration with the Institute of Chemical, Environmental and Bioscience Engineering of the Vienna University of Technology. The research aimed to advance the SIT for tsetse by exploring alternative ionizing radiation sources. The general objective was to identify new radiation technologies and associated protocols that can offer comparable or enhanced performance while mitigating some of the limitations associated with traditional gamma-ray sources. This includes considerations such as safety, cost-effectiveness, and environmental impact and involves in a particular emphasis on exploring the application of ionizing radiation, including X-rays, in SIT.

Beyond identifying alternative sources of irradiation to gamma rays, this research also focusses on refining standing irradiation protocols. This includes optimizing parameters such as dose fractionation and irradiation atmosphere/temperature to enhance the precision and effectiveness of sterilization for tsetse. This comprehensive approach to refining irradiation protocols and exploring alternative sources underscores our commitment to advancing the science of insect irradiation for both scientific and operational applications within SIT programs, to improve outcomes, reduce risks, and promote sustainability in insect pest management.

8.2 Specific objectives

The first objective was to evaluate the suitability of the blood irradiator Raycell Mk2 for sterilizing Fruit flies, Tsetse and Mosquitoes. As part of this collaborative investigation, experiments were conducted on the dose-response of male *G. p. gambiensis* irradiated with X-rays as pupae with the results presented in Chapter 2 and published in *Insects*.

The second objective was to assess the effectiveness of two X-rays sources (Blood irradiator Raycell Mk2 and Rad Source 2400) in comparison with a gamma-ray source (Model Foss 812) for the sterilization of male *G. p. gambiensis* as pupae. In addition to the dose-response, a comprehensive assessment of the effects of X- and gamma-rays radiation on adult emergence, flight propensity, survival under stress and feeding regime, as well as mating competitiveness in semi-field conditions was undertaken. The results are presented in Chapter 3 and has been published in *Scientific Reports*.

The third objective was to investigate the potential hormetic effects of radiation-dose fractionation on *G. p. gambiensis* sterility, survival as well as the flight quality parameters. The first experiment explored the effects of splitting the optimal radiation dose into (i) small and big fractions or (ii) nearly equal fractions separated by 1-, 2-, 3-day intervals. In a second experiment, an equal fractionation separated by 4-, 8-, and 24-hour interval was investigated. The results are presented in Chapter 4 and have been published in *Parasite*.

Finally, the fourth objective was to investigate the effects of radiation under low temperature and nitrogen-enriched environment on the sterility and survival of male *G. p. gambiensis* when exposed to gamma- or X-rays. Experiments were conducted to determine the dose-response of male tsetse irradiated as pupae under hypoxic conditions with gamma- or X-rays using Rad Source 2400 and Model Foss 812 and as pupae or adults in chilled/non-chilled conditions using a X-Rad 320 Precision model, respectively. The results are presented in Chapter 5.

Ultimately, the research aimed to provide valuable information and recommendations for advancing insect pest management practices through an understanding of insect sterilization protocols and the exploration of innovative radiation sources, including X-rays, for more sustainable and effective applications of the SIT. Consequently, Chapter 6 was dedicated to discussing the main results in relation to their applications in the field and their contribution to the cost-effectiveness and simplification of SIT, which could serve as drivers for the engagement of governments and stakeholders to implement SIT as a part of the Area-Wide Integrated Pest Management of tsetse in West Africa.

Chapter 2

Suitability of Raycell MK2 Blood X-ray Irradiator for the Use in the Sterile Insect Technique: Dose Response in Fruit Flies, Tsetse Flies and Mosquitoes

insects

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Article

Suitability of Raycell MK2 Blood X-ray Irradiator for the Use in the Sterile Insect Technique: Dose Response in Fruit Flies, Tsetse Flies and Mosquitoes

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Simple Summary: The sterile insect technique (SIT) is an environment-friendly, species-specific pest control method by which target insects are mass-produced in a factory and are made infertile by irradiation—usually with gamma rays. However, gamma sources are becoming more difficult and expensive to purchase, and the regulations surrounding these types of irradiators are becoming stricter. Therefore, there is now increasing interest in alternatives, such as X-ray irradiators. Following a recent technical evaluation of a blood X-ray unit, the aim of this research was to assess the biological responses of a selection of major SIT target insect species to irradiation in the X-ray unit as compared to gamma ray irradiation. It was found that all the insects responded similarly to X-rays as to gamma rays and that the X-ray unit is suitable for small- to medium-sized SIT programs.

Abstract: The sterile insect technique (SIT) is based on the inundatory field release of a target pest following their reproductive sterilization via exposure to radiation. Until recently, gamma irradiation from isotopic sources has been the most widely used in SIT programs. As isotopic sources are becoming increasingly expensive, especially for small programs, and regulations surrounding their procurement and shipment increasingly strict, irradiation capacity is one of the limiting factors in smaller or newly developing SIT projects. For this reason, the possibility of using X-ray irradiators has been evaluated in the recent decade. The availability of "off-the-shelf" blood X-ray irradiators that meet the technical requirements for insect irradiation can provide irradiation capacity for those SIT projects in which the acquisition of gamma ray irradiators is not feasible. Following the recent technical characterization of a Raycell MK2 X-ray blood irradiator, it was found in this study, that MK2 instruments were suitable for the sterilization of fruit flies, tsetse flies and mosquitoes, inducing comparable, even slightly higher, sterility levels compared to those achieved by gamma ray irradiation. This, together with its estimated processing efficiency, shows that MK2 irradiators are suitable for small- to mid-sized SIT programs.

Keywords: *Ceratitis capitata*; *Anastrepha ludens*; *Glossina palpalis gambiensis*; *Aedes aegypti*; *Anopheles arabiensis*; X-ray; gamma ray; sterility; SIT

1. Introduction

Irradiation-induced sterilization of insects is an integral part of the sterile insect technique (SIT) [1] in which target pest species are produced in mass-rearing facilities, and males are made infertile before releasing them into a field site. Successful mating between the sterile males and wild females lead to a progressive decline in the target pest population density over successive generations and, thus, reduces crop loss and preserves animal, as well as human, health [1–3]. The Insect Pest Control Laboratory (IPCL) of the Joint FAO/IAEA Center of Nuclear Techniques in Food and Agriculture in Seibersdorf, Austria, houses several species and strains of fruit flies, tsetse flies and mosquitoes and has been driving research for the development of a SIT package against these insect pests of crops, livestock and human health. The most notable research toward the development or optimization of a SIT package against these pests in the past ten years was reviewed in [4].

The process of reproductive sterilization is one of the mandatory components of the SIT, and exposure to gamma radiation from isotopic sources is, to date, the most efficient, reliable and widespread method to achieve sterility in insects [5], especially in large SIT programs. However, the regulatory challenges and costs of procuring and transporting radioactive sources are very high and still rising, making the acquisition of 60Co-based irradiators a limiting factor for many SIT facilities, especially in the earlier phases of the projects [6]. The feasibility of using X-ray irradiators has been evaluated in the most recent decade, and it has been found that X-ray irradiation, in general, induces sterility in insects similarly to gamma ray irradiation in tested insects [7–13]. Furthermore, the availability of "off-the-shelf" blood X-ray irradiators with competitive purchasing costs that meet the technical requirements for insect irradiation can now provide irradiation capacity for those SIT projects in which the acquisition of gamma ray irradiators is not feasible. A recent technical characterization of a Raycell MK2 X-ray blood irradiator (Best Theratronics Ltd., Kanata, ON, Canada) showed that this irradiator provided a dose uniformity ratio of under 1.2, an average dose rate of 7.7 Gy/min, and 2 L of irradiation capacity [6], thereby meeting the FAO/IAEA-recommended minimum criteria for insect irradiation with X-rays [14]. Following this initial assessment, the current study aims to complete an evaluation of an MK2 instrument by providing biological dosimetry data in the form of dose response curves for select insect pest species. The Plant Pests, Livestock Pests and Human Disease Vector groups of the IPCL [4] perform three separate experiments to assess the suitability of MK2 irradiators for the sterilization of fruit flies, tsetse flies and mosquitoes, respectively. The experimental set-up reflects the insect species, strains and life stages, sample preparation and irradiation processes and doses used, as expected to be used in SIT programs.

2. Materials and Methods

2.1. Irradiation Set-Up

All samples of insects were irradiated at a standard reference point, which was the center of a 2 L irradiation canister provided with the MK2 instrument used, as well as according to the dose rates and dose distribution map determined and described in Gómez-Simuta et al. [6]. Fruit fly and tsetse fly pupae were irradiated in instant rice for improved absorbed dose homogeneity in the sample and sample canister, as instant rice presents a similar density as insect pupae and, thus, serves as appropriate dummy material. Dose rates were, thus, measured in rice, and dose times were calculated accordingly. Mosquito pupae density is closer to water, whereas that of adults is closer to air. Dose time was, therefore, calculated according to dose rates measured in water and air, respectively. Where insects were additionally irradiated with a gamma ray irradiator for direct comparison, either a Foss Model 812 gamma irradiator (Foss Therapy Services Inc., North Hollywood, CA, USA) or a Gammacell 220 irradiator (Nordion Ltd., Kanata, ON, Canada) was used. These had dose rates of 56 Gy/min and 74 Gy/min, respectively.

2.2. Dosimetry

To ensure the accuracy of the irradiation dose given in each radiation event, Gafchromic HD-V2 or MD-V3 dosimetry films (International Specialty Products, Wayne, NJ, USA) were packed in small (2 × 2 cm) paper envelopes, which were placed near each insect sample. Gafchromic HD-V2 dosimetric films were previously indicated to be appropriate for the dose response of X-ray and gamma ray irradiation [6]. A DoseReader 4 instrument (Radiation General Ltd., Budapest, Hungary) appropriate for Gafchromic™ film [15] was used to read the films 24 h after irradiation. The standard operating procedure for Gafchromic™ film dosimetry [15] was followed to determine the absorption dose for each radiation event. The calibration used had a global uncertainty of 4.29%.

2.3. Dose Response of *Ceratitis capitata* and *Anastrepha ludens* Pupae under Hypoxic Conditions

2.3.1. Strains and Rearing

The *Ceratitis capitata* VIENNA 8 genetic sexing strain (GSS) was developed at the IPCL [16]. This GSS was characterized by a pupal color mutation in which a wildtype copy of the markers was attached to the Y chromosome so that males expressed the wild phenotype (brown pupae), and females expressed the mutant phenotype (white pupae sensitive to temperature). Female embryos could be eliminated at the embryo stage by exposing the eggs to high temperature (34 °C) for 24 h [16]. The *Anastrepha ludens* GSS was developed in Mexico [17] and transferred to the IPCL in 2017. The males expressed a brown pupae phenotype, while the black pupae phenotype was expressed in females; then, the sexes could be separated at the pupal stage by using a pupal color-sorting machine.

The laboratory rearing conditions of the flies were 24 ± 1 °C, 60 ± 5% relative humidity (RH) and a photoperiod of 14 h light: 10 h dark. The adult flies were fed with a standard adult diet [18,19], which consisted of sugar and hydrolyzed yeast in a ratio of 3:1 and water ad libitum. The flies in this study were kept in 30 × 30 × 45 cm (length, width and height, respectively) cages that were covered with muslin cloth and had openings for experimental handling. The larvae of the flies were maintained on a carrot-powder-based diet. The pupae were separated according to the color of the pupa, as described above.

2.3.2. Irradiation Procedure and Assessment of Sterility

Ceratitis capitata: Batches of brown pupae (males) two days before emergence were placed in plastic bags one hour before irradiation to achieve hypoxia, as described by Schwarz et al. [20] and the FAO/IAEA/USDA product quality control manual for fruit flies [21]. Two samples were irradiated separately in a Foss Model 812 Gamma Irradiator and in a Raycell MK2 irradiator at doses of 80, 90, 100, 125 and 145 Gy. Samples of pupae undergoing the same handling without irradiation were kept as controls.

After irradiation, pupae were kept in Plexiglas cages for fly emergence. Twenty-four hours after emergence, for each replicate, 20 sterile males and 20 fertile females were placed into a 30 × 30 × 30 plexiglass cage for sexual maturation, mating and oviposition. During the peak of the oviposition period, a sample of eggs was counted, placed on a piece of cloth, transferred to a larval diet and placed in an incubator at 28 °C for egg hatching. After 48 h, the number of unhatched eggs was recorded, and the number of pupae collected at the end of the experiment was registered. Five repetitions were performed for each dose and both the gamma and X-ray treatments.

Anastrepha ludens: Samples of *A. ludens* were collected, prepared and irradiated as described above for *C. capitata*. Only the dose of 80 Gy that is used in operational programs for the induction of reproductive sterility in this species was assessed for both the gamma ray (Foss Model 812) and X-ray (MK2) treatments. After irradiation, the pupae were returned to the insectary, and the dose response was assessed as described above for *C. capitata*. Nine repetitions were performed for each irradiator.

2.4. Dose Response of Glossina palpalis gambiensis Pupae

2.4.1. Strain and Rearing

The *Glossina palpalis gambiensis* colony used in this assessment was established at the IPCL in 2009 from pupae derived from the Centre International de Recherche-Developpement sur l'Elevage en zone Subhumide (CIRDES) colony in Burkina Faso. Initially, the strain was colonized at Maisons-Alfort (France) in 1972 using pupae collected in Guinguette (Burkina Faso) and transferred to CIRDES in 1975 [22]. The last wild material introduced into the colony was collected at Mare aux Hippopotames in 1981. The colony, as well as the pupae and adults used in the assessment, were maintained at a constant temperature and relative humidity (RH) of 24 ± 0.5 °C and 75–80%, respectively, and under subdued and indirect illumination with a 12 h light: 12 h dark photoperiod [23,24]. The colony and experimental flies were fed three times per week on defibrinated bovine blood using an artificial membrane feeding system.

Pupae that were produced in the colony were collected daily and sex-sorted with a newly developed Infrared Pupae Sex Sorter (NIRPSS) at 23–24 days following larviposition. The NIRPSS was preconditioned with the following melanization parameters: T1 of 252, T2 of 0.10 and T3 of 10. The male pupae were selected from the same cohort of pupae classed as unmelanized when the unmelanized ratio (unmelanized pupae/total pupae sorted) was below 38%.

2.4.2. Irradiation Procedure and Assessment of Sterility

Depending on the replication, fifty to seventy-five male *G. p. gambiensis* pupae were placed in a 60 mm × 13 mm petri dish without filling it, and this was placed in the middle of a cylindrical sample canister (2.0 l, 167 mm (Ø), 97 mm(H)) accompanying the Raycell MK2 instrument used. The remaining volume of the sample canister was filled with rice. The pupae were then exposed to radiation doses of 70, 90, 110 and 130 Gy. The control group was selected from pupae that were not irradiated. All irradiated and control pupae were handled and kept under similar conditions.

The irradiated and control pupae were incubated at 24 ± 0.5 °C and 75–80% RH until emergence. The teneral males were collected and kept in small cages (110 mm (Ø); 45 mm (H)) and fed as described above until sexual maturity. The females that emerged, due to a sorting error, were discarded. The seven- to eight-day-old irradiated and control males were mated in standard colony cages (Ø 20 cm) with three- to four-day-old virgin females at a 1:1 or slightly below male (N = 431): female (N = 592) ratio for four days, and their mortality was monitored daily. Males and females were then separated by chilling at 4 °C. The females were transferred to 20 cm diameter cages, and their daily production and mortality rates were recorded for 60 days. Six replications were performed for all doses.

2.5. Dose Response of Aedes aegypti and Anopheles arabiensis Pupae and Adults

2.5.1. Strains and Rearing

The *Aedes aegypti* strain originated from field collections in Juazeiro (Bahia), Brazil, and was transferred to the ICPL from the insectary of Biofabrica Moscamed, Juazeiro, Brazil, in 2016. Both the *Aedes* strains were maintained following the "Guidelines for Routine Colony Maintenance of *Aedes* mosquitoes" [25]. The Dongola strain of *Anopheles arabiensis*, originating from Dongola, Northern State, Sudan, was donated by the Tropical Medical Research Institute, Khartoum, Sudan, in 2010 and was maintained at the IPCL following the anopheline mass-rearing guidelines [26].

2.5.2. Irradiation Procedure and Assessment of Sterility

Eggs of *Aedes aegypti* from one cohort were collected and split in half to be hatched two days apart (one batch for collecting adults and one for collecting pupae for irradiation simultaneously). Males that emerged within an 8 h window were collected, counted into batches of 30, and placed in 15 × 15 × 15 cm Bugdorm® cages (MegaView Science Co. Ltd., Taichung, Taiwan). The next day, the adult males were transferred to and irradiated in

small 2 cL plastic cups closed with sponges. At the time of irradiation, the adults were 24–32 h old.

Pupae from the same cohort were collected in 4 h windows to ensure a uniform pupal age of 40–44 h, which is the most radioresistant age in this species. The pupae were sexed based on pupal size using a glass pupal sorter [27], and sex was verified under a stereomicroscope. All males were kept for irradiation, and females were transferred to individual tubes for emergence to ensure virginity for later mating. Male pupae were batched into groups of 30 in 2 cL plastic cups with excess water removed for irradiation.

The irradiation doses were selected according to the expected dose required to induce 50–100% sterility: 20, 55, 70 and 90 Gy. Both the pupae and adults in each technical repetition were irradiated simultaneously in a Raycell MK2 irradiator. Six repetitions were performed for all doses. Controls received the same handling but were not irradiated.

Anopheles arabiensis pupae were collected and sexed visually using a stereomicroscope. Females were placed in individual tubes for emergence to ensure virginity and were kept for later mating. Male pupae were counted into batches of 30 and were placed inside 2 cL plastic cups with excess water removed for irradiation. Male adults were knocked down in a cold room, counted into batches of 30 and placed into plastic tubes for irradiation. At the time of irradiation, male pupae were 24–28 h old, and adults were 24–30 h old. Both pupae and adults were irradiated in a Raycell MK2 irradiator with doses of 75, 90, 100, 110 and 120 Gy. Controls received the same handling but were not irradiated. Additional sample batches including controls were collected, sexed and prepared for irradiation with the same procedures but were irradiated in a GC220 gamma ray irradiator, with 55, 70, 95 and 110 Gy.

Following irradiation of both species, the males of each treatment group were placed in separate 15 × 15 × 15 cm Bugdorm® cages. Thirty virgin females were added to each cage when the adults reached 2–3 days of age and were allowed to mate for 3 days before they were provided with 2 bloodmeals on consecutive days (days 6 and 7 after emergence). Oviposition cups were added to each cage on day 8 for mass egg collection (on days 9 and 10 after emergence) and were hatched following routine rearing protocols [25,26]. The total numbers of hatched and unhatched eggs were counted using a stereomicroscope. Any nonhatched eggs were either opened with a dissection needle, or if there were many, bleached to determine fertility status [28].

2.6. Statistical Analyses

The tsetse pupae emergence rate was analyzed using a generalized linear mixed model, where the dose was considered a fixed effect and the replicates as random effects. An emmeans comparison with the Tukey method was used to assess the differences between the irradiation dose treatments. The induced sterility of the tsetse flies was calculated by subtracting from 100% (pupae production in the control group) the treatment production relative to the control group, which was obtained by dividing the pupae produced in each irradiation dose treatment by the pupae produced in the control group.

Sterility in the fruit flies was calculated as the percentage egg hatch of the control group hatch rate. A Wilcoxon rank sum test with continuity correction was used to compare hatch rates of gamma- and X-ray-irradiated fruit flies. The residual fertility (RF) for mosquitoes was calculated as a proportion of the control fertility of each treatment group (RF = HRtx/HRc), where HRtx was the hatch rate of the treatment (tx) group, and HRc was the hatch rate of the control (c) group. Induced sterility (IS) was calculated by subtracting the RF from 1.

To analyze the dose response of pupae versus adults for *Ae. aegypti* and *An. Arabiensis*, a binomial GLMM fit by maximum likelihood (Laplace approximation) was used for egg hatch rates (considered as response variables), life stage (2 levels: pupae and adults) and irradiation log (dose) (4 levels: 20, 55, 70 and 90 Gy), and their interactions were considered fixed effects, with repetition as a random effect.

3. Results

3.1. Dosimetry

The dosimetry confirmed that all doses received lay within a 4.29% error range.

3.2. Sterilization Efficiency of Raycell MK2

All insect species used in this study responded to the X-ray irradiation in the MK2 instrument as expected, with induced sterility levels comparable to those achieved in alternative X-ray and gamma ray irradiators using the same doses. When comparing the dose responses in pupae of all five species, *Ae. aegypti* were the most radiosensitive, becoming nearly fully sterile (99.8% IS) at doses of 55 Gy and above. *A. ludens* were fully sterile at 80 Gy, whereas *An. arabiensis* and *C. capitata* showed similar dose response curves and needed at least 100 Gy to achieve above 99.9% IS. Finally, *G.p. gambiensis* needed a dose of 110 Gy or above to reach 99.6% IS (Figure 1).

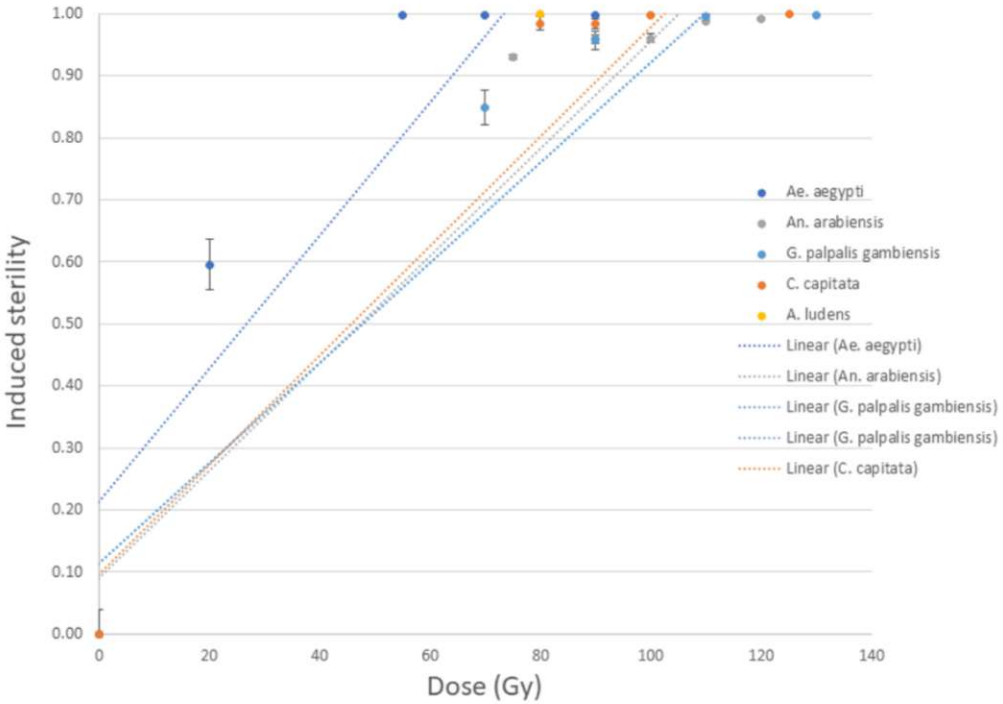

Figure 1. Induced sterility of pupae of five insect species in response to increasing irradiation doses in an MK2 irradiator. *A. ludens* was subjected to only one dose.

3.2.1. *Ceratitis capitata* and *Anastrepha ludens* Pupae

Ceratitis capitata pupae showed slightly higher levels of sterility (<2%) following irradiation with X-rays in the MK2 irradiator compared to irradiation with gamma rays (FOSS 812), (p = 0.036); a dose of 100 Gy resulted in 99.7% and 98.7% induced sterility (IS), respectively. Both the 125 Gy and 145 Gy doses gave full sterility, regardless of irradiator type. The dose responses following irradiation doses of 80, 90 and 100 Gy compared to the same doses of gamma irradiation are shown in Figure 2.

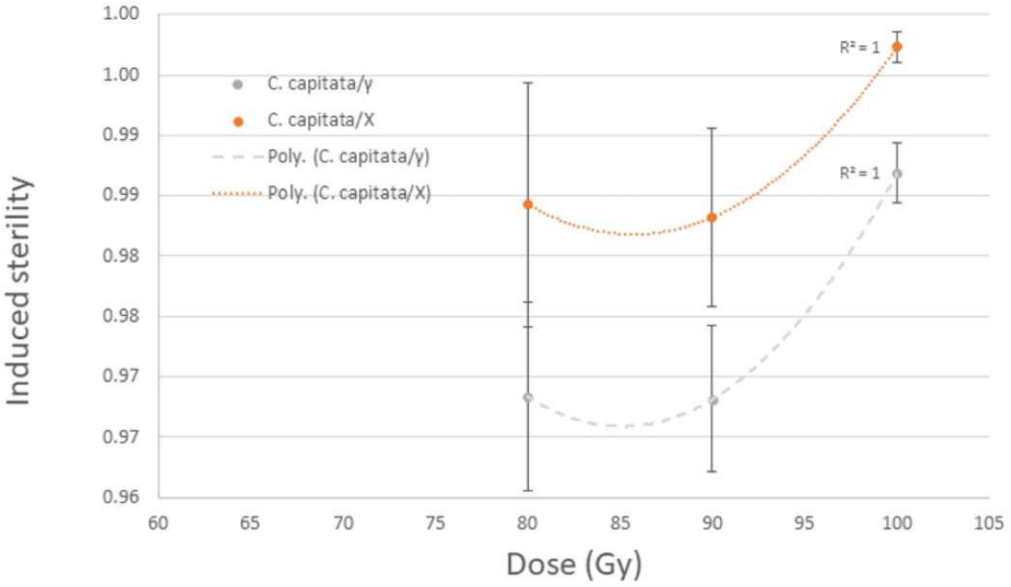

Figure 2. Dose responses of *C. capitata* following irradiation with 80, 90 and 100 Gy with X rays in an MK2 irradiator compared to γ-rays (Foss Model 812).

Anastrepha ludens irradiated as pupae in hypoxic conditions with 80 Gy with both X-rays (MK2) and gamma rays (FOSS 812) resulted in 100% sterility.

3.2.2. *Glossina palpalis gambiensis* Pupae

After exposure to radiation, the pupae were incubated until emergence. A decrease in emergence rate from 89.7% to 83.8% was observed as the dose increased. A significant difference in the emergence rate was observed (X2 = 14.332, df = 4, $p = 0.006$) and was higher in pupae irradiated with 90 Gy compared to those irradiated with 110 and 130 Gy. The total number of eggs aborted was higher in females mated with irradiated males than those mated with fertile males at all doses ($p < 0.001$), and this was inversely correlated to the pupae production. The number of eggs aborted by females mated with males irradiated at 90 Gy and 110 Gy was higher than the number in females mated with males irradiated with 70 Gy. The fecundity of females mated with irradiated males decreased from 0.012 to < 0.001 as the irradiation doses increased from 70 to 130 Gy. In contrast, the mean induced sterility in females mated with irradiated males increased from 84.9 ± 6.7 to 95.7 ± 3.6, 99.6 ± 0.6 and 99.8 ± 0.5% as the irradiation doses increased from 70 to 90, 110 and 130 Gy, respectively, when using the MK2 instrument (Figure 3).

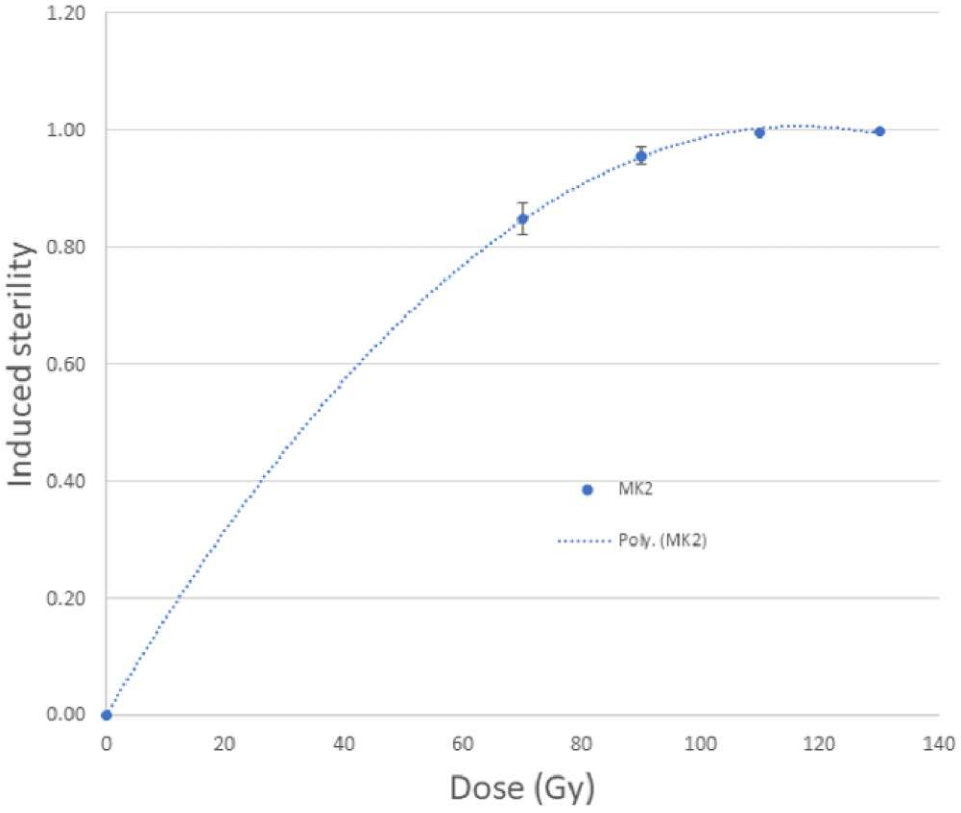

Figure 3. Dose response curve of *Glossina palpalis gambiensis* pupae irradiated with X-rays in an MK2 irradiator.

3.2.3. *Aedes aegypti* and *Anopheles arabiensis* Pupae and Adults

As expected, the hatch rates of both *Ae. aegypti* and *An. arabiensis* reduced significantly with increasing dose (df = 4, $p < 2.2 \times 10^{-16}$). When irradiated in the MK2 instrument, *Ae. aegypti* male pupae and adults presented with sterility levels exceeding those observed following irradiation with gamma rays (Figure 4). In these species, no difference in radiosensitivity between the two developmental stages could be observed following exposures in both irradiators, as a dose of 55 Gy or above led to very high sterility of over 99.9% (Figure 4), (df = 1; $p = 0.172$).

Both male adults and pupae of *An. arabiensis* responded to X-ray irradiation in the MK2 irradiator similarly to gamma ray irradiation. Induced sterility was, however, slightly higher following X-ray irradiation (Figure 5). The adult stage in this species was also more radiosensitive than the pupal stage (df = 1; $p = < 2.2 \times 10^{-16}$), which corroborates data for the same strain irradiated with gamma rays (GC220) (Figure 5).

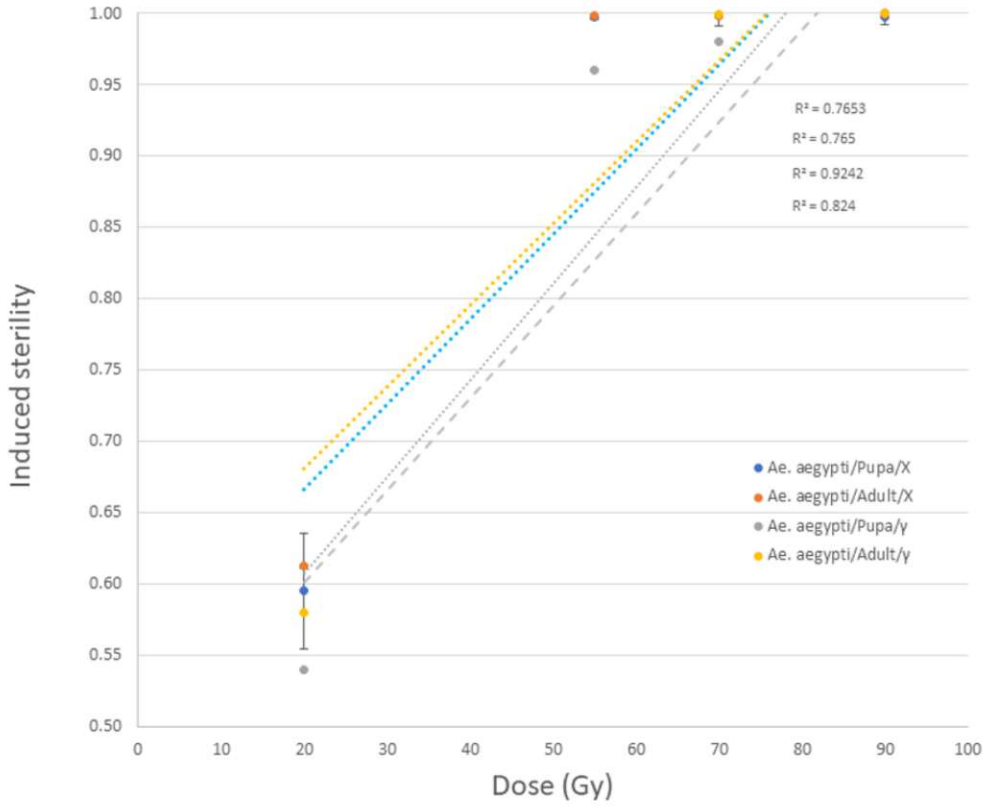

Figure 4. Dose response data of *Ae. aegypti* male pupae and adults irradiated with MK2 (X-ray) irradiator compared to the same strain irradiated in a GC220 instrument (gamma ray, (Yamada et al., 2022 [35])).

4. Discussion

The sterility data obtained from the five insect species tested in this report confirmed the relative biological effectiveness of MK2 irradiators compared to other X-ray and gamma ray irradiators. In the two tested fruit fly species, *C. capitata* was more sensitive following irradiation in the MK2 instrument than in the gamma ray irradiator, and *A. ludens* was fully sterile at the tested dose following irradiation in both irradiators. In a relevant study by Mastrangelo et al. [9], a (at the time) new generation of X-ray irradiator (RadSource2400) was evaluated in which the dose responses of *C. capitata* and *Anastrepha fraterculus* were assessed. It was also found that the exposure of the two fruit fly species to X-rays resulted in higher levels of sterility compared to gamma rays. In this case, 99% sterility in *C. capitata* was achieved with mean doses of 91.2 Gy with X-rays and 124.9 Gy with gamma rays, whereas 40–60 Gy was sufficient to sterilize *A. fraterculus* for both radiation treatments, which corroborates the results in this study. At present, most sterilization of insects is accomplished using gamma radiation, and considering that a dose of 80 Gy of gamma radiation was used in Anastrepha's mass-rearing laboratories in some countries (such as Mexico and Guatemala) [5], our result of 100% sterility in *A. ludens* was achieved with 80 Gy of X-rays, which supports the suggestion that the use of X-rays could be an alternative technology for SIT with regard to biological dosimetry.

The MK2 irradiator was equally successful at sterilizing *G. p. gambiensis* pupae. Doses of 90 and 110 Gy were sufficient to induce 95.7 and 99.6% sterility in females that mated with exposed males. In other biological dosimetry tests for this species, gamma irradiation has been predominantly used for both adult and pupae sterilization, and for both life stages, a dose of 110 Gy only has induced sterility levels of 93.4% in adults [29] and 89.7% in pupae [30]. It has been reported in other studies that pupae are more sensitive to radiation than adults. When *Glossina brevipalpis* were treated as adults, a dose of 40 Gy induced 93% sterility in females, and the same dose when applied to pupae induced a sterility of 97% [31]. This variation in sensitivity between life stages was also seen for the subspecies of *Glossina palpalis palpalis* [32], for which a dose of 120 Gy was needed for adults and a dose of 60 Gy for pupae to induce sterility of 95%. The low induced sterility for pupae exposure to a dose of 110 Gy recorded by Ilboudo et al. [30] might be because of an error in the dose, as their dosimetry indicated an absorbed dose of 81 Gy. As an X-ray dose of 90 Gy was sufficient to induce sterility of 95.7%, further assessments to verify and compare the dose response of this species using two X-ray and one gamma ray irradiator are in progress.

In the two tested mosquito species, similarly, a lower X-ray dose was needed to reach the same level of sterility when compared to gamma irradiation, corroborating historical data where gamma ray irradiation has been applied. For *An. arabiensis* (Dongola strain), 110 Gy were required for >99% sterility in adults and 120 for pupae using the same Gammacell 220 machine with a cobalt-60 source and a dose rate of 16 Gy/min at the time of the experiment [33]. The GC220 irradiator was then introduced in 2010. Yamada et al. [34] observed higher residual fertility (14% and between 4 and 7%) in pupae of the same strain following irradiation in the same GC220 instrument with dose rates of 93 Gy/min (in earlier repetitions) and 84 Gy/min (in later repetitions), respectively [34]. In this study, using the same GC220 irradiator with a dose rate of 74 Gy/min at the time of the experiment, 110 Gy was sufficient to fully sterilize adults. However, pupae showed 4.6% residual fertility at the same dose. The sterility achieved in the MK2 irradiator in this particular strain of *An. arabiensis* showed fertility data lower than but closest to the data of Helinski et al. in 2006 [33]. This was likely because the dose rates of the MK2 are lower (average of 7.7 Gy/min) and closer to the GC220′s 16 Gy/min in 2006 than the other higher dose rates used in subsequent studies [35].

The dose response results for *Ae. aegypti* confirm former reports that *Aedes* spp. are generally more radiosensitive than Anophelines. In this particular experiment, male adults and pupae showed the same responses to the irradiation doses. However, a previous study showed that adults could be slightly more radiosensitive than pupae, although usually not significantly so [36]. This was not evident with the doses used in the MK2 irradiator, as doses of 55 Gy and above resulted in nearly full sterility. Other biological dosimetry tests performed in this strain of *Ae. aegypti* using a different X-ray irradiator (RS2400) with a dose rate of 9.11 Gy/min gave a very similar response curve (Yamada, unpublished data), whereas irradiation with gamma rays at lower dose rates induced higher sterility levels [37], and inversely, those with higher dose rates resulted in lower sterility levels [34].

These results, combined with the comparison of X-ray and gamma ray irradiation of *C. capitata*, support the findings of Yamada et al. [35], where it was shown that dose-dependent dose rate effects altered the dose response in mosquitoes and, likely, in other insects. At high doses, the higher the dose rate, the higher the residual fertility (and the lower the induced sterility). These new findings support our hypothesis that the increased sterilization efficiency of the X-ray irradiators is due to a dose rate effect. However, it is important to also investigate the effects of energy independent of dose and dose rate on insect dose response.

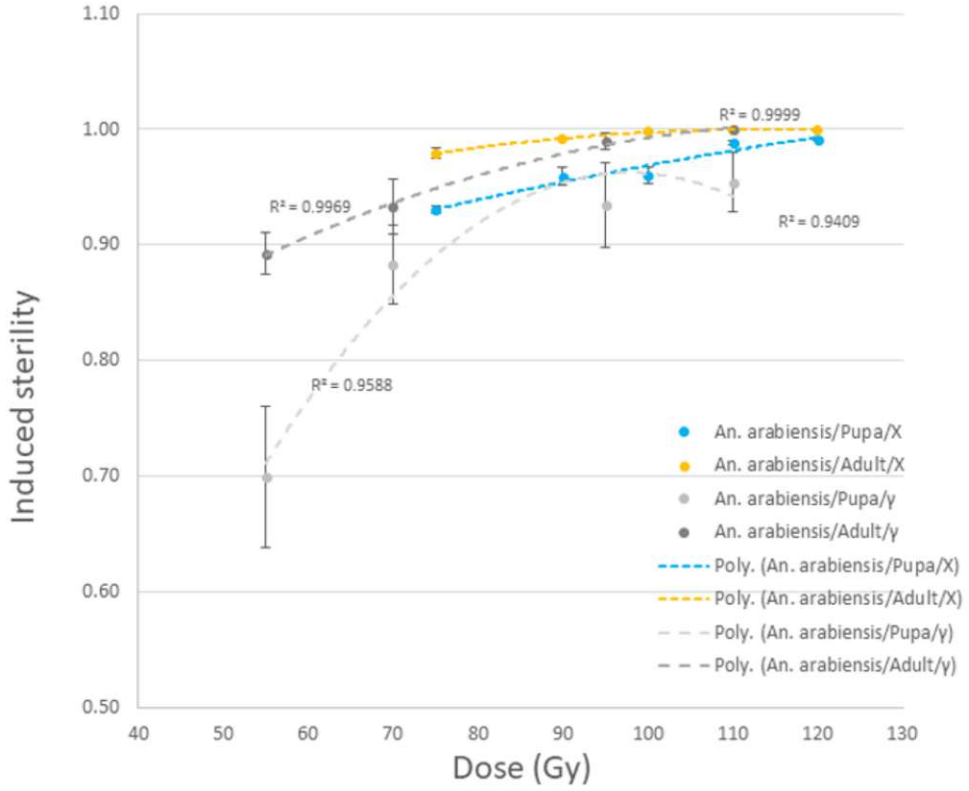

Figure 5. Dose response data of *An. arabiensis* male pupae and adults irradiated with MK2 (X-ray) irradiator compared to the same strain irradiated in GC220 irradiator (gamma ray).

Apart from the relative biological effectiveness of an irradiator, processing efficiency is important for the assessment of its suitability for operational SIT programs. The requirements for this depend on the size and production capacity of the program and, thus, can vary. The largest SIT programs currently are those controlling fruit flies; the highest-producing facility is the El Pino facility in Guatemala, which has a production capacity of 3.6 billion sterile males per week. For the sterilization of such quantities, high-dose-rate, high-capacity, self-shielded or panoramic irradiators are needed. Other programs that require smaller production numbers, such as those in Hawaii, Costa Rica, Australia and many pilot facilities, can be run adequately with smaller irradiators, such as self-shielded GC220 instruments or, alternatively, blood X-ray irradiators such as the MK2 machine. Using the full volume of the 2 L canister, a fruit fly SIT program can sterilize approximately 13.4 million *A. ludens* and 25 million *C. capitata* pupae per week with one 8 h shift per day, with the potential to increase the processing capacity to 26.9 and 50 million per week, respectively, with the implementation of a second shift per day. Of course, these numbers can be increased by procuring more than one X-ray unit.

The current protocol of pupae irradiation that is used for the SIT program against tsetse flies in Senegal indicates that the pupae are irradiated inside specialized boxes designed for pupae shipment [38]. The recommended density of pupae inside a box is 1500 [38–40], and four boxes can fit inside the 2 L canister (i.e., 6000 tsetse pupae). Thus, with a processing

time (exposure time for 110 Gy plus sample loading) of 19 min, three loads per hour can be irradiated. Therefore, around 1.1 million tsetse pupae can be treated in a 5-day week with two 6 h shifts. For the successful SIT program on Unguja Island (Zanzibar) in an area of 1650 km^2, the largest number of flies that were ever released in one week was 102,557 [41]. Thus, the processing capacity of the MK2 irradiator more than meets the requirements of similar-sized tsetse SIT programs. If the full capacity of the 2 L canister were to be used, it could hold around 48,000 tsetse pupae. The output could, thus, be increased to around 8.6 million tsetse sterile males each week. However, the handling and packing protocols would need adjusting so as to not damage the pupae during irradiation. Additionally, in SIT programs against tsetse flies (and mosquitoes), blood used for feeding the colonies requires sterilization with irradiation at 1 k Gy to minimize contamination with pathogens (https://www.iaea.org/sites/default/files/guidelines-for-blood-processing-procedures.pdf, accessed on 16 September 2022). This can also be accomplished in an MK2 irradiator in just over 2 h.

For mosquito SIT programs against *Aedes* spp., in theory, around 75 million adult mosquitoes can be treated in a 5-day week with two 6 h shifts, as the full volume of the 2 L canister can hold 250,000 Aedes adults, and a processing time (exposure time for ~60 Gy plus sample loading) of 12 min allows for five loads per hour. The processing capacity of *An. arabiensis* would be less, as double the dose and, thus, double the time (~20 min) is needed for the exposure itself, thus allowing for only three loads per hour. Additionally, this species is slightly larger, and fewer adults can be compacted into the same space [42]. These numbers are adequate for current pilot SIT trials, the largest of which releases around 10 million sterile *Ae. albopictus* males per week. However, it is anticipated that large-scale area-wide control programs may require much higher irradiation capacity, for which multiple self-contained X-ray irradiators would be needed if it is not possible to house an industrial, high-throughput irradiator. It is also important to note that adult mosquitoes need to be immobilized by, for example, chilling [35,42,43] to be compacted in a container and not sustain injuries when moving around while packed. The chilling needs to remain for the duration of the irradiation exposure if irradiation times exceed 4–5 min, by which time adults start to become active at room temperature.

The MK2 irradiator, in common with other X-ray sources, has several advantages over isotopic sources: lower capital cost, much lower transportation costs and much simpler regulation and access control. As the generation of X-rays relies on electrical power, the radiation can also be easily turned off by removing the power. Servicing is more straightforward, as there is no radiation to contend with, and replacement tubes can be supplied by regular carriers. The supply of replacement cobalt-60 sources is both expensive and problematical. There have been an increasing number of cases of denial or delay of shipments of radioactive material [44–48], and there are stringent regulations and rising costs. The lower energy of X-ray systems means that it is much easier to block radiation, and typical X-ray systems are self-shielded and do not require a special room to house them. Finally, the skills needed for the handling of high-level radioactive sources are scarce, whereas the skills for handling the high-voltage systems required for X-ray are available in most countries.

The downside is, if the electrical supply is not reliable, the system does not function. All X-ray systems require good cooling to prevent the tubes from overheating, which can be difficult in remote locations, and X-ray tubes are rather fragile and susceptible to damage during transport. X-ray dose rates are often much lower than those from isotopic irradiators, and the lower energy and, in some cases, beam configuration restrict the volume that can be irradiated. In addition, X-ray systems are more likely to suffer failures due to the fragility of the tubes and the complexity of the electronics, high-voltage systems and external cooling units.

X-ray systems, therefore, offer advantages to small SIT programs with their lower costs and simpler regulation but are not yet able to compete with isotopic irradiators for larger programs. Although some SIT programs have implemented e-beam technology for

high-throughput irradiation (for instance, 600 million *C. capitata* are irradiated per week in Spain), currently available industrial e-beam systems are very expensive, and purchase is not feasible for most facilities. Small, compact electron beam systems and flat panel X-ray technology show promise for the future but are not yet ready for use.

5. Conclusions

Overall, MK2 irradiators were suitable for the effective and reliable sterilization of three target insect groups of SIT. The observed biological responses to the X-ray irradiation were comparable to gamma ray irradiation—in this case, irradiation in an MK2 machine resulted in higher sterility levels than those obtained in the two tested gamma irradiators. Together with its good DUR and processing efficiency, the unit met the requirements for small- to medium-scale SIT programs for fruit flies, tsetse flies and mosquitoes. Further research on the effects of dose rate and energy can further the understanding of the differences between X-ray and gamma ray irradiation.

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Chapter 3

X-rays are as effective as gamma-rays for the sterilization of *Glossina palpalis gambiensis* Vanderplank, 1911 (Diptera: Glossinidae) for use in the Sterile Insect technique

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X-rays are as effective as gamma-rays for the sterilization of *Glossina palpalis gambiensis* Vanderplank, 1911 (Diptera: Glossinidae) for use in the sterile insect technique

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An area-wide integrated pest management strategy with a sterile insect technique (SIT) component requires a radiation source for the sterilisation of male insects. Self-contained gamma irradiators, which were exclusively used in past SIT programmes, are now facing increasing constraints and challenges due to stringent regulations. As a potential alternative, new generation high output X-ray irradiators have been proposed. The feasibility of using X-ray irradiators was assessed by comparing the effects of both gamma- and X-ray irradiators on biological parameters of *Glossina palpalis gambiensis* (Vanderplank, 1911), that are important for SIT applications. The gamma irradiator Foss Model 812 and two X-ray irradiators, the Rad Source 2400 and the blood irradiator Raycell Mk2 were used. *Glossina palpalis gambiensis* males were exposed to radiation as pupae. A radiation dose of 110 Gy or above induced more than 97% sterility in females that mated with the irradiated males for all the irradiators. Adult emergence rate, flight propensity, survival and mating performance did not differ between gamma- and X-rays irradiators. These results suggest that irradiating pupae with a dose of 110 Gy is optimal for both gamma-and X-ray irradiators used in this study, to achieve a sterility of approximately 99%. Similar research on other tsetse species could gradually phase out the use of gamma-ray irradiators in favour of X-rays irradiators, especially for smaller SIT programmes.

Insect pests that transmit pathogens and parasites to livestock are responsible for the loss of billions of dollars in agriculture and livestock production globally. Furthermore, climate change will likely contribute to a change in the traditional geographic distribution of insect-borne pathogens.

In Africa, tsetse flies (Diptera: Glossinidae) are the cyclical vectors of *Trypanosoma* spp. that cause Human African Trypanosomosis (HAT), known as sleeping sickness, and African Animal Trypanosomosis (AAT) known as nagana. Sleeping sickness caused devastating epidemics during the twentieth century and if left untreated, the disease can lead to death[1]. Nagana remains a neglected disease that causes a reduction in animal health and productivity[2,3]. As such, it represents a major obstacle to the expansion of livestock breeding and livestock-based industries in humid and sub-humid zones in 10 million km^2 of sub-Saharan Africa[4].

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A variety of techniques have been developed to suppress or eradicate populations of tsetse flies. Although insecticide-impregnated targets/traps, live bait technologies and the sequential aerosol technique have been successful in clearing some areas containing tsetse flies, most of these areas have later been re-invaded as most of these programmes were not implemented on an area-wide basis[5]. Area Wide-Integrated Pest Management (AW-IPM) with a Sterile Insect Technique (SIT) component can potentially eliminate a population of a specific insect species within a circumscribed target area. In the 1930s, E.F. Knipling suggested that sterile males could be used to reduce or eradicate wild populations of specific pest insects[6,7]. This idea led to the largest and most successful AW-IPM programme, integrating a SIT component, implemented over a period of 50 years, i.e., the eradication of the New World screwworm *Cochliomyia hominivorax* (Coquerel, 1858) (Diptera: Calliphoridae) from the southern USA, Mexico, the rest of Central America and Panama[7,8] . The SIT was successfully used, in combination with other control methods, to eradicate, suppress or contain several insect pest populations including the eradication of the pink bollworm *Pectinophora gossypiella* (Saunders, 1844) (Lepidoptera; Gelechiidae) in the southern USA and northern Mexico, the containment of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann, 1824) (Diptera; Tephritidae) in Guatemala and Mexico[9,10] and suppression of the codling moth, *Cydia pomonella* (Linnaeus, 1758) (Lepidoptera; Tortricidae) in the Okanagan Valley of British Colombia, Canada[11] and the suppression of *Ceratitis capitata* (Wiedemann, 1824) in South Africa, Israel and Jordan[12,13]. This technique was demonstrated on the Island of Unguja, Zanzibar in 1994–97 where a population of *Glossina austeni* (Newstead, 1912) was eradicated using a combination of control tactics, i.e., insecticide impregnated targets, pour-on treatments on livestock and SIT[2,14]. The same approach was successfully used against a population of *G. p. gambiensis* (Vanderplank, 1911) in the Niayes of Senegal[15].

The SIT consists of the production of the target insect in large production facilities, the sterilisation of the male insects using ionizing radiation and the sustained and sequential release of the sterile insects in the target area[16]. The success of the SIT depends on the effective sterilization of the released male insects and the ability of these males to compete with their field counterparts for mating with wild females. Since the start of research on the development of the SIT package for the management of tsetse fly populations, the sterilization was mostly achieved using ionizing radiation[16,17]. Self-contained gamma irradiators that have a Cobalt-60 (^{60}Co) source remain the most practical and extensively used radiation source in SIT programmes, whereas Cesium-137 (^{137}Cs) sources have largely been phased out[18–20]. Gamma irradiators are, however, facing growing constraints and difficulties in terms of transport, import legislation, safety concerns and costs, limiting them only for programmes that produce large enough quantities of sterile insects[21–25]. As a result, some gamma irradiator manufacturers have been obliged to stop manufacturing these self-contained ^{60}Co irradiators[23]. In the last two decades, efforts have been undertaken to assess whether a new generation of high output X-ray irradiators could likewise be used in these SIT programmes. As X-ray irradiators are not dependent on an isotopic source to produce radiation and their use is perceived as having fewer safety concerns, they have been proposed as an alternative to gamma irradiators in SIT programmes[23]. Regardless of the source of the radiation, the suitability of the type to be used for SIT programmes depends on properties such as relative biological effectiveness, penetrability, availability, safety and cost[26]. Before X-ray irradiators can be incorporated in SIT programmes, the feasibility to use these sources for insect sterilization needs to be determined and validated. Results obtained with mosquitoes, fruit flies and lastly the tsetse fly species *G. p. gambiensis* showed that X-ray irradiators can potentially be used for the sterilization of male insects of these target species and can serve as a cheaper and more practical alternative especially in smaller SIT programmes[20,23,24,27–29]. Indeed, X rays induced 99.8% of sterility in *Aedes aegypti* with doses of 55 Gy and above, 100% of sterility in *Anastrepha ludens* with 80 Gy, 99.9% in *Anopheles arabiensis* and *Ceratitis capitata* with 100 Gy, 99.6% in *G. p. gambiensis* at 110 Gy[30]. Compared to gamma-rays, X-rays induced comparable, and in some cases, slightly higher sterility levels in these fruit flies and human diseases vectors.

Following this recently demonstrated suitability of a blood X-ray unit for tsetse sterilization, the objective of the current study was to assess the dose responses of the target species *G. p. gambiensis* in the Niayes region of Senegal after exposure of pupae to radiation with two X-rays irradiators, in comparison with one gamma-ray irradiator. The biological parameters assessed included adult emergence rate, adult fly survival, induced sterility after mating of the treated males with non-irradiated females, flight propensity and mating performance or competitiveness. Our findings will supply the needed background to support the possibility of using X-ray sterilisation in the tsetse control programmes.

Results

Dosimetry

Gafchromic film was used to measure the absorbed dose. Supplementary Table S1. show the means, the 95% confidence interval, and the difference between the absorbed and targeted dose for the dose response evaluations, flight quality control and mating performance experiments. With the exception of the 130 Gy dose with the Foss Model 812 in the dose response evaluation (5.8%), the doses as measured with Gafchromic film did not differ significantly from the target doses at 5%. However, dose response curves were based on the absorbed dose.

Experiment 1: dose response of pupae exposed to gamma- and X-rays

Emergence rate and female contamination

Adult emergence rate did not show significant differences based on the irradiators ($\chi^2 = 2.5275$; df = 1; $p = 0.1119$) or the radiation type ($\chi^2 = 2.3864$; df = 1; $p = 0.1224$). Furthermore, there were no significant effects on the emergence rate due to interactions between irradiators and doses ($\chi^2 = 10.6905$; df = 8; $p = 0.2199$). However, the radiation dose had a significant negative effect on the emergence rate ($\chi^2 = 25.248$; df = 4; $p < 0.0001$) which decreased with increasing radiation dose (Supplementary Fig. S1). The emergence rate was significantly higher in the control group as compared to the 110 Gy ($p = 0.0218$) and 130 Gy ($p = 0.0002$) treatment groups. Additionally,

the emergence rate was significantly higher in the 70 Gy treatment group than the 130 Gy treatment group ($p = 0.0014$). Female contamination rate was $9.4 \pm 4.7\%$. All females that emerged were not further used in the experiment and discarded.

Female and male survival

The survival curves of the non-irradiated females mated with the irradiated males are shown in Supplementary Fig. S2. The mean survival time was 57.1 ± 14.3 days. No statistically significant difference was observed in the survival time of flies between the irradiators ($\chi^2 = 0.19$; $df = 2$; $p = 0.9085$), nor the radiation type ($\chi^2 = 0.126$; $df = 1$; $p = 0.7227$) or the interaction between the irradiators and the radiation doses ($\chi^2 = 3.3659$; $df = 8$; $p = 0.9093$). The best model showed that only the radiation dose had a significant effect on survival ($\chi^2 = 21.99$; $df = 4$; $p = 0.0002$) (Supplementary Fig. S2). Females that mated with males irradiated as pupae with 70, 110 and 130 Gy survived significantly longer than females mated with non-irradiated males ($p_{70/0Gy} = 0.0146$; $p_{110/0\ Gy} = 0.0008$; $p_{130/0\ Gy} = 0.0060$). In the case of females that mated with males irradiated with 90 Gy, no statistically significant difference in lifespan was observed when compared to the control group ($p = 0.3020$).

The mean survival of the males under normal feeding conditions was 26.7 ± 16.7 days. No significant effect was observed in male survival probability between the irradiators ($\chi^2 = 0.3439$; $df = 2$; $p = 0.842$) or the radiation type ($\chi^2 = 0.3052$; $df = 1$; $p = 0.5807$). The interaction between the irradiators and the doses did not significantly affect the survival ($\chi^2 = 5.2319$ $df = 8$; $p = 0.7325$). The results of the best Coxme model showed that the radiation dose had a significant effect on the survival of the males ($\chi^2 = 345.06$; $df = 4$; $p < 0.0001$). In addition, survival decreased proportionally with increasing dose (Fig. 1).

Fecundity of females that mated with irradiated males

A total of 1770 non-irradiated virgin females were mated with irradiated and non-irradiated males and on day 18 (average age of first larviposition), 1658 were still alive, giving an overall survival rate of 93.7%. Supplementary Table S3 and Table S4 contain the adult emergence from irradiated pupae and the reproduction parameters of females mated with the irradiated and non-irradiated males. The three-parameter Weibull (W1.3) model was the best fitting model to describe the fecundity dose response curves. The graph was developed using the summarized data and predicted data from the experiments (Supplementary Fig. S3). Table 1 shows the results from the Weibull 3-parameter model. When comparing the three parameters i.e., upper limits, slopes and effective doses, no significant difference was found between the irradiators (Supplementary Table S2). Similar results were found when comparing the estimated effectives doses that reduced 50, 95 and 99% of fertility ($p > 0.05$).

The number of eggs aborted during the 60 days was similar in females mated with males irradiated with all the three irradiators ($\chi^2 = 1.8823$; $df = 1$; $p = 0.1565$).The GLMM with a negative binomial family showed that the number of eggs aborted varied significantly with dose ($\chi^2 = 691.78$; $df = 4$; $p < 0.0001$) while the irradiators had no significant effect (Fig. 2). The number of aborted eggs was higher in females mated with the irradiated males than females mated with non-irradiated males ($p < 0.0001$), and this was inversely related with pupae production (Supplementary Table S3). Additionally, the number of eggs aborted by females mated with males irradiated with 90 Gy and 110 Gy was higher ($p_{90Gy} = 0.0177$; $p_{110Gy} = 0.0024$) than those aborted by females mated with males irradiated at 70 Gy.

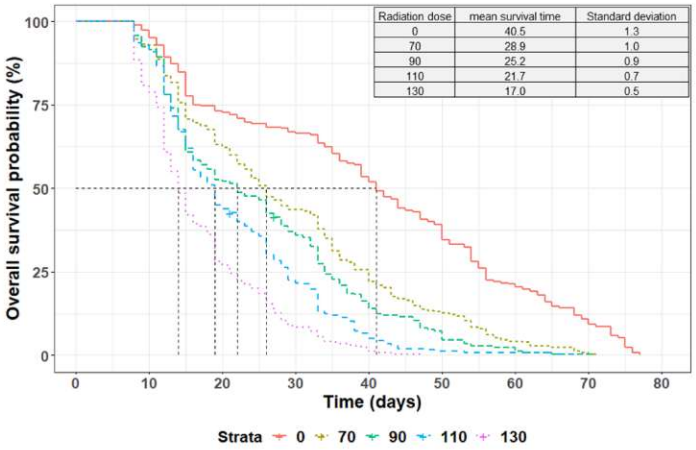

Figure 1. Survival curves of non-irradiated males *vs.* males irradiated with 70, 90, 110 and 130 Gy. The x-axis line shows the survival time in days and the black vertical lines indicate the median survival time (50% survival point) while the table display the mean survival time for each treatment.

Irradiator	Curve equation	D50 (95% CI)	D95 (95% CI)	D99 (99% CI)
Foss Model 812	$y = 0.09\exp(-\exp(2.03(\log(x) - 54.04)))$	45.11 (31.31–58.92)	92.79 (81.21–104.38)	114.69 (87.54–141.84)
Rad Source 2400	$y = 0.09\exp(-\exp(1.85(\log(x) - 47.75)))$	39.17 (22.98–55-35)	86.41 (75.82–96.99)	109.02 (81.54–136.49)
Raycell Mk2	$y = 0.09\exp(-\exp(1.75(\log(x) - 45.94)))$	37.26 (22.30–52.24)	85.92 (75.42–96.42)	109.82 (82.74–136.90)
	compParm, $p > 0.05$	Kruskall-Wallis, $\chi^2 = 2$; df = 2; $p = 0.3679$		

Table 1. Weibull W1.3 model curves equations and the respective effectives doses that reduce fecundity of 50, 95 and 99% (95% CI). The curves equations are expressed as $y(x) = 0 + (d-0)\exp(-\exp(b(\log(x) - e)))$ where the lower limit is fixed at 0, d is the upper limit, b is the slope and e is the effective dose; x represents the radiation dose needed to reduce a given fecundity.

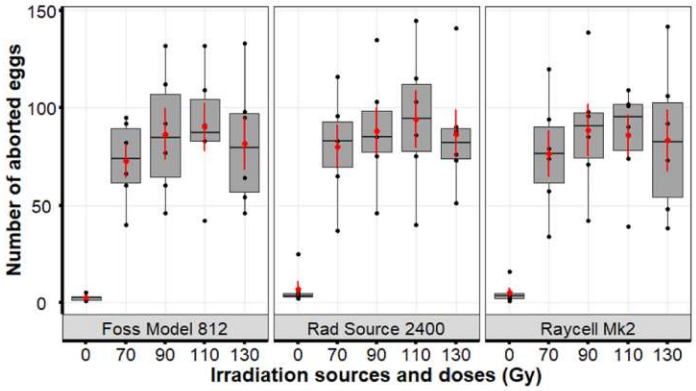

Figure 2. Number of ovulated eggs aborted during the 60-day experiment. Females that mated with males irradiated aborted a greater number of eggs as compared with those mated with non-irradiated males. The boxplot shows the median, and upper and lower quartiles while the means and the standard errors are shown in red.

The emergence of the pupae produced by females mated with non-irradiated males was higher than those produced by females mated with irradiated males except those mated with males irradiated with 90 Gy in the Raycell Mk2 (95.8%) (Supplementary Table S3). The development time of flies in the produced pupae varied from 31 to 37 days. The male to female sex ratio of the emerged flies was about 1:1 (Supplementary Table S3).

Dissection results of surviving females on day 60 indicate that the insemination rate ranged from 0.99 to 1 for the females mated with non-irradiated males in comparison to a range from 0.91 to 0.99 for the females mated with irradiated males (Supplementary Table S4). Although the insemination rate was slightly higher in females mated with non-irradiated males than in those mated with irradiated males, radiation did not suppress the ability of males to transfer sperm, and there was no difference between the irradiators. The spermathecae fill score in females mated with non-irradiated as well as irradiated males was predominated by the score of 0.25, 0.50 and 0.75 for all irradiation doses and irradiators. Examination of the status of the uterus on day 60 in the dissected females showed a clear difference in its contents between females mated with non-irradiated males and females mated with irradiated males. The uteri of females that mated with males irradiated was mostly empty due to abortions or contained a recently ovulated egg in embryonic arrest. In contrast, the uteri of females that mated with non-irradiated males, contained recently ovulated eggs or viable instar larvae, while only a few were empty due to abortion (Supplementary Table S4).

Induced sterility

Sterility induced in females mated with males irradiated as pupae averaged 82.2 ± 7.1% for the lowest dose of 70 Gy, while it averaged 99.8 ± 0.5% for 130 Gy and induced sterility increased with increasing dose (Fig. 3). Thus, 97.7%, 98.8% and 99.6% sterility were induced in the females mated with males irradiated with 110 Gy as pupae in the Foss Model 812, Rad Source 2400 and Raycell Mk2, respectively. Modelling the dose–response showed a good fit to the Weibull two parameters model (W2.2) (Table 2), and the model combining irradiators and doses was confirmed through the lack of fit test ($F = 0.8724$; $Df = 57$; $p = 0.6749$). There was no significant difference in the curves parameters between the irradiators as indicated in Supplementary Table S5. Specifically, the slopes of the curves and the effective median radiation doses were not significantly different between the irradiators ($p > 0.05$) (Supplementary Table S5). However, the parameter e was superior for the Foss Model 812 irradiator

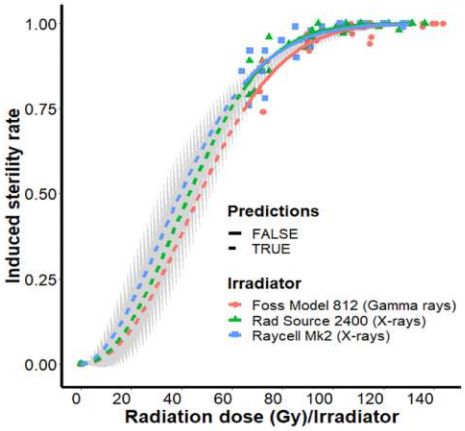

Figure 3. Weibull dose–response curves for the induced sterility in *Glossina palpalis gambiensis* pupae radiated with Foss Model 812 (Gamma rays), Rad Source 2400 (X-rays) and Raycell Mk2 (X-rays). The dashed lines indicate the predictive (Predictions = TRUE) irradiation doses and sterility while the solid lines represent the experimental data (Predictions = FALSE). The grey part indicates the 95% confidence interval.

Irradiator	Curve equation	D50 (95% CI)	D95 (95% CI)	D99 (99% CI)
Foss Model 812	$y = \exp(-\exp(2.17(\log(x)-55.96)))$	47.26 (39.75–54.78)	92.7 (86.06–99.44)	113.06 (98.06–128.07)
Rad Source 2400	$y = \exp(-\exp(2.14(\log(x)-51.09)))$	43.06 (34.45–51.67)	85.24 (79.35–91.13)	104.18 (89.89–118.46)
Raycell Mk2	$y = \exp(-\exp(1.86(\log(x)-48.14)))$	39.52 (31.7–47.27)	86.94 (81.03–92.85)	109.60 (95.16–124.04)
	compParm, $p > 0.05$	Kruskall-Wallis, $\chi^2 = 2$; df = 2; $p = 0.3679$		

Table 2. Weibull W2.2 model curves equations and the respective effectives doses that induce 50, 95 and 99% of sterility (95%CI). The curves equations are expressed as $y(x) = \exp(-\exp(b(\log(x) - e)))$ where the lower limit was fixed at 0, *d* the upper limit was fixed at 1, *b* is the slope and *e* is the effective dose; *x* represents the radiation dose needed for a given induced sterility.

as compared with the Raycell Mk2 ($p = 0.0834$). This indicates that for achieving the same level of sterility, a lower irradiation dose is required for the Raycell Mk2 as compared with the Foss Model 812 irradiator. The estimated effective doses showed that the doses of Raycell Mk2 and Rad Source 2400 were lower (around 5 Gy less) than that of Foss Model 812. Indeed, the real data showed that Raycell Mk2 and Rad Source 2400 induced more than 95% sterility wi 90 Gy whereas a dose of 110 Gy Foss Model 812 was needed to reach the same level (Supplementary Table 3).

Experiment 2: emergence rate, flight propensity, and survival

Mean adult emergence rate of the irradiated and control group pupae resulted in an overall average of 91.2 ± 5.5%. The statistical analysis showed that there was no significant difference in the emergence rate of irradiated pupae as compared with the control group ($\chi^2 = 3.77$; df = 3; $p = 0.2878$) (Supplementary Fig. S4). Prior to irradiation, all pupae were sex sorted using the Near Infra-Red Pupae Sex Sorter and the average female contamination observed was 5.25 ± 3.3%.

Flight propensity of males irradiated as pupae compared to those of the control group varied significantly ($\chi^2 = 16.243$; df = 3; $p = 0.0010$). Indeed, the flight propensity of the control group was significantly higher than those irradiated with both the Raycell Mk2 ($p = 0.0242$) and the Rad Source 2400 ($p = 0.0005$) while there was no significant difference with those irradiated with Foss Model 812 ($p = 0.0568$). No significant difference was detected among the irradiators (Fig. 4).

The survival time of males under feeding stress was significantly different between the treatments, i.e., between the irradiated males and the control group ($\chi^2 = 47.55$; df = 3; $p < 0.0001$). When computing a pairwise comparison, there was no significant difference between the survival time of the males irradiated with Foss Model 812 and both Rad Source 2400 ($p = 0.1463$) and Raycell Mk2 ($p = 0.2916$), nor between the X-rays irradiators ($p = 0.9939$). (Supplementary Table S6). Irradiated males from all the irradiators survived significantly longer than the non-irradiated males (Fig. 5).

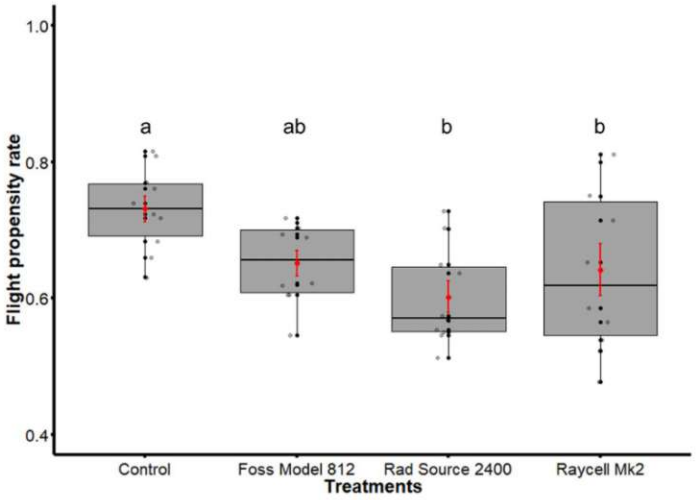

Figure 4. Flight propensity of males irradiated with the Foss Model 812, the Rad Source 2400 and the Raycell Mk2 *versus* non-irradiated males. The boxplot shows the median, upper and lower quartiles while the means and the standard errors are shown in red. Different letters indicate a significant difference between the treatments.

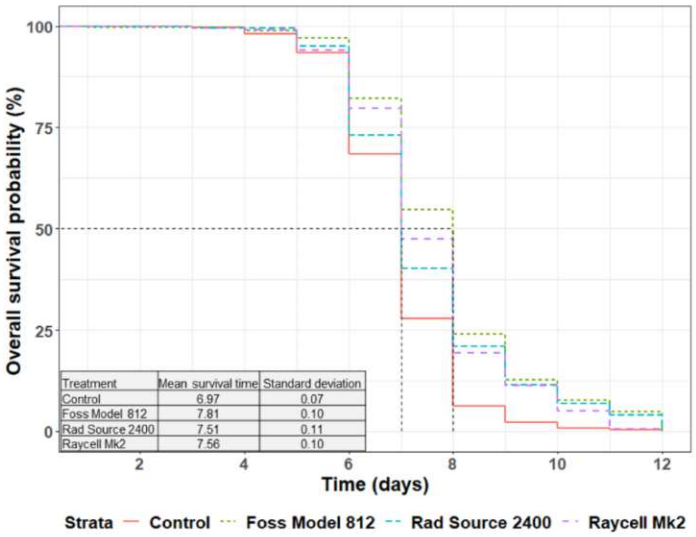

Figure 5. Survival curves of males irradiated with the Foss Model 812, the Rad Source 2400 and the Raycell Mk2 *versus* non-irradiated males. The x-axis line shows the survival time in days and the black vertical lines indicate the median survival time (50% survival point) while the table display the mean survival time for each treatment.

Experiment 3: male mating performance

Environmental conditions and fly behaviour in the field cage

The temperature during this assessment ranged from 21.1 to 27.5 °C, with the mean being 24.5 ± 1.5 °C. The relative humidity varied from 48.8 to 88.9%, with the mean being 71.1 ± 8.7%. Light intensity ranged from 112 Lux at the bottom of the cage to 2520 Lux at the top of the cage, with averages of 780.3 ± 330.0 Lux, 852.1 ± 449.8 Lux and 1357.3 ± 474.7 Lux at the bottom, mid/foliage and top of the cage, respectively. After the females were released, most flies aggregated at the top of the cage. Similar to the females, most of the released males also aggregated in the upper part of the cage, and immediate matings were usually observed. Subsequently, 81.8% of the mating pairs were collected at the upper half of the cage, where the average light intensity was 1239.83 ± 551.18 Lux (Supplementary Table S7).

Overall mating, relative mating index (RMI) and relative mating performance (RMP)

Out of 390 possible pairs, 297 mating couples were formed, giving an overall propensity of mating (overall proportion of released females that mated) of 0.76 (Table 3). The RMI in Table 3 showed that radiation significantly decreased the mating ability of the males ($\chi^2 = 87.32$; df = 2; $p < 0.0001$). The RMI of the non-irradiated males was significantly higher as compared to the RMI of the males irradiated with the Foss Model 812 ($p < 0.0001$) and the Rad Source 2400 ($p < 0.0001$). No significant difference was observed between the two irradiators ($p = 0.8634$) (Fig. 6).

The RMP defined as the difference between the numbers of mating pairs of irradiated males to non-irradiated males as a proportion of the total number of mating pairs, was − 0.57 ± 0.24 for the males irradiated with the Foss Model 812 as compared with − 0.58 ± 0.25 for those irradiated with Rad Source 2400 (Table 3).

Treatment	Possible pairs	Actual mated	Overall proportion (PM)	Relative Mating Index	Relative mating performance	Mating latency (min)	Mating duration (min)	Spermathecae value (Mean ± SD)	Insemination rate
Control	–	213	–	71.39 ± 15.94^a	–	35.39 ± 46.92^a	86.59 ± 34.15^a	0.88 ± 0.20^a	0.99 ± 0.03^a
Foss Model 812	–	44	–	14.78 ± 8.85^b	− 0.57 ± 0.24	70.82 ± 59.43^b	69.30 ± 36.41^b	0.82 ± 0.26^{ab}	0.97 ± 0.07^a
Rad Source 2400	–	40	–	13.83 ± 10.34^b	− 0.58 ± 0.25	58.68 ± 51.97^b	58.85 ± 30.84^b	0.78 ± 0.27^b	0.89 ± 0.28^a
Global	390	297	0.76	–	–	43.77 ± 51.34	80.29 ± 35.52	0.86 ± 0.22	0.95 ± 0.17

Table 3. Mean (± SD) of the main parameters of the mating performance experiment. Superscript letters indicate the p-values and different letters indicate a significant difference between the treatments.

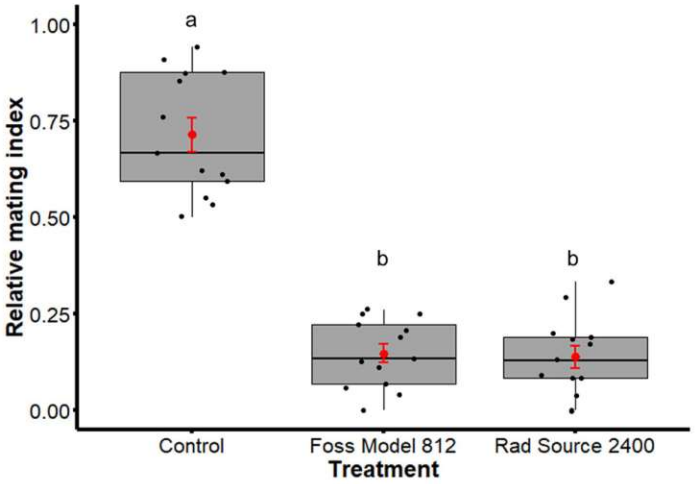

Figure 6. Comparison of the relative mating index (RMI) between non-irradiated males and males irradiated with Foss Model 812 and Rad Source 2400. The boxplot indicates the median, upper and lower quartiles while the means and the standard errors are highlighted in red. Different letters indicate a significant difference between the treatments.

Mating latency and duration

Mating latency ($\chi^2 = 26.71$; df = 2; $p < 0.0001$) and mating duration ($\chi^2 = 38.96$; df = 2; $p < 0.0001$) varied significantly between the treatments. Non-irradiated males and irradiated males were released simultaneously, and non-irradiated males were significantly faster to engage in mating as compared with those irradiated with the Foss Model 812 ($p < 0.0001$) and the Rad Source 2400 ($p = 0.0034$). However, there was no significant difference between the mating latency of the males irradiated with both irradiators (Supplementary Fig. 5). The couples formed by the non-irradiated males mated longer than the couples formed by males irradiated with the Foss Model 812 ($p = 0.0009$) and the Rad Source 2400 ($p < 0.0001$). However, the mating duration of males irradiated with both irradiators did not differ significantly ($p = 0.6948$) (Supplementary Fig. S6).

Insemination rate and spermathecae fill

Despite the significant difference in mating latency and duration between non-irradiated and irradiated males, no significant difference ($\chi^2 = 1.33$; df = 2; $p = 0.2770$) in insemination rate was observed. Among the treatments (irradiation vs control), mating duration, and their interaction in the linear model used to explain the spermathecae fill, only the treatment had a significant effect ($\chi^2 = 7.3139$; df = 2; $p = 0.0258$). However, using the pairwise comparison with adjusted p-values revealed that there was no significant difference between the treatments (Supplementary Table S8 and Table S9).

Discussion

The established use of gamma irradiation for tsetse sterilization has prompted a search for alternatives, with X-rays, which, like gamma rays, induce DNA damage in insects through chromosome breaks, demonstrating high effectiveness in tsetse fly sterilization[30]. In the present study, the effectivity of a range of radiation doses (70–130 Gy) generated by two X-ray irradiators (Rad Source 2400 and Raycell Mk2) was compared with that of the same range generated by a gamma irradiator (Foss Model 812) for the sterilization of *G. p. gambiensis* pupae.

Adult male flies, that were irradiated as pupae with 110 Gy of gamma- and X-ray irradiators, induced more than 97% sterility in non-irradiated females. In addition, induced sterility increased as irradiation dose increased independent of the irradiator. The 97% sterility was higher than the 93.4% sterility obtained when adults of the same species were irradiated with the same dose of gamma-rays five decades ago[31]. Given the limitations of separating the sexes during the pupal stage at that time, Tazé et al.[31] employed biological material consisting of adult males identified post-emergence. These adult males are recognized for their reduced sensitivity to irradiation compared to the pupae utilized in our experiment. The difference in sensitivity to radiation between tsetse development stages has been demonstrated in *G. brevipalpis* (Newstead, 1911)[32] and *G. p. palpalis* (Robineau-Desvoidy, 1830)[33], where pupae were found to be more sensitive to the radiation than adult flies.

It was also higher than the 89.7% sterility obtained in a recent study that used pupae of *G. p. gambiensis*[34].

The pupae in the study of Ilboudo et al.[34] were "under-dosed" as dosimetry revealed an absorbed dose of 81 Gy instead of 110 Gy. Furthermore, the absence of dosimetry and the potential for under-dosing, coupled with the protective effects of chilling[35] could provide an explanation for the 120 Gy suggested by Pagabeleguem et al.[36] when a re-evaluation of the dose response of male *G. p. gambiensis* has been done. These observations accentuate the importance of the implementation of an accurate and reliable dosimetry system in SIT facilities, as radiation dose is central to most radiobiological work[37].

More than 95% sterility was induced in species such as *G. brevipalpis*[32,38], *G. austeni* (Newstead, 1912)[39], and *G. fuscipes fuscipes* (Newstead, 1911)[38] with respective doses of 40 Gy, 80 Gy and 80–100 Gy. These doses differ from our finding of 110 Gy for *G. p. gambiensisis* (Vanderplank, 1911), indicating species-specific variations in radiosensitivity. Therefore, the optimal dose in SIT programmes needs to be specified for each species, revisited periodically, and it remains to be seen whether the same range of radiosensitivity will be observed with X-ray irradiation. In addition to endogenous factors of insects that affect their radio-sensitivity, exogenous factors, such as handling, oxygen level, ambient temperature, dose-rate, and many others before and during irradiation, could influence the radio-sensitivity[40].

The results of our study indicate that gamma- and X-ray irradiators induced similar sterility when *G. p. gambiensis* were irradiated as pupae, which suggests that X-ray irradiation can be an acceptable alternative to gamma irradiation. This finding is in agreement with a study on the navel orange worm which concluded that X- and gamma-rays treatments were biologically equivalent at similar doses[41]. These observations are ddditionally supported by studies that show that X-ray irradiation can induce an acceptable level of sterility in several insects, such as mosquitoes[27,28,42], Lepidoptera[43] and fruit flies[20,41,44].

In the current study, a dose of 90 Gy was sufficient to induce more than 95% sterility in females that had mated with males irradiated with one of the X-ray irradiators. This efficiency of X-rays might be related to their characteristics and the different dose rate of X-ray machines as compared with the Foss Model 812, since DNA damage due to radiation in multicellular organisms is dependent on the environmental dose rate[45]. Concerning the characteristics, gamma- and X-rays are waves in the electromagnetic spectrum, and gamma-rays have the shortest wavelength that are typically, but not always, shorter than those of X-rays (range from 10 pico- to 10 nm). These rays can be differentiated by their origin, i.e., gamma-rays are produced during nuclear decay of the nuclei of atoms, whereas X-rays are produced by electrons. Gamma-rays have a stronger ionizing ability. X-rays have less penetrating power as compared with gamma-rays (https://pediaa.com/difference-between-x-rays-and-gamma-rays/) and are widely used in the medical field. Secondly, in the current study, the dose rate of the Foss Model 812 was higher (between 76.4 and 68.9 Gy/min) than the dose rates of the X-ray irradiators (14.1 ± 0.7 Gy/min and 8.23 Gy/min for the Rad Source 2400 and the Raycell Mk2, respectively). The available literature on the impact of dose rate on the biological responses of insects shows that opinions are divergent. While several studies conclude that dose rate is a negligible parameter[46], some showed that dose rate could have

an influence on dose response of the insects, i.e., by increasing biological damage with increasing dose-rate[47–49]. A recent study, however, on mosquitoes showed that a low dose rate can achieve greater sterility than a high dose rate at high doses, while the inverse is seen in lower doses[49].

Dissection results to determine uterus content in relation to ovarian development can be used to assess the effectiveness of sterile males in tsetse SIT programmes[50]. Similar to the results obtained with gamma-rays, females that mated with males subjected to X-rays as pupae had eggs in embryonic arrest in their uterus or showed an empty uterus probably due to the abortion of an embryo or larvae. The present results, independent of the irradiators, are in agreement with that obtained with *G. brevipalpis*[32] and *G. austeni*[39] following gamma radiation. The detectable imbalance between intra-uterine content and ovarian development reflects that there are higher proportions of dominant lethal mutations in the sperm of the males[38] as the radiation dose increases.

Females mated with irradiated males (either gamma- or X-rays) survived longer than those mated with non-irradiated males, and this may be a result of their reproductive status. Lipolysis is required for milk production during tsetse pregnancy and is an indispensable and energy consuming process for the females mated with non-irradiated males.Unlike other insects, female tsetse flies undergo viviparous reproduction, producing a single instar larva in the uterus at any time, which is nourished by milk produced by the female's milk gland[51]. The absence of lactation and the stressful pregnancy cycle in females mated with irradiated males is the main reason for the prolonged female lifespan[52,53]. This result is of particular importance in tsetse control programmes and shows how accurate the sex-sorting system must be to eliminate the females from the release batches, as a longer life span implies more blood meals taken from host animals with a potential risk of transmission of trypanosome species by the released flies.

The current study shows that the survival of males that were offered blood meals decreased with increasing doses irrespectively whether gamma- or X-ray irradiators were used. In addition to dominant lethal mutations obtained in the sperm of males, irradiation causes undesirable somatic damage that is expressed as the development of abnormalities including a reduction in lifespan[26].

Even though irradiating male pupae with 110 and 130 Gy induced more than 97% sterility in the non-irradiated females, the ultimate selection of the radiation dose for SIT programmes will depend on the competitiveness of the irradiated males[54,55]. Assessing flight quality and mating performance is therefore of particular importance. Based on the results of the dose–response curves generated in the current study, a dose of 110 Gy was selected to assess its effects on adult emergence, flight propensity, and male mating performance in a walk-in field cage.

Irradiation with either gamma- or X-ray irradiators showed that irradiation dose, regardless of the irradiator, had no significant negative effect on adult emergence. This agrees with results obtained in studies with the same species and the same radiation dose using gamma radiation[34,56]. As expected, with the exception of poor rearing conditions, only excessive irradiation doses or inappropriate handling of pupae may also reduce the emergence rate[57]. Flight propensity did not differ significantly between males irradiated with gamma- or X-rays irradiators, nor between those irradiated with the two X-ray irradiators. This result strengthens the hypothesis that at similar doses, the gamma- and X-rays have similar biological/physiological effects[41].

Survival of irradiated males with gamma- and X-ray irradiators under stress was higher than the survival of non-irradiated males. Similar results were obtained with *G. pallidipes*[58]. The opposite was observed in a recent study[34] although the median survival in our study (7 days for non-irradiated and irradiated flies respectively) was almost double of the one observed in the study of Ilboudo et al. (4.75 and 4.55 days for non-irradiated and irradiated males, respectively). The difference could be due to the sex-separation methodology of irradiated pupae that were sorted in the pupal stage with the NIRPSS in our study in comparison to chilled-sorting after adult emergence in the other one. In the absence of feeding, survival seems to be an inverse function of the body's use of energy. It was observed that the irradiated males were less active and more lethargic as compared with the control group, the latter being very mobile and consequently using more energy. This difference in activity between irradiated and non-irradiated males could explain the longer survival of irradiated males. While these laboratory results could be perceived as a potentially negative consequence that may reduce mating or mate-seeking in the wild, the presence of various wild animals as feeding hosts could counterbalance this effect, increasing the likelihood of encounters with wild females and competition with wild male counterparts[59,60]. Indeed, when monitoring the experiment on survival under starvation, the irradiated starved flies attempt to bite the operator, suggesting that in the field, they will be active in the presence of hosts.

Mating performance in relation to the level of induced sterility is critical in the evaluation of the produced sterile males. As with several other parameters, none of the parameters characterising mating performance varied significantly between gamma- and X-ray irradiators except the average spermathecae fill. The overall mating propensity of 0.76 in the current study was higher than the one found in previous experiments with the same species and with the same irradiation target dose (0.57)[34], and similar non-irradiated males (0.64)[61]. On the contrary, the PM was smaller than that of 0.83 found with non-irradiated males of *G. p. gambiensis*[62]. Other results indicated an overall propensity of 0.57 with *G. brevipalpis*[32], while 0.63 and 0.41 were obtained with *G. austeni*[39]. These dissimilarities may be a result of a difference in treatments and environmental conditions as fly behaviour is indeed influenced by these[63]. However, the relative mating index indicates that the irradiated males are still competitive, and the small deficit as compared with non-irradiated male flies can be mitigated in SIT programmes by an increase in the sterile to wild male ratio. In addition, the insemination rate and spermathecal fill of females mated with males irradiated as pupae with gamma- and X-rays irradiators and with non-irradiated males were similar, as found in previous studies with *G. p. gambiensis, G. brevipalpis, and G. austeni*. These results confirm that irradiation does not affect their reproductive competence in the field[31,32,34,39].

As males irradiated with X-rays can compete and inseminate similarly to males irradiated with gamma-rays and non-irradiated males under semi-field cage conditions, the use of X-rays seems to have potential for tsetse SIT programmes. However, treatment capacities are very relevant. Yamada et al.[30] showed that it is possible to irradiate 1.1 million tsetse pupae in 5 days and two 6-h shifts per day using the Raycell Mk2 irradiator with 4

boxes of 1,500 pupae that can fit in the canister. Considering the same parameters, 35 boxes could fit in the five Rad Source 2400 canisters, while the Foss Model 812 chamber can hold 7 boxes. Considering the dose rates, the Foss Model 812 treatment capacity would be 7 times that of the Raycell Mk2, whereas it is almost equal to that of the Rad Source 2400 (1.2 times), the Rad Source 2400 capacity being 6 times that of the Raycell Mk2.

These results show that X-ray irradiators are well suited for tsetse pupae irradiation in large programmes, such as the one at "Insectarium de Bobo-Dioulasso-Campagne d'Eradication de la mouche Tsetse et de la Trypanosomose (IBD-CETT)", which in 2022 shipped an average of 56,000 pupae per week to the Senegal eradication project in the Niayes (http://projet-tsetse-niayes.cirad.fr/). The highest number of sterile male tsetse released in one week was 102,557 during the successful SIT programme against *G. austeni* on Unguja Island (Zanzibar). Despite different treatment capabilities, X-ray irradiators are available and suitable for all sizes of SIT programme for irradiating pupae, unless bacterial decontamination of the blood by irradiation is considered[64]. In view of the constant low dose rate of X-ray irradiators, about 60 L and 600 L of blood could be irradiated with the Raycell Mk2 and the Rad Source 2400, respectively, in a 5-day week and two 6-h shifts daily, while the Foss Model 812 could irradiate about 900 L in the same period of time. In practical terms, the CIRDES and SEIBERDORSF strains of *G. p. gambiensis* in the IBD-CETT colony had an average size of ~ 500,000 females in 2022 and required 186 L of blood per week for feeding three times per week (IBD-CETT, unpublished data). Hence, X-ray irradiators could be used for blood irradiation, the Rad Source 2400 being more appropriate. While a single Rad Source 2400 unit is suitable for both blood and pupae irradiation in a programme of this size, four (04) Raycell Mk2 units with 2 L canister or two of 4.8 L canister option (http://www.theratronics.ca/product_raycell_mk2.html) would be necessary to do the same work.

X-ray irradiators have several economic and technical advantages, which include, lower capital cost, much lower transportation costs, and less stringent regulations regarding required infrastructure and safety of staff. According to Hendrichs, the average transport costs of a radioisotope source is US$50,000, which is 10 times the cost of shipping an X-ray irradiator. Regarding environmental safety, X-rays are emitted only when an x-ray machine is turned on compared to gamma-rays that are continuously emitting radioactive materials. In addition, the lower energy delivered by X-rays compared to gamma-rays requires less self-shielding, so X-ray irradiators are lighter than radionuclide irradiators.

In conclusion, this study showed that the results obtained with X-ray sterilization are quite comparable to those obtained with gamma-ray sterilization. The same dose of 110 Gy is optimal as an effectively induced sterility, counterbalancing the conservation of male biological parameters. Our findings showed that X-ray irradiators are suitable to be used in SIT programmes especially using *G. p. gambiensis*. Extending the same evaluation to other species will allow decision making about the exclusive use of X-ray technology in tsetse SIT programmes. Pending the adoption of X-ray irradiators, centres currently using gamma irradiators could test different irradiation conditions such as dose fractionation or hypoxic irradiation in order to continuously improve the quality of sterile males.

Methods

Biological material selection

G. p. gambiensis experimental flies were obtained from a colony maintained at the Insect Pest Control Laboratory (IPCL), FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria. The colony was established at the IPCL in 2009 from pupae obtained from the colony of the "Centre International de Recherche-Développement sur l'Elevage en zone Subhumide" (CIRDES) in Burkina Faso. Originally, this strain was colonized at Maisons-Alfort, France in 1972 using pupae collected in Guinguette, Burkina Faso and transferred to CIRDES in 1975[61]. Additional wild material collected at the Mare aux Hippopotames was introduced into the colony in 1981.

The colony (pupae and adults) was maintained at a constant temperature of 24 ± 0.5 °C, a relative humidity (RH) of 75–80%, under subdued/indirect illumination, and with a 12 h light/12 h dark photoperiod[66,67]. Similar to the colony flies, the experimental flies were offered a blood meal three times per week on defibrinated bovine blood using an artificial membrane feeding system[66], except the flies subjected to the survival under starvation test.

Pupae produced in the colony were collected daily and sorted by sex with a newly developed Near Infrared Pupae Sex Sorter (NIRPSS), preconditioned with the melanisation parameters set at T1 of 252, T2 of 0.10 and T3 of 10, 23–24 days post larviposition[68]. Male pupae were selected from the pupae classed as unmelanized when the unmelanized ratio (unmelanized pupae/total pupae sorted) was below 38%. Throughout all the experiments, we selected pupae that were 23–24 days old, and the total number of pupae irradiated depended on the specific type of experiment, with detailed information provided in each experiment description. The irradiation time was determined based on the doses and the dose rate of the irradiators, as described in the "Irradiation facilities and procedures" section.

Irradiation facilities and procedures

The current study compared the gamma-ray irradiator, the Foss Model 812 and the X-ray irradiators, i.e., the Rad Source 2400 irradiator (Rad Source technology Inc., Buford, GA) and the Raycell Mk2 irradiator (Best Theratronics Ltd., Canada). Characterization and dose mapping of the two machines showed that both are suitable for the sterilization of male insects and hence, for application in SIT programmes[24,27,28].

Foss Model 812 gamma irradiator

The samples were exposed to gamma-rays in normoxia in a ^{60}Co Foss Model 812 with a dose rate between 76.4 and 68.9 Gy/min from the beginning to the end of the experiments. The irradiation set up was made by using

the middle position of the radiation chamber on the turntable 3, the three sources A, B, and C being activated. The pupae were placed in a plastic vial (25 mm (ø) × 50 mm (H)) with an aerated upper part which was inserted in a Plexiglas tube on a Plexiglas support in a metal canister (250 mm high and a diameter of 190 mm). The canister and the samples were placed in the middle position of the irradiation chamber, to receive the central dose.

Rad Source 2400 irradiator
This self-shielded low energy X-ray irradiator contains five horizontal cylindrical canisters that rotate around an X-ray tube and has an operating voltage of 150 keV, an operating current of 45 mA, a dose rate of 14.1 ± 0.7 Gy/min and a dose uniformity of 1.3[69]. The radiation canister was a cylinder (178 mm in diameter by 167 mm in length) with a squared space delimited in the centre containing a 100 × 100 × 100 mm Plexiglas to hold a Petri dish containing the pupae during irradiation[28]. The surrounding space was filled with rice, the density of which is close to that of the pupae, to follow the protocol that was used for the dose rate measurements. The pupae were placed in a small Petri dish (60 mm × 13 mm) and glued to a bigger one (90 mm × 15 mm) vertically between the Plexiglas in the centre of the square space.

Raycell Mk2 irradiator
This irradiator has two X-ray tubes that are located opposite from each other and that have an operating low-voltage of 160 keV with a shielded chamber of irradiation and a separate heat exchanger. The target dose was controlled by setting and monitoring the irradiation time, based on the central dose rate that was 8.23 Gy/min. The cylindrical canister had a volume of 2.0 L with a diameter 167 mm and a height of 97 mm with a central position. The irradiation was done under the same conditions as described above for the Rad Source 2400.

In all irradiators, the samples were placed in the centre of the canisters and the irradiation temperature was measured before and after each irradiation by using an RS PRO thermometer (RS Components Ltd., Northants, UK).

Dosimetry monitoring

The quality of insect sterilisation is of paramount importance for the successful application of the SIT. Considering the different irradiators used for irradiation, it was essential to measure and confirm the actual radiation doses given to the samples. To ensure that the irradiation dose was actually absorbed by the samples with the intended target dose, an accurate and reliable dosimetry system was needed.

The dosimetry system used Gafchromic™ type HD-V2 (#lot 02202001) radiochromic dosimetry films (International Specialty Products, NJ, USA) following standard operating procedures[70]. These films have an appropriate dose–response for X- and gamma-rays irradiation[24]. Three 1 × 1 cm Gafchromic dosimetry films, individually enclosed in paper envelops, were included, and irradiated with each batch. The change in colour, as measured with a DoseReader 4 (Radiation General Ltd, Hungary), in reaction to the radiation dose over time, indicated the absorbed dose at 24 h post irradiation by reading two wavelengths, 458 nm and 590 nm[70]. The calibration used for the Rad Source 2400 and the Raycell Mk2 had a global uncertainty of 4.3% arising from multiple factors, including the dosimetry system (1.6%), the dose rate measurement (0.6%), the calibration (0.2%), the lot-non homogeneity (1.3%), the read-out temperature (0.4%) and the temperature of the dosimeter during the irradiation. The uncertainty of the Foss Model 812 was 2.9%.

Experiment 1: dose response of pupae to gamma- and X-rays

The dose-responses of *G. p. gambiensis* pupae exposed to 70, 90, 110 and 130 Gy of radiation generated with the Foss Model 812, the Rad Source 2400, and the Raycell Mk2 was determined. Six biological replicates were conducted, each containing an average of 60 pupae for each of the four (04) irradiated treatments per irradiator, as well as the control batch subjected to the same environmental conditions.

After irradiation, the Petri dishes containing the irradiated pupae were placed under cages (~ 126 mm (Ø); 88 mm (H)) for emergence. The female flies that emerged due to the pupae sex-sorter error were subsequently discarded. The emerged irradiated males were mated on day 7–8 post emergence, in standard colony cages (20 cm diameter) with 3–4 day-old virgin non-irradiated females from the IPCL colony at a male: female ratio of 1:1 or 1:2. After 4 days, the flies were separated under chilled conditions (4 °C), and the females were transferred to standard colony cages on individual dishes , while the males were transferred to small cages (110 mm (Ø); 45 mm (H)). Male survival was monitored daily for 90 days. Female survival and pupae production/abortions were monitored daily for 60 days. Thereafter, female flies were dissected to determine their reproductive status, i.e., presence/absence of egg/larvae in the uterus and spermathecae fill level. The pupae produced were allowed to emerge and their sex was recorded. Pupae that did not emerge were dissected to determine their development stage.

Female fecundity was expressed as the number of pupae produced per mature female day as calculated for each treatment by adding the number of flies alive each day, starting on day 18 after emergence, until the end of the experiment on day 60. Induced sterility was determined by calculating the pupae production of the treatments as a proportion of the production of pupae of the control (non-irradiated) group (100%).

Experiment 2: flight quality control: emergence rate, flight propensity and survival

The effects of irradiation as compared with the control group on adult emergence, flight propensity and male survival under feeding stress were evaluated. The dose of 110 Gy was selected for this evaluation as it induced at least 95% sterility in the dose–response evaluation. Evaluations were conducted with fifty pupae aged 23–24-day-old for each of the control group and the three irradiators. The pupae were placed in small Petri dishes (60 mm × 13 mm) that were placed within 90 mm × 15 mm Petri dishes. A black cylinder (of 100 mm height,

94 mm inner diameter and 3 mm thickness), with the interior walls coated with talcum powder to prevent the flies from crawling out, was placed over the Petri dish containing the pupae[71]. This experiment was conducted under standard colony conditions.

Emergence from the cylinder was monitored daily and the number of flyers (i.e., flies that succeeded to fly out of the tube) were sex-sorted and recorded after chilling them in a cold room at 4 °C. Before transferring the flyers in the emergence cages to the cold room, the top of the cylinder was closed with a Petri dish to prevent the flyers from re-entering the tube. The non-flyers, remaining in the tube were also removed and recorded. The daily mortality (except for weekends) of the flyers was monitored under starvation conditions until the last fly had died. Then biological replicates have been done.

Experiment 3: male mating performance

In SIT programmes, the mating performance of irradiated males is defined as their ability to compete with wild males to mate with wild females. To compare the effect of gamma- and X-ray irradiators on male mating performance against non-irradiated males, 23–24-days-old pupae, collected over 24 h, were sorted with the NIRPSS and exposed to 110 Gy of gamma-rays (Foss Model 812) or X-rays (Rad Source 2400). Among the two X-ray irradiators assessed in the previous experiments, the Rad Source 2400 was chosen to obtain the sex-ratio requirement of 3 males to 1 female for the mating competitiveness experiment. This decision was made due to its availability at certain SIT facilities, including the Centre International de Recherche-Developpement de l'Elevage en zone Subhumide. Considering the female contamination rate and the mortalities prior to the test date, a minimum of 50 pupae were selected for the aforementioned two treatments, as well as for the control group.

The irradiated pupae were kept in emergence cages, and the males that emerged were randomly marked with a small dot of blue, yellow, or red acrylic paint on the thoracic tergum using a wooden toothpick. Flies were offered a blood meal as described above, with the exception of the last feeding. The last feeding was divided into two parts, each lasting5 minutes, occurring two days and one day before the field cage test.

The mating experiments were carried out in a cylindrical walk-in field cage of 2.9 m in diameter and 2.0 m high. The cage is made of cream polyester netting with a flat floor and ceiling. The cages were deployed in the ecosphere of the IPCL, where a field environment can be simulated and temperature and humidity conditions can be set between 21 and 27 °C and 48 and 88% RH, respectively. A bergamot orange tree of ~ 2 m high, *Citrus aurantium*, was placed in the centre of the cage. Temperature and relative humidity were recorded every 15 min throughout the experiment with an RS PRO RS-172 Temperature and Humidity Data Logger (RS Components Ltd., UK) with an accuracy of ± 1 °C and ± 3.5% for temperature and relative humidity, respectively. Light intensity at the top of the cage, the bottom of the cage, and at the tree mid/foliage level was recorded every 15 min using a dual display light meter (VWR International, LLC, USA) with an accuracy of ± 4.5%. All experiments were carried out between 10:00 h and 15:00 h.

Thirty virgin non-irradiated 3–4-day-old females were released in the middle of the cage five minutes before the release of thirty 8–9-day-old males from each group, i.e., non-irradiated males and two groups of males irradiated with the Foss Model 812 and the Rad Source 2400. Non-irradiated and irradiated males were released simultaneously to compete for mating with non-irradiated females at a 3:1 male: female ratio, i.e., 90 males and 30 females.

The observer remained inside the cage for the 3-h duration of the experiment, keeping her/his movements to a minimum. The time when each mating couple occured was recorded, as well as the location and light intensity at that location. Mating couples were collected individually into small vials and surveyed until their separation, while also recording their duration of mating. After 3 h, all the remaining flies in the cage were collected, and the unmated females were kept separately at – 5 °C and dissected to confirm their virginity. The unmated males were discarded. Mated females were removed at the end of the mating and dissected in a phosphate buffered saline solution to assess the spermathecae filling score[72]. The experiment was repeated thirteen times.

The mating indices used were Mating Latency (ML), Mating Duration (MD), Propensity of Mating (PM), Relative Mating Index (RMI), and Relative Mating Performance (RMP)[32,61,73]. The ML was defined as the time from the end of male release to the time when the first couple is successfully formed, and MD was defined as the difference between starting time and the end of mating. The PM was defined as the pairs collected during a test as a proportion of the total possible pairs, whereas the RMI was considered as the number of males of each treatment mated as a proportion of the total number of males that mated. The RMP was determined as the difference between the number of couples of irradiated to sterile males as a proportion of the total number of couples.

Data analysis

Data analysis was done in RStudio (RStudio, Inc. Boston, MA, United States, 2016) using the R software version 4.1.2. through different fitted models and controlled for the overdispersion.

To analyse some of the data from the dose–response experiment, Generalized Linear Mixed Models (GLMM) under the package lme4[74] were used with the relevant family after the model overdispersion verification[75]. Thus, adult emergence and insemination rate from the experiment 3 were analysed using this model with binomial family, where the irradiators nested in the radiation type and doses were considered as the fixed effect and the replications as random effect. The same model was used with Poisson family to analyse the effect of irradiators and doses on the number of aborted eggs. To analyse spermathecae fill a GLMM with gaussian family was used after Tukey's Ladder of Powers transformation of data.

To analyse the effect of gamma- and X-ray irradiators on male sterility/residual fertility, the dose response model with "drm" function was used under the Dose–Response Curves (drc) package[76]. The best model was selected with the "mselect" function based on the log likelihood value, Akaike's Information Criterion (AIC), known as the estimated residual standard error or the p-value from a lack-of-fit test as criteria.

The best model for the fecundity data was the Weibull three-parameters type 1 model (W1.3) given by the expression $y(x) = 0 + (d-0)\ \exp(-\exp(b(\log(x) - e)))$, while the induced sterility fitted with the Weibull model with two parameters (W2.2) expressed by $y(x) = \exp(-\exp(b(\log(x) - e)))$. The first model assumes that the lower limit is 0 (fecundity tended to zero at high doses), and d represents the upper limit of the fecundity, while b assigns the slope, e denotes the median effective irradiation dose (ED_{50}) and x is the absorbed radiation dose (Gy). In the second model, the lower limit was fixed at 0, and the upper limit d is fixed at 1, being the full sterility of 100%. The curves parameters were then compared between the irradiators using the compParm function. The estimated effective doses that reduce 50, 95 and 99% of the fertility and induce the same levels of sterility for the three irradiators were determined with the ED function and then compared using the Kruskal–Wallis test.

To analyse the survival time, the Cox Mixed Effects Models ("survival" package, "coxme" function) fit by maximum likelihood was used. In this analysis, the survival time served as the response variable, and the treatments (irradiation with three different irradiators as well as a non-irradiated control group) and doses were included as fixed effects and the replications as random effect. Multiple comparisons were done using the estimated marginal means ("emmeans" function in package "emmeans") with the Tukey p-value adjustment method. The survival graphs were constructed using "ggsurvplot" with "survimer", "ggplot2," and "ggpubr" packages.

For the flight quality control and the mating performance, the data were analysed using GLMM with binomial family and the overdispersion test. Adult emergence rate and flight propensity were modelled considering the treatments (irradiated with three irradiators and non-irradiated) and doses as fixed effects and replications as random effect. Male survival was analysed using the Cox Mixed Effects Model fit by maximum likelihood as in the dose response section.

The mating latency, duration and the spermathecae fill data were analysed using a Generalized Linear Model with gaussian family after Tukey's Ladder of Powers transformation of data. The effect of the treatments as irradiated and non-irradiated and doses on the mating index was analysed with a Poisson family. During the data analysis, multiple comparisons were done using the estimated marginal means where a significant difference was found at the global level.

Data availability

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions
K.B.A.: methodology, investigation, data curation, formal analysis, visualization, writing—original draft preparation; N.A.: investigation, review and editing; M.H.: data duration, formal analysis, review and editing. S.O.: investigation, review and editing; S.P.: supervision, data duration, review and editing; OGMS: supervision, review and editing; M.L.R.: supervision, review and editing; V.J.M.B.: conceptualization, methodology, writing—review and editing; d.B.C.J.: conceptualization, methodology, supervision, validation, writing—review and editing; all authors contributed to the manuscript final version.

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Chapter 4

Radiation dose fractionation and its potential hormetic effects on male *Glossina palpalis gambiensis* (Diptera: Glossinidae): a comparative study of reproductive and flight quality parameters

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Radiation dose fractionation and its potential hormetic effects on male *Glossina palpalis gambiensis* (Diptera: Glossinidae): a comparative study of reproductive and flight quality parameters

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Abstract – One of the most critical factors for implementing the sterile insect technique for the management of tsetse is the production of large quantities of highly competitive sterile males in the field. Several factors may influence the biological quality of sterile males, but optimizing the irradiation protocols to limit unwanted somatic cell damage could improve male performance. This study evaluated the effect of fractionation of gamma radiation doses on the fertility and flight quality of male *Glossina palpalis gambiensis*. Induced sterility was assessed by mating irradiated males with virgin fertile females. Flight quality was assessed using a standard protocol. The male flies were irradiated as pupae on day 23–27 post larviposition with 110 Gy, either in a single dose or in fractionations of 10 + 100 Gy and 50 + 60 Gy separated by 1-, 2- and 3-day intervals or 55 + 55 Gy separated by 4-, 8-, and 24-hour intervals. All treatments induced more than 90% sterility in females mated with irradiated males, as compared with untreated males. No significant differences were found in emergence rate or flight propensity between fractionated and single radiation doses, nor between the types of fractionations. Overall, the 50(D0) + 60(D1) Gy dose showed slightly higher induced sterility, flight propensity, and survival of males under feeding regime. Dose fractionation resulted in only small improvements with respect to flight propensity and survival, and this should be traded off with the required increase in labor that dose fractionation entails, especially in larger control programs.

Key words: Tsetse pupae, Gamma-ray irradiation, Sterility, Flight propensity, Sterile insect technique.

Résumé – Fractionnement de la dose de rayonnement et ses effets hormétiques potentiels sur les *Glossina palpalis gambiensis* mâles (Diptera : Glossinidae) : une étude comparative des paramètres de reproduction et de qualité de vol. L'un des facteurs les plus critiques pour la mise en œuvre de la technique de l'insecte stérile pour la gestion des glossines est la production de grandes quantités de mâles stériles hautement compétitifs sur le terrain. Plusieurs facteurs peuvent influencer la qualité biologique des mâles stériles, mais l'optimisation des protocoles d'irradiation pour limiter les dommages indésirables aux cellules somatiques pourrait améliorer les performances des mâles. Cette étude a évalué l'effet du fractionnement de la dose d'irradiation gamma sur la fertilité et la qualité de vol des mâles de *Glossina palpalis gambiensis*. La stérilité induite a été évaluée en accouplant des mâles irradiés avec des femelles vierges et fertiles. La qualité du vol a été évaluée à l'aide d'un protocole standard. Les mouches mâles ont été irradiées sous forme de pupes agées de 23 à 27 jours après la larviposition avec 110 Gy, soit en dose unique, soit en fractions de 10 + 100 Gy et 50 + 60 Gy séparées par 1, 2 et 3 jours ou 55 + 55 Gy séparés par des intervalles de 4, 8 et 24 heures. Tous les traitements ont induit plus de 90 % de stérilité chez les femelles accouplées avec des mâles irradiés par rapport aux mâles non traités. Aucune différence significative n'a été trouvée dans le taux d'émergence ou la propension au vol entre les doses

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d'irradiation fractionnées et uniques ni entre les types de fractionnements. Dans l'ensemble, la dose de 50 (J0) + 60 (J1) Gy a montré une stérilité induite, une propension à voler et une survie légèrement plus élevées chez les mâles sous régime alimentaire. Le fractionnement de dose n'a entraîné que de légères améliorations en ce qui concerne la propension à voler et la survie, et cela devrait être compensé par l'augmentation nécessaire du travail qu'implique le fractionnement de dose, en particulier dans les programmes de contrôle de grande envergure.

Introduction

Trypanosomes are parasitic protozoa that cause the debilitating diseases African Animal Trypanosomosis (AAT) in livestock, which is also known as Nagana, as well as Human African Trypanosomosis (HAT), also known as sleeping sickness in humans. AAT and HAT are mainly transmitted by tsetse flies, the sole cyclical vectors in sub-Saharan Africa [39, 63] where they are distributed over approximately 10 million km^2 [43]. The disease causes significant losses in animal production and makes fertile land inaccessible for cultivation. As a result, it is responsible for huge economic losses, making it a major limiting factor for sustainable agricultural development in affected areas [17, 35]. Thus, several methods have been developed to control the disease and its vectors.

One of the control methods is the sterile insect technique (SIT), a method of pest control that involves the release of large numbers of sterilized insects, usually males, to compete with wild males for mating opportunities with wild females. The released sterile males mate with wild females and subsequently no offspring is produced, resulting in a reduction of the pest population over time [24]. The SIT is implemented as part of an area-wide integrated pest management (AW-IPM) program where it is integrated with other control measures to manage an entire pest population in a circumscribed area. Control tactics that can be integrated with the SIT, and that are currently acceptable from an environmental point of view, are the sequential aerosol technique (SAT), the use of traps and insecticide impregnated targets, and the live bait technique. The SIT has been successfully used against various insect pests species [32, 36, 53, 64], including tsetse flies in central Nigeria [51], in Sidéradougou (Burkina Faso) [47], on the Island of Unguja, Zanzibar [57], and in the Niayes of Senegal [58].

In all past tsetse programs that had an SIT component, producing the required quantities of sterile males was one of the main challenges. The SIT can only be successful when the colony-reared and released sterile males are as competitive as their wild counterparts to mate with wild females [61]. To produce high-quality sterile males for use in SIT programs, factors and processes such as the rearing, sterilization, transport, handling and release must be considered and properly managed as they are the critical factors that can affect the quality of the sterile males [67, 69].

To address these challenges, programs that have an SIT component continuously aim at improving the protocols for insect rearing and handling, shipment, and irradiation processes. Improvement of irradiation protocols can include insects live stage radiation sensitivity refinements, radiation under various atmosphere and environmental conditions, or dose fractionation. Several studies have investigated the effects of irradiation under different conditions on the quality of sterile males produced for SIT programs. Some of these studies have shown that irradiation under nitrogen (hypoxia or anoxia) or other modified atmospheres can improve the quality of the sterile males [10]. Other studies based on dose fractionation showed the same trend with improved male performance, *i.e.*, induced sterility, longevity, or mating competitiveness in insect pests of crops, livestock, and vectors of human diseases [52]. Fractionation of the radiation doses applied to the Indian meal moth, *Plodia interpunctella* showed a significant improvement of male longevity, mating competitiveness, and sterility [14], and similarly, a significant improvement was obtained in male *Aedes aegypti's* survival and mating competitiveness [67]. Dose fractionation applied to *Glossina morsitans* increased their residual fertility as compared with a similar but continuous single dose [21]. Early stage pupae of *Glossina tachinoides* that were irradiated with fractionated and single doses under nitrogen atmosphere resulted in a similar induced sterility but survival was better [59]. Therefore, administering split doses lower than the optimal dose, separated by an ideal time interval, could induce a hormetic effect, resulting in the improvement of such parameters [56].

As only a limited number of studies on the effect of dose fractionation on tsetse quality have been carried out, the effect of an optimal dose of 110 Gy fractionated into 10 + 100 Gy and 50 + 60 Gy, separated by 1-, 2-, and 3-day intervals or a fractionated doses of 55 + 55 Gy separated by 4-, 8-, and 24-hour intervals, as compared with a single dose, was investigated on male *Glossina palpalis gambiensis*.

Materials and methods

Tsetse strain, rearing and samples selection

A *G. p. gambiensis* strain has been maintained at the Insect Pest Control Laboratory (IPCL) of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Seiberdorf, Austria since 2009. The colony was established from pupae provided by the Centre International de Recherche-Développement sur l'Élevage en zone Subhumide (CIRDES) in Bobo Dioulasso, Burkina Faso. The original colony was established at Maisons-Alfort (France) in 1972 from wild pupae collected in Guinguette (Burkina Faso, Latitude: 11.56165885, Longitude: – 4.16220681) and transferred to CIRDES in 1975 [41]. In 1981, additional wild pupae collected in Mare aux Hippopotames (Burkina Faso, Latitude: 11.2043219, Longitude: – 4.4382593) were introduced into the colony.

The colony, experimental pupae, and flies were maintained under standard laboratory conditions in a climate-controlled room at a constant temperature of 24 ± 1.0 °C, a relative humidity (RH) of 75–80%, and under subdued/indirect illumination in a 12 h light/12 h dark photoperiod [26, 30]. The colony flies were offered a blood meal of defibrinated bovine blood three

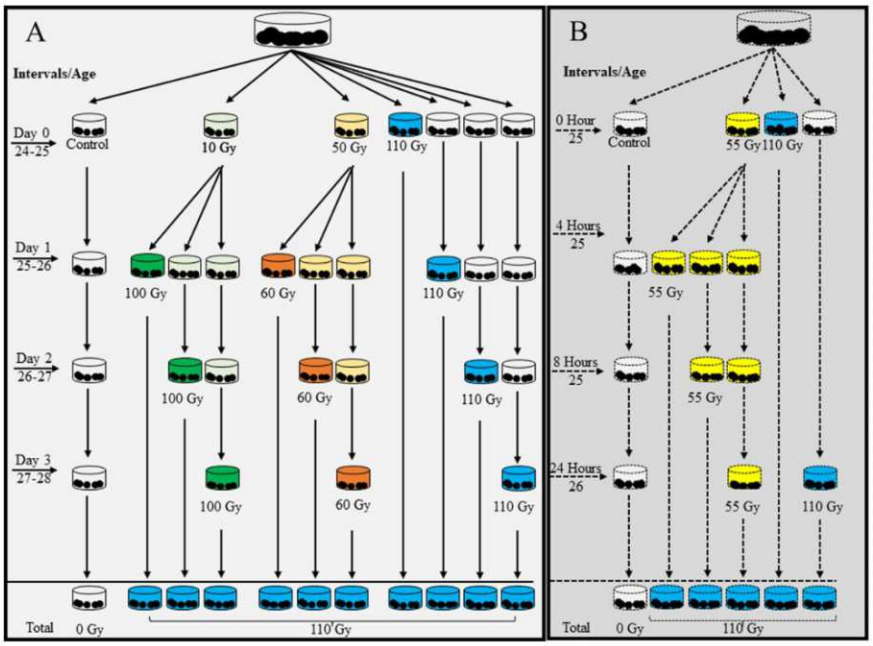


Figure 1. Flowchart of fractionation procedures for the irradiation *of male Glossina palpalis gambiensis* pupae. Pupae were irradiated with dose of 110 Gy, administered as a single dose or fractionated into 10 + 100 Gy and 50 + 60 Gy separated by 1-, 2- and 3-day intervals (A) or using equal fractionated doses of 55 + 55 Gy separated by 4-, 8- and 24-hour intervals (B). The first fraction was administered on day zero/hour zero, and the second fraction on days 1, 2 and 3, or 4, 8 and 24 hours later. The control pupae that were not irradiated were handled in the same conditions.

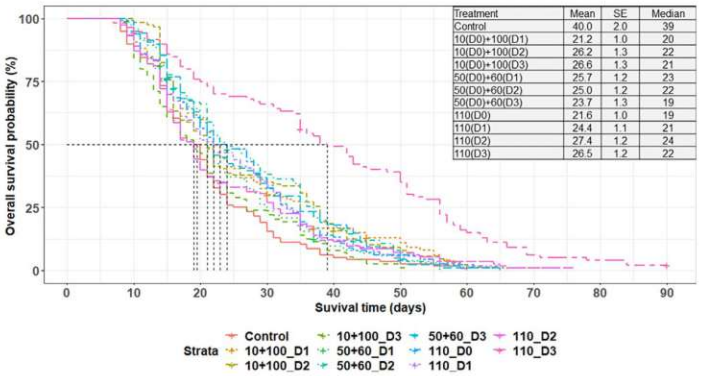
times per week, using a TPU 4 *in vitro* feeding system [30]. Males and females of the flies in experiment 1 were fed manually at the same frequency.

Pupae were collected twice daily (9:00 and 15:00) and incubated at 24.0 ± 1.0 °C and 75.0 ± 5.0 RH. The pupae were then sex sorted with the newly developed Near Infrared Pupae Sex Sorter (NIRPSS) 23–24 days post larviposition based on the melanization ratio (unmelanized pupae/total pupae sorted) [2]. Male pupae were selected from the pupae classified as non-melanized when the unmelanized ratio was below 35%, whereas the melanized pupae were returned to the colony.

Irradiation procedures and dosimetry

A Foss 812 gamma irradiator (Foss Therapy Services Inc., Pacoima, CA, USA) was used to assess the effects of dose fractionation on male *G. p. gambiensis* pupae. This Co-60 Self-Contained Irradiator comprises three sources and three turntables, yielding a combined power of 22,500 Curies per operation. The irradiation setup was executed by activating all three sources and utilizing turntable 3. This set up involved a dose rate decline from 69.49 Gy/min at the onset of the experiment to 62.15 Gy/min at the end. The canister set-up was based on a Plexiglas tube attached to double 9 cm × 9 cm × 1.5 cm Petri dishes so that the sample in a small plastic vial was inserted into the tube to be in the central position of the irradiation chamber. The GAFchromic dosimetry system, with HD-V2 for the high and MD-V3 for the low doses, was used according to the IAEA standard operating procedures [27]. During each irradiation, three MD or HD films placed in 2 cm × 2 cm paper envelopes were included in the sample vial. An optical density reader (wavelengths of 458 nm and 590 nm) was used to read the irradiated films 24 hours after irradiation.

Previous dose response experiments with *G. p. gambiensis* pupae using both gamma and X-rays indicated that 110 Gy was sufficient to induced more than 97% sterility in females [54, 65]. For the treatment groups, *G. p. gambiensis* pupae were therefore exposed to a total dose of 110 Gy. First, two types of fractionations, *i.e.*, 10 + 100 Gy and 50 + 60 Gy separated by 1, 2 and 3 days were evaluated in comparison with single doses following the same time interval (Fig. 1A). Pupae were exposed to irradiation on days 23–27 post larviposition. An additional experiment was conducted after reviewing the results from the small and nearly half-fractionations. Pupae of 25 days old were exposed to 55 + 55 Gy administered with 4-, 8-, and 24-hour intervals, in comparison with exposure to a single dose of 110 Gy administered on day 0 and 24 h later (Fig. 1B). All replicates had a control group of pupae which was not irradiated and subjected to the same environmental conditions.



Treatment	Mean	SE	Median
Control	40.0	2.0	39
10(D0)+100(D1)	21.2	1.0	20
10(D0)+100(D2)	26.2	1.3	22
10(D0)+100(D3)	26.6	1.3	21
50(D0)+60(D1)	25.7	1.2	23
50(D0)+60(D2)	25.0	1.2	22
50(D0)+60(D3)	23.7	1.3	19
110(D0)	21.6	1.0	19
110(D1)	24.4	1.1	21
110(D2)	27.4	1.2	24
110(D3)	26.5	1.2	22

Figure 2. Survival of male *Glossina palpalis gambiensis* irradiated as pupae with single doses (110 Gy) as compared with those irradiated with fractionated doses of 10 + 100 Gy and 50 + 60 Gy with 1-, 2- or 3-day intervals (D1, D2, D3). Control males were not irradiated. All flies were maintained under standard feeding conditions and were monitored under feeding regime. The vertical black lines indicate the median survival time, representing the duration when the likelihood of survival decreased by 50%. The table accompanying the graphs displays the mean survival time, its standard error (SE), and the median.

Assessing adult emergence rate, survival under feeding regime, and induced sterility

After irradiation, control and irradiated pupae were transferred to 35 mm diameter Petri dishes and maintained in emergence cages in an incubation room. At emergence, only males were maintained and fed under the conditions described above.

For each treatment, 6–7-day-old males were mated with 3–4-day-old virgin females at 1:1 ratio or slightly less, male (n = 1,265) to female (n = 1,430), in standard colony cages (20 cm Ø). After 4 days, males and females were separated under low temperature (4 °C) conditions. The females were kept in standard colony cages and placed on individual dishes, whereas the males were transferred into small cages (110 mm Ø). Daily observations were made of male (90 days) and female (60 days) mortality as well as pupae production and egg/larvae abortions. Females were dissected when they were 60 days old to determine their reproductive status. The content of the spermathecae was scored as 0 (empty), 0.25 (quarter), 0.50 (half), 0.75 (three-quarters) or 1 (full), and the content of the uterus was examined to determine the reproductive stage that could be a post-larviposition/blockage or the discovery of aborted eggs and immature larval stages (I, II, III). This experiment was replicated five times.

Evaluating flight quality using fractionated doses: adult emergence rate, flight propensity, and survival under stress regime

All treatment groups described in Figures 1A and 1B were used for the flight quality control test, as described by Seck *et al.* [50].

Pupae were placed in small Petri dishes (35 mm Ø) in the center of 90 mm Ø petri dishes surrounded by a black cylinder (10 cm high and 9.4 cm Ø). To prevent the emerging flies from crawling out of the cylinder, the inner walls were coated with unscented talc. The flight tube with pupae was then kept in a BugDorm cage of 30 cm × 30 cm × 30 cm (MegaView Science Co., Ltd., Taichung, Taiwan). The flies that were able to escape the tube were considered flyers and were sexed and recorded daily (except on weekends). At the end of the emergence period (approximately five days), the un-emerged pupae were recorded. The flies that were not able to escape the flight tube were classed as non-flyers. Emergence was defined as the number of emerged flies divided by the total number of initial pupae, while flight propensity was calculated as the number of flying flies divided by the number of emerged flies. Thereafter, the survival of the "flyers" was monitored daily without feeding. Ten replicates were performed for each treatment for 10 + 100 Gy and 50 + 60 Gy fractionations, while six were done for the equal fractionation of 55 + 55 Gy.

Statistical analysis

Data were statistically analysed using R studio, version 4.4.2. Generalized linear mixed models were used with the appropriate family after checking overdispersion [11]. Overdispersed models were rebuilt by using the alternative family function. When modeling the emergence rate and flight propensity, the treatments and the pupae age on their respective full irradiation days were treated as mixed effects, while the replicates were considered random effects. The emmeans function under the emmeans package was used for the pairwise comparison between the treatments or ages. In the fractionated dose irradiation trial, age is automatically considered as a variable, based on the magnitude of the time intervals. In this study, to detect the effect of radiation treatment (single or fractionated doses), pairwise comparisons were made between treatments irradiated on the same days. To detect the effect of age, comparisons were made between different ages within each treatment. The response to radiation dose, measured as a proportion of induced sterility, was analyzed using the Kruskal–Wallis rank sum test.

Table 1. Reproduction parameters of *Glossina palpalis gambiensis* females mated with males irradiated as pupae with fractionated (10 + 100 Gy and 50 + 60 Gy) and single doses (110 Gy), separated with 1-, 2- and 3-day intervals (D1, D2, D3). Mature female days were calculated for each treatment by adding the number of flies alive each day, starting on D18 after emergence (age of maturity) until the end of the experiment on D60.

Treatment radiation dose (Gy, days)	Parental pupae emergence (%)	Mature female days*	No. of aborted eggs and instar larvae				No. of pupae produced	Mean ± sd fecundity	Mean ± sd induced sterility (%)	Produced pupae emergence / females (%)	Age at emergence (days)
			E	I	II	III					
0	81.0 ± 6.3	4559	38	4	1	0	393	0.087 ± 0.010	0.0	94.4 (45.8)	31
10(D0) + 100(D1)	76.8 ± 4.3	4426	531	0	0	0	18	0.005 ± 0.002	95.4 ± 1.3	75.3 (44.4)	31
10(D0) + 100(D2)	71.0 ± 7.9	4985	588	1	0	0	21	0.004 ± 0.002	94.7 ± 1.9	69.1 (23.8)	32
10(D0) + 100(D3)	73.8 ± 15.2	4505	497	0	1	0	32	0.007 ± 0.005	90.5 ± 7.5	81.5 (40.6)	31
50(D0) + 60(D1)	78.4 ± 7.4	5219	582	2	0	0	18	0.003 ± 0.002	95.0 ± 4.8	67.5 (22.2)	31
50(D0) + 60(D2)	73.3 ± 10.5	5197	595	1	0	0	23	0.005 ± 0.002	94.4 ± 2.0	71.7 (34.8)	32
50(D0) + 60(D3)	70.6 ± 6.8	4931	565	0	1	0	23	0.005 ± 0.002	93.6 ± 5.0	66.4 (43.5)	31
110(D0)	73.0 ± 7.5	5385	587	3	1	0	22	0.004 ± 0.002	95.2 ± 3.5	72.0 (18.2)	31
110(D1)	80.5 ± 5.4	5052	565	5	0	0	21	0.004 ± 0.003	94.4 ± 5.0	72.9 (28.6)	32
110(D2)	78.9 ± 4.3	5496	646	2	1	0	26	0.005 ± 0.004	93.1 ± 5.4	83.3 (34.6)	30
110(D3)	74.8 ± 8.1	5011	573	2	1	0	32	0.006 ± 0.003	91.0 ± 5.4	94.0 (40.6)	31

Prior to the analysis, the normality of the data was checked using the Shapiro–Wilk normality test. Finally, radiation dose fractionation effects on male survival time were analyzed using the Cox mixed-effects model ("coxme" function in "survival" package) fit by maximum likelihood, with the treatments as fixed effects and the replicates as random effects.

Results

Dosimetry

The dosimetry results indicated that the absorbed doses varied less than 5% from the target doses (Supplementary Table 1).

Assessing adult emergence rate, survival under feeding regime, and induced sterility

Adult emergence rates ranged from 69.2 ± 6.3% to 79.6 ± 5.0%. The generalized linear model that fitted the emergence rate data with the treatment as a fixed effect showed that there was a significant difference between the treatments (χ^2 = 17.9130; df = 7; p = 0.0124). While computing a pairwise comparison using the adjusted p-values of the Tukey method, no significant differences were observed (Supplementary Figure 1). Neither the fractionation/single dose administration nor the age of pupae at the time of irradiation influenced the rate of adult emergence. A total of 1,427 females were available at the beginning of the 60-day survival assessment, and 1,213 flies remained at the end, resulting in an overall mortality rate of 15.0%. Survival of the males that were kept under a standard feeding regime was evaluated for 90-days and they survived on average for 25.7 ± 14.1 days. Overall, survival was significantly affected by the irradiation (χ^2 = 101.74; df = 10; p < 0.0001), with the fertile untreated males surviving longer than those irradiated with a single or fractionated doses (Fig. 2). Supplementary Table 2 shows that, when considering each type of irradiation treatment (fractionated or not), males derived from pupae that received their second dose when more mature (with 2- and 3-day intervals) survived on average longer as compared with those that were irradiated with the second dose only after 1 day (10 + 100 Gy). Similarly, males irradiated with a single dose on days 26–27 post larviposition survived longer than those that received the dose on days 24–25 post larviposition. Flies irradiated with single dose survived similarly to those irradiated with fractionated doses when considering the same age on irradiation days (χ^2 = 10.0000; df = 10; p = 0.4405). However, males that were exposed to 50 + 60 Gy with a 1-day interval exhibited a slightly longer survival time (25.7 ± 1.2 days) compared to those exposed to a single dose (24.4 ± 1.1 days) or a fractionated dose of 10 + 100 Gy with a 1-day interval (21.2 ± 1.0 days), as illustrated in Table 1 and Figure 2.

The dose-response assessment showed that all irradiation treatments resulted in sterility levels greater than 90% as compared with the control group. Residual fertility of females mated with males irradiated with a single or fractionated doses was low, irrespective of fractionation proportions (10 + 100 Gy or 50 + 60 Gy) and intervals between exposures (0-, 1-, 2- and 3-days) (Fig. 3; Table 1). There were no significant differences in the level of induced sterility between females that had mated with males that had received a single dose, fractionated doses or between the two fractionation proportions (χ^2 = 5.3698; df = 9; p = 0.8010). Across all types of irradiations, whether treatment was with a single or fractionated dose, induced sterility decreased with pupal age, as shown in Figure 3 and Table 1.

The number of eggs aborted was significantly higher in females mated with irradiated males than those mated with fertile males (Table 1), irrespective of whether the radiation doses were fractionated or given as a single dose. Dissections of the females conducted after the 60-day experimental period revealed that, regardless of whether the radiation doses were fractionated or given in a single dose, the irradiated males retained their ability to mate and transfer sperm to the females, as evidenced by the insemination rate (>90%) and the spermathecae fill (Table 2). The content of the uterus during the dissection revealed a difference between the females that had

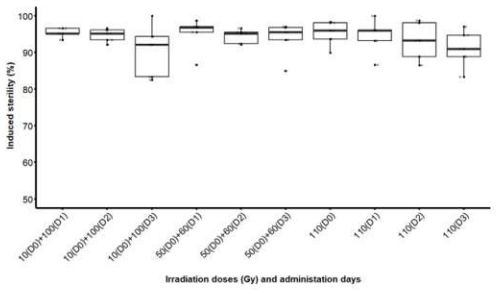


Figure 3. Induced sterility in female *Glossina palpalis gambiensis* mated with males that were irradiated as pupae with a single dose (110 Gy) or fractionated doses of 10 + 100 Gy and 50 + 60 Gy with 1-, 2- or 3-day intervals (D1, D2, D3). Females from the control group had mated with untreated fertile males. The boxplot shows the median and upper and lower quartiles. Dots represent experimental data.

mated with fertile males and those that had mated with irradiated males. Females that had mated with fertile males had either a recently ovulated egg or a larva in the uterus, and some had recently deposited the third instar larvae (post larviposition). Conversely, females that had mated with irradiated males displayed an empty uterus and the next ovarian follicle was not yet mature, which strongly suggests that the eggs or instar larvae had recently been aborted (Table 2).

Evaluating flight quality using fractionated doses: adult emergence rate, flight propensity, and survival under stress regime

Fractionation of 10 + 100 Gy and 50 + 60 Gy

The emergence rate of pupae irradiated with a single or fractionated doses did not differ significantly, neither from the emergence rate of the pupae of the control group (Fig. 4), nor from the two types of fractionations (χ^2 = 6.3813; df = 10; p = 0.7823). Thus, the type of irradiation treatment (single or fractionated dose) and the age of the pupae during the second dose (1, 2, and 3-day interval) did not have a significant impact on adult emergence rates.

The propensity of emerged flies to escape the flight cylinder was similar with various treatments (χ^2 = 7.4597; df = 10; p = 0.6815) (Fig. 5). This means that there was no notable variation in the flight propensity of flies that emerged from pupae irradiated with either a single or fractionated doses, nor between the 10 + 100 Gy and 50 + 60 Gy fractionations, as compared with the control group. These findings were consistent across all irradiation time intervals. However, it should be noted that a slightly higher propensity to fly was observed in flies irradiated with fractionated doses of 50(D0) + 60(D1) Gy with a 1-day interval as compared with those irradiated with a single or fractionated doses of 10 + 100 Gy with the same time interval and a single dose on day 0.

As for survival under feeding stress, there was an overall significant difference between the treatments (χ^2 = 36.9590; df = 10; p = 5.753e-05 (Fig. 6). However, pairwise comparison of the survival of flies that emerged from pupae irradiated with both fractionation types and the single dose on the same day did not reveal any statistically significant differences (Supplementary Table 3). Nevertheless, regarding pupae age in each treatment, flies that emerged from pupae irradiated with a single dose on day 25–26 post larviposition showed significantly higher survival rates than those that emerged from pupae irradiated with a single dose on day 24–25 post larviposition and day 26–27 post larviposition (Supplementary Table 4). In addition, males irradiated with a single dose or a fractionated dose of 10 + 100 Gy with a 1-day interval survived longer than the fertile males (Supplementary Table 5).

Equal fractionation of 55 + 55 Gy

Splitting the optimal radiation dose into two equal doses of 55 Gy separated by 4-, 8- and 24-hour intervals did not significantly affect the adult emergence rate and there were no significant differences between the irradiated and the non-irradiated groups (χ^2 = 3.6979; df = 5; p = 0.5937) (Fig. 7).

Similar to the adult emergence rate, splitting the optimal radiation dose into two equal doses did not significantly impact the flight propensity (χ^2 = 8.8826; df = 5; p = 0.1138) (Fig. 8).

Under feeding stress, the median survival time, representing the duration when the likelihood of survival decreased by half across all treatments, was observed to be 7 days. The Cox mixed-effects model fit by maximum likelihood showed that the survival time varied significantly across the treatment (χ^2 = 23.656; df = 5; p = 0.0003). Pairwise comparison using the Tukey method for adjusting p-values showed that males from pupae irradiated with a single dose of 110 Gy at 0 h and 24 h after sorting, as well as those irradiated with equal fractionated doses of 55 Gy separated by 24 h, survived significantly longer than the non-irradiated group (Fig. 9, Supplementary Table 6). Nevertheless, as illustrated in the first fractionations, there was no notable difference in the survival rates of male individuals emerging from pupae irradiated with either a single dose or equal fractionated doses, regardless of the time interval between the two equal doses (Supplementary Table 6).

Discussion

Sterilization of insects using ionizing radiation is a crucial step in the successful implementation of the SIT as part of AW-IPM programs [4]. Research and development (R&D) plays a crucial role within the framework of these programs, as they are essential in improving the cost-effectiveness of all aspects of the SIT application, including radiation biology [60]. Through R&D, protocols can be refined and optimized, resulting in enhanced program efficiency and effectiveness [13]. These efforts aim to advance the SIT application and related technologies, leading to their wider adoption and use. Insect radiation protocols are continuously improved under the SIT operational program framework. Aspects that were recently or are currently being investigated are the feasibility of using the SIT as a new pest management method for several new species [8, 18, 23], the use of X-rays as an alternative to

Table 2. Reproductive status of *Glossina palpalis gambiensis* females that mated with males exposed as pupae to fractionated (10 + 100 Gy and 50 + 60 Gy) and single (110 Gy) doses, separated by 1-, 2- and 3-day intervals (D1, D2, D3), dissected after an experimental period of 60 days.

Irradiation treatments (Gy, days)	No. of live females at day 60	Insemination rate (%)	Spermathecae fill score					Uterus content or status at day 60						
								No. of recently ovulated eggs	Viable instar larvae			Empty due to		Blockage
			0	0.25	0.50	0.75	1		I	II	III	Larviposition	Abortion	
0	95	100.0	1	1	8	64	22	19	13	8	25	26	5	0
10(D0) + 100(D1)	96	99.3	1	1	11	57	29	13	0	2	0	0	84	0
10(D0) + 100(D2)	110	96.5	4	6	6	56	37	10	1	0	2	0	95	1
10(D0) + 100(D3)	100	90.9	10	9	17	48	16	7	0	0	2	0	90	1
50(D0) + 60(D1)	116	96.7	4	3	20	63	23	16	1	0	2	0	94	0
50(D0) + 60(D2)	115	97.2	3	1	12	71	23	33	0	0	1	0	75	1
50(D + 60(D3)	110	98.9	1	2	15	69	22	14	0	1	2	0	88	4
110(D0)	121	96.7	4	2	17	59	37	21	0	0	2	0	96	0
110(D1)	113	96.2	4	2	9	62	34	11	0	1	1	0	98	0
110(D2)	126	97.5	3	1	12	56	54	12	0	0	1	0	112	0
110(D3)	111	100.0	0	0	5	63	41	11	0	0	5	0	93	0

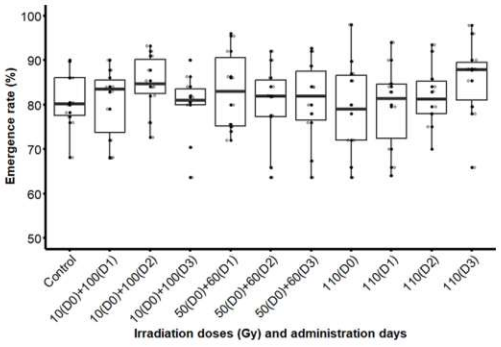


Figure 4. Adult emergence rate of male *Glossina palpalis gambiensis* irradiated as pupae with a single dose (110 Gy) or a fractionated dose of 10 + 100 Gy and 50 + 60 Gy with 1-, 2- or 3-day intervals. The control group consisted of non-irradiated pupae. The boxplot shows the median and upper and lower quartiles. Dots represent experimental data.

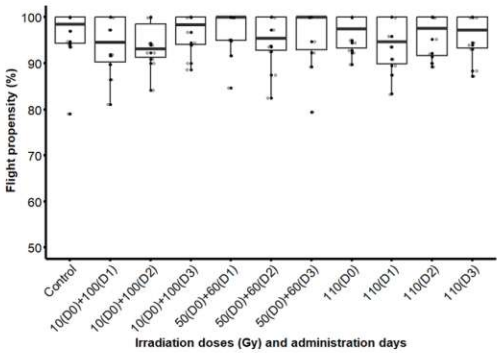
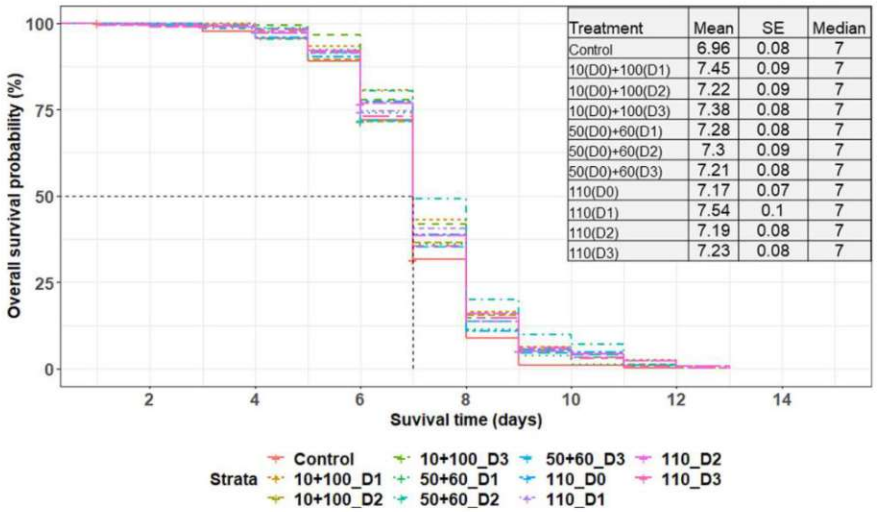


Figure 5. Flight propensity of male *Glosinna palpalis gambiensis* irradiated as pupae with a single dose (110 Gy) or a fractionated dose of 10 + 100 Gy and 50 + 60 Gy with 1-, 2- or 3-day intervals. The control group consisted of non-irradiated males. The boxplot shows the median and upper and lower quartiles. Dots represent experimental data.

gamma rays [23, 65, 69], the effect of radiation dose rate on the biological response [38, 70], the effect of radiation on insects symbionts of interest [22, 40], the radiation sensitivity of the different live stages of insects [3, 45], and the effect of dose fractionation [67] and of different atmospheric and environmental conditions on radiation sensitivity and somatic damage [9]. The objective of these studies is to optimize sterilization protocols to provide the highest level of sterility with the lowest level of somatic damage [31, 46]. The positive effect of irradiation fractionation on sterility has been demonstrated in several insects species [62, 67].

In the current study, *G. p. gambiensis* pupae were irradiated with a dose of 110 Gy that was recommended as optimal for this species some 50 years ago [34, 54], either as a single dose or as fractionated doses, and the effects were assessed on induced sterility and flight quality. Two types of fractionations were selected based on previous studies [15, 67]: for the first, the doses were almost an equal split of the optimal dose (50 + 60 Gy), while for the second, the first dose administered was low (10 Gy) followed by a high dose (100 Gy). The different fractionations were separated by 1-, 2-, and 3-days. The results obtained revealed that the rate of induced sterility by a single or fractionated doses did not significantly differ, regardless of the time intervals. Similar results were obtained with *G. tachinoides* when younger pupae (15 days post larviposition) were irradiated with fractionated doses under a nitrogen The sensitivity of insects to the induction of dominant lethal mutations depends on their developmental stageatmosphere [59]. In contrast, this result differs from that of Yamada *et al.* [67] which showed that fractionated doses led to complete sterility in *Aedes aegypti* that mated with irradiated males [67]. Dose fractionation assessments with the Indian meal moth *P. interpunctella* showed that the percentage of sterile pairs was dependent both on the dose and on the pattern of fractionation [14].



Treatment	Mean	SE	Median
Control	6.96	0.08	7
10(D0)+100(D1)	7.45	0.09	7
10(D0)+100(D2)	7.22	0.09	7
10(D0)+100(D3)	7.38	0.08	7
50(D0)+60(D1)	7.28	0.08	7
50(D0)+60(D2)	7.3	0.09	7
50(D0)+60(D3)	7.21	0.08	7
110(D0)	7.17	0.07	7
110(D1)	7.54	0.1	7
110(D2)	7.19	0.08	7
110(D3)	7.23	0.08	7

Figure 6. Survival curves of male *Glossina palpalis gambiensis* irradiated as pupae with a single (110 Gy) or fractionated doses of 10 + 100 Gy and 50 + 60 Gy, separated by 1-, 2- and 3-day intervals (D1, D2, D3). The control group consisted of non-irradiated males. All flies were maintained under standard rearing conditions and were monitored under feeding stress. The black vertical line indicates the median survival time, representing the duration when the likelihood of survival decreased by 50%. The table accompanying the graphs displays the mean survival time, its standard error (SE), and the median.

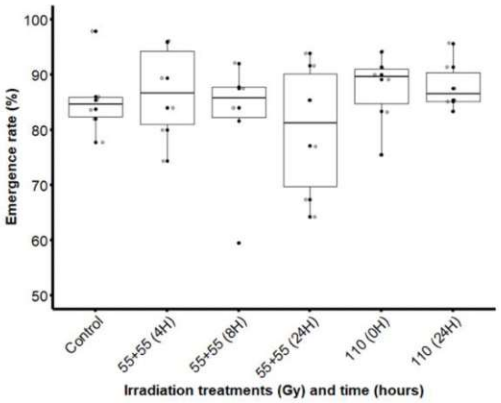


Figure 7. Adult emergence rate of male *Glossina palpalis gambiensis* irradiated as pupae with a single dose (110 Gy) or equal fractionated doses of 55 + 55 Gy with 4-, 8- or 24-hour intervals. The control group consisted of non-irradiated pupae. The boxplot shows the median and upper and lower quartiles. Dots represent experimental data.

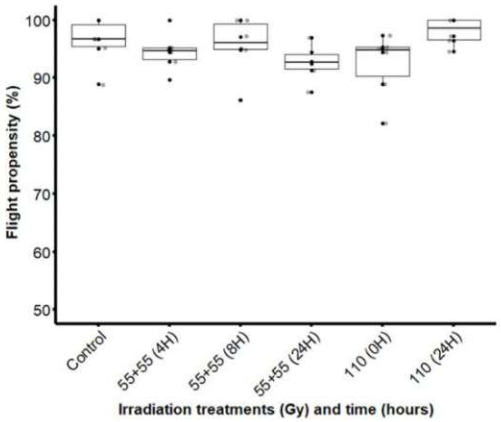


Figure 8. Flight propensity of male *Glossina palpalis gambiensis* irradiated as pupae with a single dose (110 Gy) or equal fractionated doses of 55 + 55 Gy with 4-, 8- or 24-hour intervals. The control group consisted of non-irradiated males. The boxplot shows the median and upper and lower quartiles. Dots represent experimental data.

LaChance and Graham reported that exposing *Musca domestica* L. (housefly) (Diptera), *Oncopeltus fasciatus* (Dallas) (milkweed bug) (Hemiptera), *Anagasta kuehniella* (Zeller) (meal moth) (Lepidoptera), and *Heliothis virescens* (Fab.) (Tobacco budworm) (Lepidoptera) to a single dose of radiation or fractionated into two equal exposures separated by an 8-hour interval did not decrease the frequency of induced lethal mutations [37]. These observations suggest that the time interval might be a crucial parameter when doses are fractionated. The inconsistency of the data from the different studies may be explained by the species and developmental stage- specific impact of radiation on insects. The sensitivity of insects to

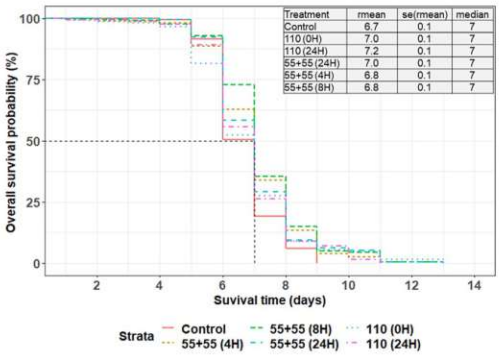


Figure 9. Survival curves under feeding stress of male *Glossina palpalis gambiensis* irradiated as pupae with a single (110 Gy) or equal fractionated doses of 55 + 55 Gy, separated by 4-, 8- and 24-day intervals. The control group consisted of non-irradiated males. All flies were maintained under standard rearing conditions and were monitored under feeding stress. The black line indicates the median survival time, representing the duration when the likelihood of survival decreased by 50%. The table accompanying the graphs displays the mean survival time, its standard error (SE), and the median.

the induction of dominant lethal mutations depends on their developmental stage [5, 28, 33], necessitating the identification of a suitable radiation dose that can effectively achieve the desired level of sterility while preserving the overall quality of the released insects [49]. Previous dose-response studies with tsetse species showed a different sensitivity when pupae or adults were irradiated [6, 7, 42, 44, 54]. In the current study, it was clear that irradiating *G. p. gambiensis* pupae using any of the dose fractionation combinations resulted in a decrease of induced sterility with increasing age. The reason for these age- or development stage-related differences may be attributed to changes in age-dependent gene expressions. Several genes involved in resistance to toxic chemicals were upregulated in aging *Drosophila*, reflecting an overall increase in the defense response system with age [12]. Moreover, our results showed that there was no significant difference in reproductive parameters such as the rate of egg abortion, insemination rate, and the presence of sperm in the spermathecae of females mated with males from pupae exposed to a single or fractionated doses, irrespective of the time intervals between the fractionations. Our results showed similarities with the results obtained from an experiment carried out on *G. tachinoides*, where fractionated doses were administered during the mid-pupal stage [59]. When assessing the impact of dose fractionation on adult emergence rate, flight propensity, and survival time, there were no significant differences between the fractionations and the single dose, nor between the two types of fractionated doses given in the same time interval. It was hypothesized that the phenomenon of radiation hormesis would lead to an expected improvement in lifespan as exposing an organism or cell to low doses of an agent can produce positive biological responses, whereas a higher dose of the same agent can decrease the beneficial effects [16]. However, contradictory results available in the literature provide uncertainty that hormesis is a "real phenomenon". Ionizing radiation administered to the nematode *Caenorhabditis elegans* did not promote subsequent resistance or increased longevity [20], while the results are different from those obtained on insects species such *Ae. aegypti* [67], *P. interpunctella* [14], and adult *Spodoptera litura* [56]. The observed increase in longevity of *G. tachinoides* when exposed to gamma radiation may be attributed to the additional nitrogen atmosphere present during irradiation [59], since radiation under hypoxic or anoxic conditions could improve the insects' biological parameters [55]. For flight propensity, our results were similar to those found for *Aedes aegypti* [67]. From these observations, the contrasting results in the hormesis investigations are believed to be the result of experimental design considerations, particularly with respect to the number of doses, the range of doses, and the selection of the endpoint [16]. Overall, while our results did not indicate any significant difference in the parameters assessed, male from pupae exposed to a fractionated dose of 50 + 60 Gy with a 1-day interval demonstrated a marginally higher level of induced sterility, flight propensity, and survival time (under feeding), when compared to those subjected to either a single dose or a fractionated dose of 10 + 100 Gy. These observations imply that the values of the fractionation and the time intervals between exposures may play a significant role in determining the response of irradiated insects. Among the available studies on fractionated-dose irradiation, although the methods used vary, the majority have employed either one low and one high dose, or two equal doses. The biological stage and the time interval between the administration of fractionated doses could be a crucial factor in determining the response of the irradiated pupae. Depending on the stage of spermatogenesis, giving a single or fractionated dose may have the same effect on the final sterility. Alexander and Bergendahl [1] demonstrated that exposure of adult *Drosophila virilis* to single or consecutive low doses had no significant effect on sterility when the spermatozoa were mature. However, when the dose was fractionated at the spermatid stage, an increase in genetic damage was observed, leading to an increase in sterility [1]. Regarding the time intervals between the administration of doses and biological stage, selecting the appropriate development stage and intervals between exposures is crucial. However, the optimal timing for this species remains unknown, especially due to the limited research on fractionated radiation doses in tsetse flies. The interval between two administrations of radiation at 1, 2, and 3 days may be too long, which can affect the immune system's ability to remain active depending on the radiation type. When the germ cells are mature and their metabolism is inactive during irradiation, recovery mechanisms cannot function. This also holds true for somatic cell damage, which increases with dose and decreases when irradiation occurs at a later stage of insect development, as the number of dividing cells decreases [31]. This is especially true as irradiation affects not only the chromatin material but also partially blocks metabolic pathways [48]. However, the same results were not obtained with the fractionated doses of 10 + 100 Gy or with the single dose on day 1 compared to that of 50 + 60 Gy, indicating the importance of the required dose to

stimulate an appropriate response in the insect organism. The 10 Gy dose administered on day zero might be too low, as low doses have been known to have a double effect, including a low probability of damage and adaptive protection against DNA damage through prevention and repair, as well as immune stimulation [29], since the fractionation is supposed to stimulate somatic cell recovery between doses [25]. As hormetic radiation doses vary considerably between species, we conducted an additional experiment to explore the effects of equal fractional doses of 55 + 55 Gy separated by intervals of 4, 8, and 24 h. Once again, no significant effects were observed. The comprehensive outcomes of our study emphasize the potential need for further investigation, as exemplified by studies conducted by Vimal *et al.* [56] or exploration of the synergy between dose fractionation and a low-oxygen atmosphere. In addition, the biological development stage should be considered. Since previous results indicate that fractionating doses on earlier pupae stages in the presence of nitrogen greatly improved *G. tachinoides* longevity [59], biological stage, exposures interval time, and radiation atmosphere should be considered in future studies. An important notion to consider when selecting the developmental stage to evaluate is the current pupal sex sorting time constraints [2]. In the absence of a genetic sexing strain for *G. p. gambiensis*, the technology of the NIRPSS can only separate pupae according to their sex at an advanced stage of development. Selecting male pupae at the beginning or in the middle of their pupal development is currently not possible. Consequently, the practice of dose fractionation might be challenging to implement in SIT operational programs. For instance, during routine irradiation and shipment of pupae, which occurred twice a week for the Senegal eradication project – specifically on Tuesdays for pupae sorted from Saturday to Tuesday, and on Fridays for pupae sorted from Wednesday to Friday – it would have been complex to implement dose fractionation under these schedules. Furthermore, dose fractionation would result in a doubling of the workload for the radiation operator, as well as repeated handling of the pupae that may cause stress [19, 66]. Thus, this increased workload and likely stress on the pupae must be carefully weighed against the potential benefits in terms of improved sterile male quality parameters.

Conclusion

The findings of our study suggest that the value of hormesis induced in fractionally sterilized males is influenced by the pupal developmental stage and fractionated doses, which is consistent with previous studies. The biological benefits generated by dose fractionation in this study are relatively modest when compared to the associated workloads and transportation constraints. As a result, it is challenging to recommend its immediate implementation without further considerations and optimizations. Alternative dose fractionation schemes could be investigated in the future to determine if there are more efficient ways to achieve better results, while reducing workload. Although current benefits may be limited, exploration of alternative methods, such as irradiation in a low-oxygen environment, may pave the way for more effective and practical irradiation protocols.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Data availability statement

Materials described in the paper, including all relevant raw data, are available in this link: https://doi.org/10.7910/DVN/TJ7NOO.

Supplementary materials

The Supplementary materials of this article are available at https://www.parasite-journal.org/10.1051/parasite/2024001/olm

Supplementary Figure 1: Adult emergence rate of *Glossina palpalis gambiensis* pupae irradiated with single dose (110 Gy) or fractionated doses of 10 + 100 Gy and 50 + 60 Gy with 1-, 2-, and 3-day intervals (D1, D2, D3) as compared to the non-irradiated pupae. The boxplot shows the median, upper and lower quartiles. Dots represent experimental data. There was no significant difference.

Supplementary Table 1: Overview of *Glossina palpalis gambiensis* radiation dosimetry data using the GAFchromic dosimetry system. Data were summarized based on radiation dose and days. The first fraction of the dose was given on Day 0 or hour 0 followed by the second part on day 1, 2 and 3 or after 4 hours, 8 hour and 24 hours. The single doses were given from day 0 to day 3 or at hour 0 and 24 hours. The data were averaged.

Supplementary Table 2: Pairwise comparison of the survival time of *Glossina palpalis gambiensis* males emerged from non-irradiated pupae and those irradiated with single dose (110 Gy) or fractionated doses of 10 + 100 Gy and 50 + 60 Gy with 1-, 2-, 3-day intervals (D1, D2, D3). Survival was monitored under conditions of feeding regime.

Supplementary Table 3: Pairwise comparison of survival time under feeding stress of male *Glossina palpalis gambiensis* from pupae irradiated with single dose (110 Gy) and fractionated doses of 10 + 100 Gy and 50 + 60 Gy separated by 1-, 2- and 3-day intervals (D1, D2, D3). Survival was monitored under conditions of feeding stress. The comparison was based on the same age to detect the possible effect of the irradiation treatments (single or fractionated doses).

Supplementary Table 4: Pairwise comparison of survival time under feeding stress of male *Glossina palpalis gambiensis* from pupae irradiated with single dose (110 Gy) and fractionated doses of 10 + 100 Gy and 50 + 60 Gy separated by 1-, 2- and 3-day intervals (D1, D2, D3). Survival was monitored under conditions of feeding stress. The comparison was based on the same irradiation treatment to detect the effect of the age of the pupae at the day of irradiation.

Supplementary Table 5: Pairwise comparison of the survival time of *Glossina palpalis gambiensis* males emerged from non-irradiated pupae and those irradiated with single dose (110 Gy) or fractionated doses of 10 + 100 Gy and 50 + 60 Gy with 1-, 2-, 3-day intervals (D1, D2, D3). Survival was monitored under conditions of feeding stress.

Supplementary Table 6: Pairwise comparison of the survival time of male *Glossina palpalis gambiensis* emerged from non-irradiated pupae and those irradiated with a single dose (110 Gy) and equal fractionated doses of 55 + 55 Gy separated by 4-, 8- and 24-hour intervals. Survival was monitored under conditions of feeding stress.

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Chapter 5

Effects of low temperature and nitrogen-enriched environment on the sterility and survival of male *Glossina palpalis gambiensis* (Glossinidae: Diptera) using gamma and x-ray sources

Effects of low temperature and nitrogen-enriched environment on the sterility and survival of male *Glossina palpalis gambiensis* (Glossinidae: Diptera) using gamma and x-ray sources.

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Summary

This study investigated the impact of a nitrogen-enriched environment and low temperature on radiation-induced adaptations in male *Glossina palpalis gambiensis*, aiming to provide insights into potential applications for pest management. Results revealed that atmospheric conditions did not significantly affect the emergence rate, contrasting with previous studies on radiation in air. Cold treatment also did not influence the emergence rate, aligning with prior observations on G. p. gambiensis. Additionally, male survival time under radiation treatment was notably extended in a nitrogen atmosphere, suggesting radioprotective effects consistent with studies on other insect species. Nitrogen also required higher radiation doses of 115 Gy and 135 Gy using Rad Source 2400 and Foss Model 812 respectively, to induce sterility in females compared to air, indicating its potential as a radioprotective agent. However, chilling of pupae and adults did not confer radioprotective effects and 75 Gy and 90 Gy were required to induce 95% of sterility in pupae under chilled (7 °C) and non-chilled (~24 °C) conditions. As for adults, 90 Gy were sufficient for sterilization in both treatments. The study also highlighted the efficacy of the X-Rad 320 in sterilization processes. Overall, the findings suggest optimal radiation doses for effective sterilization and emphasize the importance of further assessments on flight quality parameters. Standardization of protocols and acquiring necessary treatments tools are crucial for understanding radiation biology and ensuring the production of high-quality sterile males in pest management initiatives. Further investigation into the mating competitiveness of irradiated males is recommended to enhance decision-making processes in Sterile Insect Technique campaigns.

Introduction

Insect pests pose formidable challenges to the well-being of livestock, the productivity of fruit crops, and the health of human populations, both directly and through the transmission of diseases [1]. The tsetse fly serves as the sole cyclical vector for Human African Trypanosomosis and African Animal Trypanosomosis, significantly impacting agricultural development in various arable regions across Africa [2,3]. To comprehensively address the global impact of these pests, a multifaceted approach is essential, targeting both the transmitted diseases and the pests themselves. Throughout history, vector control has consistently proven to be the central strategy in the fight against vector-borne diseases, yielding positive outcomes [4].

Contemporary strategies encompass the judicious application of approved pesticides through targeted spray programs in infested areas and the deployment of insecticide bait technology designed to capture and eliminate pests selectively [5–9] . However, one of the most promising, innovative and environmentally acceptable approaches is the Sterile Insect Technique (SIT) [10,11]. Integrated seamlessly into Area-Wide Integrated Pest Management, SIT is the utilization of the sterility of insect pests, employing a form of birth control to curtail, suppress, or eradicate targeted species [10]. This technology involves mass rearing of the target species, sterilization through calibrated radiation doses, and field activities encompassing the successful release of sterilized males [12–15] . These sterile males compete with their wild counterparts for mating opportunities with females, ultimately leading to a lack of viable offspring and a reduction in the overall population.

While the success stories of eradication using SIT are notable, such as the control of New World Screwworm, the melon fly, and tsetse flies [9,11], the implementation of an SIT program is not without its challenges. Feasibility studies become paramount, particularly in ensuring the induced sterility in males of the target species does not compromise their essential behavioral characteristics [16–18]. Conventionally, ionizing radiation for insect sterilization has been predominantly reliant on gamma rays from Cobalt-60 or Cesium-137 sources. However, challenges related to safety, costs, and the stringent regulatory environment governing isotope transport have placed constraints on the use of gamma-rays sources, especially for smaller or emerging SIT programs [19,20]. This underscores the growing need for alternative radiation sources that are both cost-effective and logistically viable. Furthermore, continuous enhancements to existing radiation protocols aim to ensure the production of outputs of the highest quality, maximizing the cost-effectiveness of the SIT approach [21]. Improving standing protocols include the assessment of critical factors that may influence the insect's response to radiation such as dose rate, radiation atmosphere and temperature, dose fractionation, etc. [22–27].

In this evolving landscape, our study seeks to contribute insights by assessing the potential hormetic effect of nitrogen and cold environment radiation on *G. p. gambiensis* sterility and male survival. By employing both gamma rays and X-rays, we aim to explore alternative radiation sources and conditions that may offer advantages in terms of safety, cost-effectiveness, and regulatory compliance for SIT programs.

Material and methods

Strain and rearing

The *G. p. gambiensis* strain reared at the Insect Pest Control Laboratory (IPCL) of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Seiberdorf, Austria, has undergone a recent X-ray assessment due the ongoing interest in this species. This is especially crucial as it is nearing eradication in the Niayes [28] and may soon be targeted in the Sine Saloum region of Senegal. Originally established at Maisons-Alfort using wild material collected in Burkina Faso [29], the colony was later transferred to the Centre International de Recherche-Développement sur l'Elevage en zone Subhumide (CIRDES), back in Burkina Faso. In 2009, the IPCL colony was established from pupae received from CIRDES. Maintained under laboratory conditions, the colony experiences a constant temperature and relative humidity (RH) of 24 ± 0.5 °C and 75–80%, respectively. It is also kept under subdued/indirect illumination, following a 12-hour light/12-hour dark photoperiod (Feldmann 1994; FAO/IAEA 2006). Both the colony and experimental flies are fed three times per week using a defibrinated bovine blood diet with an artificial silicon membrane feeding system.

Pupae were collected daily and stored in an incubator set at a precise temperature of 24±0.5°C and a relative humidity (RH) range of 75–80%. These pupae were subjected to sex sorting using the newly developed Infrared Pupae Sex Sorter (NIRPSS) approximately 23-24 days post larviposition [30]. Specifically, male pupae were meticulously selected from the cohort of pupae categorized as unmelanized.

Pre-irradiation treatments and irradiation procedures

In evaluating the susceptibility of *G. p. gambiensis* to gamma (Foss Model 812) and X-rays (Rad Source 2400) radiation within a nitrogen-enriched environment, 24-25-day-old male pupae were placed in a small vial with perforations to facilitate gas circulation. This vial was then attached with reusable putty-like pressure-sensitive adhesive (UHU gmbh & co. kg, 77815 Bühl, Baden Deutschland) to the base of LocknLock Twist containers (LocknLock Co., Jung-gu, Seoul, 04527, Republic of Korea) with specific modifications, as depicted in the figure 1. One hour before the irradiation process, the treatments underwent a 1-minute nitrogen flushing, and the O2/CO2 levels were promptly measured using Dansensor CheckMate 3 (CheckMate3, Dansensor A/S, Ringsted, Denmark). Subsequently, the treatment groups exposed to radiation experienced varying doses of gamma and X-rays - 110, 120, 130, 140, and 150 Gy - all within a nitrogen environment. Additionally, two batches were subjected to both radiation sources in

the air, specifically with a 110 Gy dose. Two control groups, one in air and the other in nitrogen, were not exposed to radiation but were handled in a manner identical to the irradiated treatment groups.

Figure 1: Set up for the irradiation under nitrogen-enriched environment.

In a second experiment the dose response of pupae and adults of *G. p. gambiensis* that were exposed with the X-RAD 320 Precision irradiator to x-rays in air under chilled conditions, were assessed. Male pupae (24-25-day-old) and newly emerged teneral male flies were immobilized under chilling (<3 min at 4-5°C) and placed within a 60 mm x 13 mm petri dish, which was then attached to a larger petri dish (90 mm x 15 mm). The pupae and teneral males, co-located in these petri dishes, were positioned immediately at the center of the radiation chamber. Subsequently, they underwent exposure to radiation doses of 70, 90, 110, and 130 Gy under two distinct conditions: at room temperature and in chilled conditions at 7°C. In average, they samples were exposed to 30-45 min of chilling treatment. The control group, which was not irradiated, underwent the same handling procedures.

Assessment of the dose-response under different atmosphere and temperature treatments

The control group consisted of non-irradiated pupae, and both irradiated and control pupae were handled and maintained under identical conditions. Incubation took place at 24 ± 0.5 °C and 75–80% relative humidity until emergence. Teneral males were placed in small cages (110mm (Ø); 45mm (H)) and fed following the previously described method until reaching

sexual maturity. The flies irradiated as adults were also kept in cages and fed until their sexual maturity.

In all treatment groups, 6-7-day-old individuals were paired in standard colony cages (Ø 20 cm) with virgin females aged 3-4 days, maintaining a 1:2 male to female ratio for four days. Daily mortality monitoring was conducted. Subsequently, males and females were separated through chilling at 4°C. The females were then transferred to 20 cm diameter cages, and their daily production and mortality were recorded over 60 days. The survival of males under the feeding regime in the small cages (110mm (Ø); 45mm (H)) was monitored for 90 days.

Data analysis

Data were statistically analyzed using R studio, version 4.4.2. Adult emergence rate was modelled using a generalized linear mixed model. Male survival was analyzed using the Cox Mixed Effects Model fit by maximum likelihood ("*survival*" package, "*coxme*" function). In both analyses, the radiation environments and doses were considered as fixed effects and the replications as random effect. The dose response data has been analyzed using the dose response model under the *drc* package with the *drm* function. The best model has been selected using the *mselect* function. Subsequently, the *compParm* function was employed to compare the curve parameters, and the *ED* function was utilized to determine the effective doses. The One Inflated Beta regression model has been utilized in conjunction with the dose-response model to compare the effectiveness of different treatments. Ggplot2 was employed to construct the dose-response curves.

Results

Nitrogen and cold treatments

The results of the nitrogen treatment showed that the average level of O_2 before irradiation was 0.078%, with CO_2 at 0.008%. Following irradiation, these levels turned to 0.407% for O_2 and 0% for CO_2. The average duration of pupal exposure to nitrogen before irradiation was 94 minutes. Regarding the cold treatment, the temperature was consistently maintained at 7°C throughout the irradiation period.

Adult emergence rate

When analyzing the impact of the irradiation atmosphere on the adult emergence rate, no significant difference was found between the radiation sources ($\chi^2 = 2.6692$; df = 2; p = 0.2633) or the dose, even with an increase ($\chi^2 = 3.6091$; df = 4; p = 0.4615) or atmospheric treatment

(χ^2 = 0.1376; df = 1; p = 0.7107) (Figure 2). In comparing this parameter while considering the cold treatment using the X-Rad Precision irradiator, neither the temperature ($\chi 2$ = 0.0109; df = 1; p = 0.9168) nor the radiation dose ($\chi 2$ = 0.6707; df = 4; p = 0.9549) had a significant impact on the adult emergence rate (Figure 3).

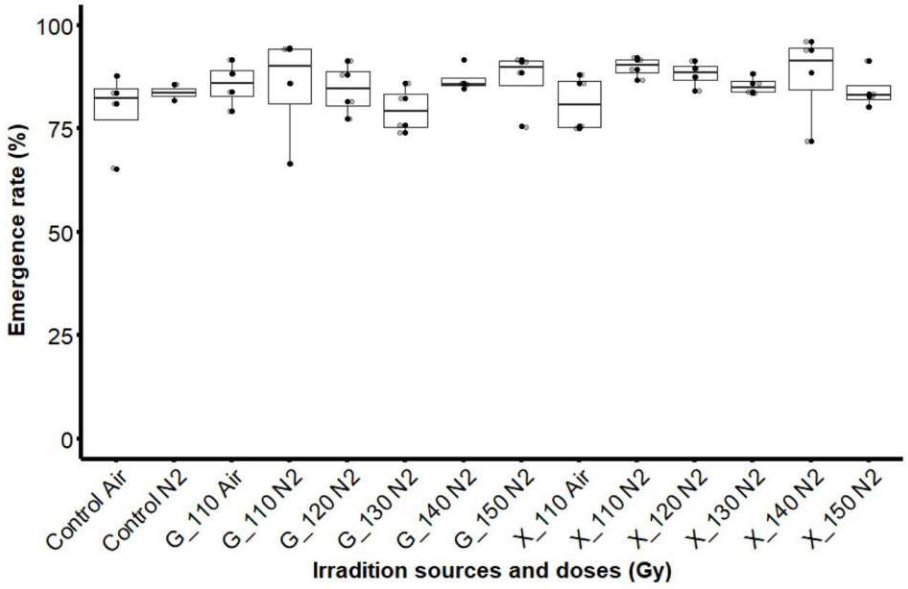

Figure 2: Adult emergence rate of male *Glossina palpalis gambiensis* irradiated as pupae with Gamma- (G) or X- (X) rays under air and nitrogen atmosphere. The control group consisted of non-irradiated pupae. The boxplot shows the median and upper and lower quartiles. Dots represent experimental data.

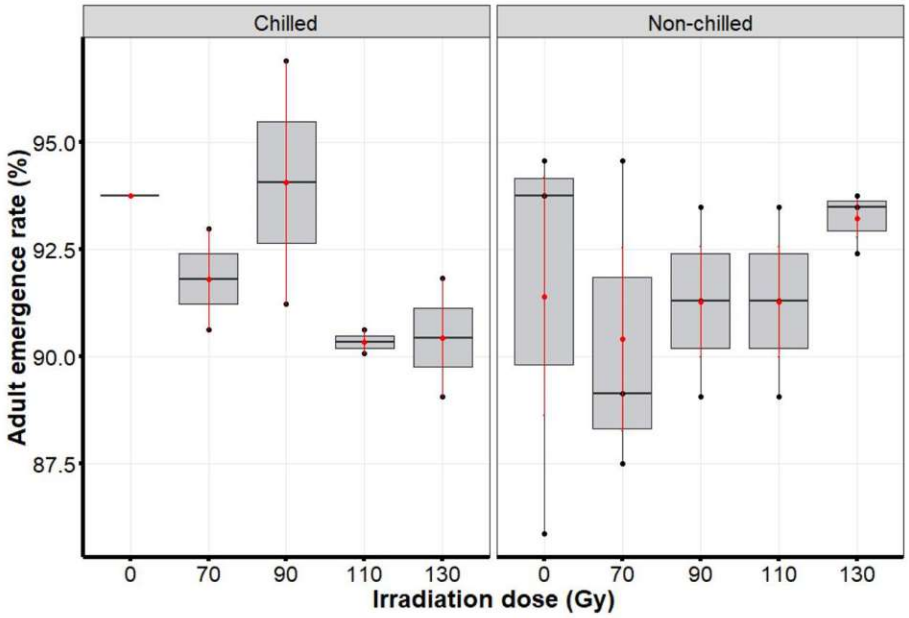

Figure 3: Adult emergence rate of male *Glossina palpalis gambiensis* irradiated as pupae chilled or not. The boxplot shows the median and upper and lower quartiles. Dots represent experimental data.

Male survival rate under feeding regime

Survival of G. p. gambiensis male irradiated as pupae in nitrogen and air

The coxme model revealed significant variations in the survival rate based on both the atmosphere treatment ($\chi^2 = 44.65$; p = 2.356e-11) and the radiation dose ($\chi^2 = 55.29$; df = 4; p = 1.138e-10). Males irradiated in nitrogen exhibited a significantly longer survival time compared to those exposed to radiation in air ($p < 0.0001$). Additionally, the control group demonstrated a longer survival time than the irradiated groups, as illustrated in Figure 4.

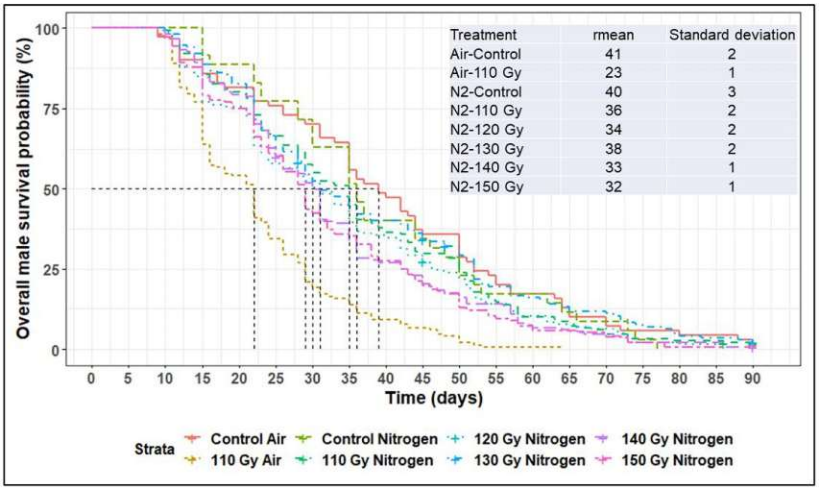

Figure 4: Survival curves of males irradiated under nitrogen compared to air-exposed males. The x-axis represents the survival time in days, and the black vertical lines denote the median survival time (50% survival point).

Survival of G. p. gambiensis male irradiated as pupae and adult under chilled and non-chilled conditions

The analysis of male survival time in *G. p. gambienisis*, irradiated as pupae, indicated that radiation temperature had no significant effect ($\chi2 = 0.7513$; df = 1; p = 0.3861). However, a notable impact was observed with the radiation dose ($\chi2 = 61.5231$; df = 4; p = 1.388e-12) when irradiated as pupae or adult. In general, the control group consistently exhibited a longer survival time than the irradiated groups and the survival time decreased with increasing doses. In addition, there was a significant difference in the survival rate according to the biological stage weather irradiated as chilled or non-chilled ($\chi2 = 22.9707$; df = 1; p = 1.645e-06). The adult survival time was longer compared to the pupae survival time for both chilled and non-chilled pupae (Figure 5).

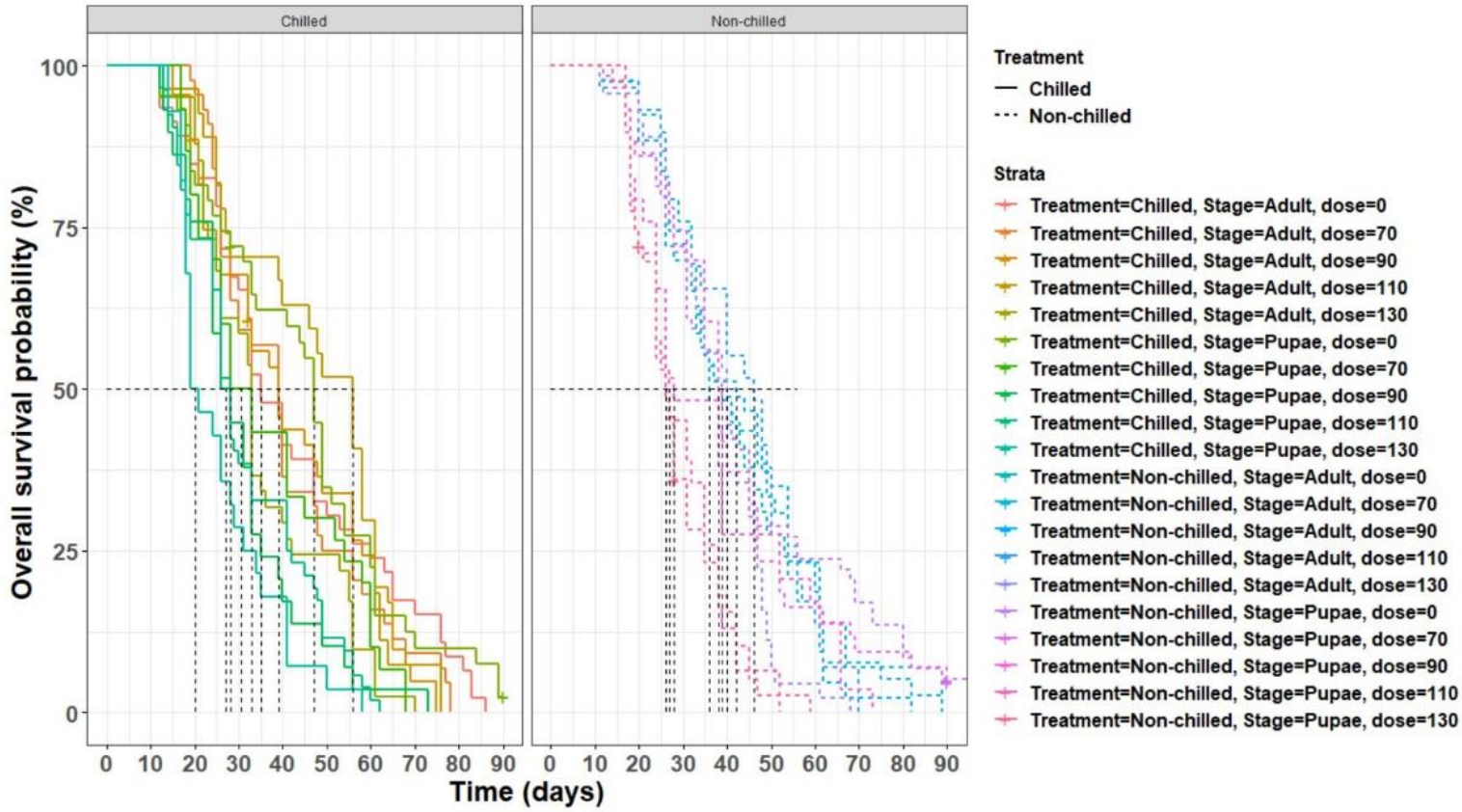

Figure 5: Survival curves of males irradiated as pupae or adult under chilled and non-chilled conditions. The x-axis line shows the survival time in days and the black vertical lines indicate the median survival time (50% survival point).

Dose-response relationship to radiation under various atmospheric

Radiation under nitrogen environment as compared to normoxia

The dose-response modeling indicated a fitting of the data with the Weibull 1.2 function, characterized by a lower limit (d) of 0 and an upper limit of 1. The equations for the induced sterility curves were expressed as $y(x) = \exp(-\exp(b(\log(x)-\log(e))))$. Notably, there was no significant difference observed between the gamma and X-ray dose-response curve parameters b ($p = 0.1556$) and inflection point e ($p = 0.3796$). Nevertheless, it was observed that irradiated pupae displayed higher sensitivity to X-rays compared to gamma-rays at higher doses, as detailed in Table 1 and illustrated in Figure 6.

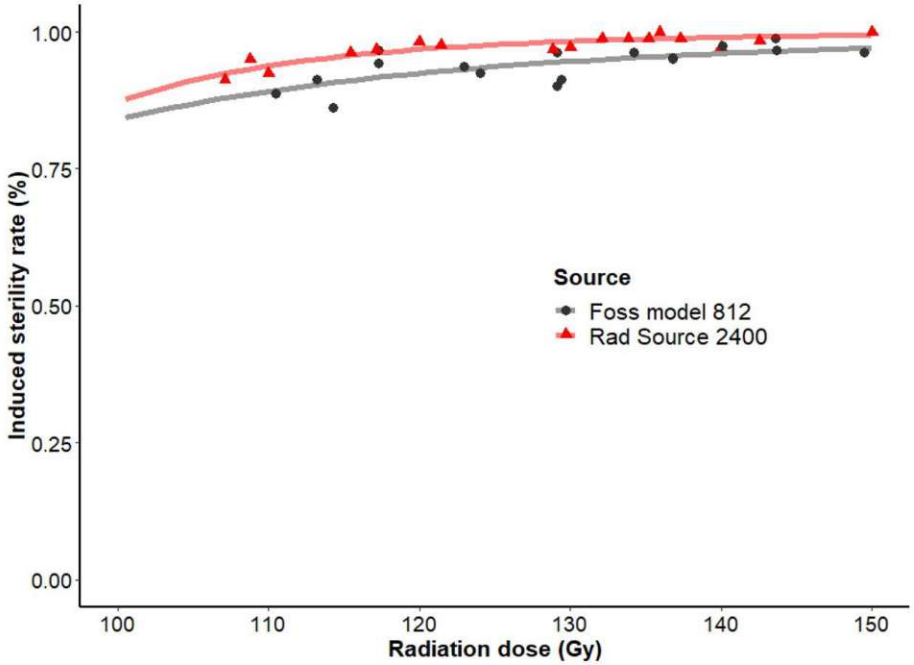

Figure 6: Weibull model 1.2 dose–response curves for the induced sterility in *Glossina palpalis gambiensis* irradiated as pupae with Foss Model 812 (Gamma rays) and Rad Source 2400 (X-rays) under nitrogen-enriched environment. The dots represent the experimental data.

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Table 1: Estimated effectives doses that induce respectively 50%, 95% and 99% of sterility in G. p. gambiensis male irradiated under nitrogen environment using gamma-ray (Foss Model 812) and X-ray (Rad Source 2400) sources.

Treatments	Effective doses (Gy)		
	50%	**95%**	**99%**
Foss Model 812	73.0	132.2	148.9
Rad Source 2400	81.4	113.2	120.9

Irrespective to the radiation source, the number of eggs aborted was significantly higher in females mated with irradiated males than those mated with fertile males (Table 2). Moreover, regardless of the radiation source, the irradiated males retained their ability to mate and transfer sperm to the females, as evidenced by the insemination rate (> 90%) and the spermathecae fill (Table 3). Analysis of uterine status on day 60 in dissected females revealed notable differences in content between those mated with non-irradiated males and those with irradiated males. Uteri from females mated with irradiated males were largely empty, either due to abortions or containing recently ovulated eggs in embryonic arrest (Table 3).

Table 2: Reproduction parameters of *Glossina palpalis gambiensis* females mated with males irradiated as pupae under nitrogen and air. Mature female days were calculated for each treatment by adding the number of flies alive each day, starting on D18 after emergence (age of maturity) until the end of the experiment on day 60.

Treatment	Mean mature female days	No. of aborted eggs and instar larvae				No of pupae produced	Mean PRC	Mean fecundity	Mean ±SD induced sterility
		E	I	II	III				
Control									
Control Air	1100	25	2	7	4	327	1.0000	0.0745	0.0
Control N2	1023	10	0	2	4	231	0.9542	0.0756	0.0
						Foss model 812			
110 Air	1204	423	1	1	0	7	0.0392	0.0030	96.1 ± 1.5
110 N_2	1028	433	0	1	1	25	0.0945	0.0073	90.6 ± 5.5
120 N_2	1099	463	1	1	0	20	0.0598	0.0044	94.0 ± 2.2
130 N_2	949	318	0	4	2	15	0.0703	0.0055	93.0 ± 3.1
140 N_2	1059	480	1	2	1	15	0.0467	0.0035	95.3 ±3.1
150 N_2	1084	448	2	1	0	12	0.0356	0.0027	96.4 ± 0.9
						Rad Source 2400			
110 Air	1071	426	1	0	1	5	0.0168	0.0013	98.3 ± 1.1
110 N_2	1092	419	3	0	1	21	0.0614	0.0046	93.9 ± 2.5
120 N_2	1106	462	0	1	0	9	0.0285	0.0021	97.1 ± 0.8
130 N_2	1068	491	0	0	0	6	0.0174	0.0014	98.3 ± 0.7
140 N_2	1093	491	0	0	0	4	0.0102	0.0008	99.0 ± 1.3
150 N_2	1112	453	1	0	1	1	0.0031	0.0002	99.7 ± 0.6

SD: Standard deviation; PRC: Production relative to the control (number of pupae produced by a treatment group/number of pupae produced by the control group

Table 3: Reproductive status of *Glossina palpalis gambiensis* females that mated with males exposed as pupae under air or nitrogen environment using gamma and X-ray sources. Females were dissected after an experimental period of 60 days.

Irradiation treatment/dose (Gy)	No of females alive at day 60	Insemination rate (%)	Spermathecae fill score					Uterus content or status at day 60						
								No of recently ovulated eggs	Viable instar larvae			Empty due to		
			0	0.25	0.5	0.75	1		I	II	III	Abortion	Larviposition	Blocage
Control Air	92	0.98	2	1	17	52	20	10	13	10	28	21	9	0
Control N2	39	0.97	1	4	2	14	18	8	4	6	12	6	3	0
Foss model 812														
110 Air	101	0.92	10	14	19	38	20	24	1	4	1	56	5	1
110 N_2	94	0.97	3	7	12	29	43	18	2	0	1	67	4	2
120 N_2	95	0.96	4	4	9	52	26	14	1	0	3	75	2	0
130 N_2	90	0.99	1	2	8	64	15	15	3	3	9	51	8	1
140 N_2	94	0.97	3	1	13	61	16	23	0	0	2	67	0	0
150 N_2	95	0.98	2	0	18	45	30	11	1	0	0	81	0	0
Rad Source 2400														
110 Air	92	0.89	10	6	21	37	18	18	0	1	0	71	1	0
110 N_2	94	1.00	0	2	11	64	17	26	0	1	1	65	1	0
120 N_2	95	0.97	3	5	16	49	22	14	1	1	1	76	0	0
130 N_2	85	0.97	3	25	2	37	18	9	0	0	0	73	2	1
140 N_2	98	0.97	2	4	10	41	41	8	0	0	0	86	4	0
150 N_2	97	0.98	2	3	20	48	24	10	0	0	0	87	0	0

Radiation under chilled and non-chilled conditions

Modelling the induced sterility data using the One Inflated Beta regression showed that there was no significant difference in the sterility according to the Temperature treatment ($p = 0.2908$) or the insect life stage ($p = 0.0627$). Using the dose-response function allowed the determination of the estimated effectives doses that induce 50, 95 and 99% of sterility (Table 4). The doses responses curves presented in Figure 7 shows that in chilled and non-chilled conditions, the pupae were slightly sensitive as compared to the adults at the lower radiation dose.

Table 4: effective radiation doses inducing 50%, 95%, and 99% sterility in females mated with males irradiated as pupae or adults under chilled and non-chilled conditions.

Stage	**Treatments**	**Estimated effective doses (Gy)**		
		50%	**95%**	**99%**
Pupae	Chilled	45.1	72.3	97.3
	Non-chilled	41.9	85.3	133.1
Adult	Chilled	57.5	88.2	115.2
	Non-chilled	50.3	86.3	121.0

Tables 5 and 6 provide insights into reproduction parameters over a 60-day experiment and following female dissection. The elevated number of aborted eggs expresses the induced sterility in females mated with irradiated males. Importantly, the insemination rate and spermathecae fill score in Table 6 infers that irradiated males retained the ability to transfer sperm to the females.

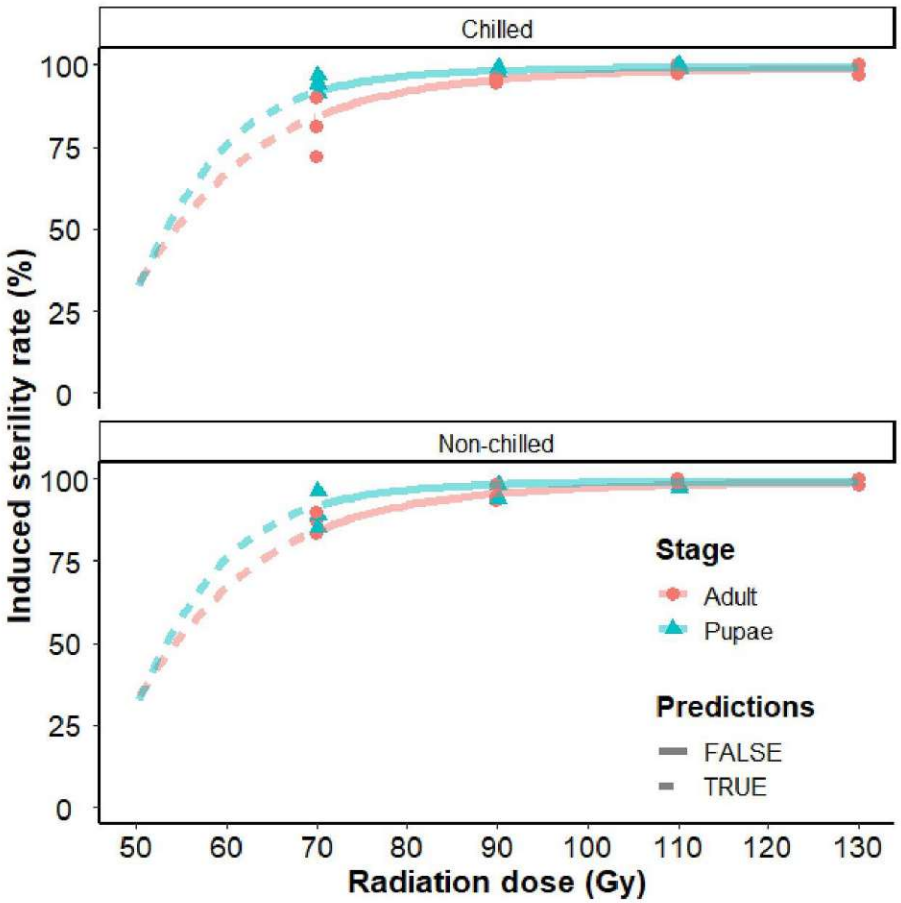

Figure 7: Dose–response curves for the induced sterility in *Glossina palpalis gambiensis* irradiated under chilled and non-chilled treatments with the X-Rad 320 Precision irradiator (X-rays). The chilling was done at 7 °C inside the irradiation chamber and non-chilled treatment was handled at room temperature (24 °C). The dots represent the experimental data.

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Table 5: Reproduction parameters of *Glossina palpalis gambiensis* females mated with males irradiated as pupae under chilled and non-chilled conditions using X-Rad 320 Precision. Mature female days were calculated for each treatment by adding the number of flies alive each day, starting on day 18 after emergence (age of maturity) until the end of the experiment on day 60.

Stage	Treatment	Radiation dose	Mature female days	No. of aborted eggs and instar larvae				No pupae	Mean PRC	Mean fecundity	Mean ±SD induced sterility
				E	I	II	III				
Adult	Chilled	0	1153	13	3	1	2	304	1.0000	0.088	0.0 ± 0.0
		70	1220	336	0	0	1	58	0.1894	0.0155	81.1 ± 9.1
		90	1235	357	2	1	0	13	0.0434	0.0035	95.7 ± 0.9
		110	1193	368	3	0	0	4	0.0122	0.0011	98.8 ± 1.3
		130	1241	370	0	0	0	3	0.0104	0.0008	99.0 ± 1.8
	Non-chilled	0	1276	8	1	2	0	297	1.0000	0.0773	0.0 ± 0.0
		70	1299	349	0	1	1	38	0.1303	0.0098	87.0 ± 3.2
		90	1314	372	0	0	1	13	0.0464	0.0033	95.4 ± 2.6
		110	1236	238	1	1	1	1	0.0055	0.0004	99.5 ± 0.8
		130	1265	380	1	1	0	2	0.0057	0.0005	99.4 ± 1.0
Pupae	Chilled	0	1220	11	2	1	4	296	1.0000	0.0806	0.0 ± 0.0
		70	1189	310	1	0	1	17	0.0598	0.0049	94.0 ± 2.7
		90	1220	363	0	0	0	4	0.0142	0.0011	98.6 ± 0.8
		110	1142	350	0	0	0	2	0.0067	0.0006	99.3 ± 0.6
		130	1159	323	0	0	0	1	0.0028	0.0003	99.7 ± 0.5
	Non-chilled	0	1222	9	1	1	2	309	1.0000	0.0845	0.0 ± 0.0
		70	1212	335	3	0	0	30	0.099	0.0084	90.1 ± 5.7
		90	1245	357	1	0	0	14	0.0463	0.0038	95.4 ± 2.4
		110	1305	279	1	0	0	5	0.0238	0.0019	97.6 ± 0.5
		130	1266	375	1	0	0	0	0.0000	0.0000	100.0 ± 0.0

SD: Standard deviation; PRC: Production relative to the control (number of pupae produced by a treatment group/number of pupae produced by the control group

Table 6: Reproductive status of *Glossina palpalis gambiensis* females that mated with males exposed as pupae or adult under chilled or non-chilled conditions using the X-Rad 320 Precision irradiator. Females were dissected after an experimental period of 60 days.

Stage	Treatment	Dose (Gy)	Insemination rate	No of females alive at day 60	Spermathecae fill score					Uterus content or status at day 60						
										No of recently ovulated eggs	Viable instar larvae			Empty due to		
					0	0.25	0.5	0.75	1	E	I	II	III	Abortion	Larviposition	Blocage
Adult	Chilled	0	0.97	74	2	5	17	29	21	8	10	9	31	3	13	0
		70	0.96	84	3	4	7	38	32	12	1	1	2	63	2	3
		90	0.93	84	6	2	14	27	35	37	1	0	0	40	1	5
		110	0.98	80	2	5	10	29	34	14	0	0	1	61	0	4
		130	0.99	85	1	6	4	44	30	12	0	0	0	73	0	0
	Non-chilled	0	0.93	78	5	2	5	38	28	10	8	9	25	11	14	1
		70	0.96	84	3	9	8	37	27	7	2	1	1	68	4	1
		90	0.97	85	3	10	10	28	34	8	0	0	0	71	3	4
		110	0.95	77	4	4	6	20	43	12	3	5	6	44	5	2
		130	0.94	81	5	3	9	31	33	10	0	0	0	69	0	2
Pupae	Chilled	0	0.99	69	1	2	5	21	40	12	19	9	11	0	16	0
		70	0.95	76	4	5	3	39	25	18	3	1	1	45	1	3
		90	0.96	82	3	4	10	37	28	11	1	0	0	69	1	0
		110	0.93	71	5	4	11	27	24	12	0	0	0	58	0	1
		130	0.88	74	9	7	7	32	19	17	0	0	0	54	0	3
	Non-chilled	0	0.99	78	1	1	5	43	28	19	8	10	29	3	9	0
		70	1.00	74	0	5	7	35	27	7	1	1	1	60	3	1
		90	0.96	81	3	4	4	27	43	23	3	0	0	49	2	4
		110	0.97	86	3	2	12	40	29	14	4	5	9	50	4	0
		130	0.96	82	3	2	12	43	22	34	0	0	1	42	0	5

Discussion

This study delved into radiation-induced adaptations in male *G. p. gambiensis*, specifically focusing on the effects of a nitrogen-enriched environment and low temperature. Utilizing gamma and X-ray sources, our investigation sought to elucidate the nuanced responses of *G. p. gambiensis* to these environmental conditions, offering insights into potential applications for pest management. In the assessment of atmospheric conditions, our findings reveal that however there was not a significant difference in the emergence rate, it did not decrease with the increasing dose. Interestingly, this contrasts sharply with previous observations of radiation in air, where higher doses were associated with a declining adult emergence rate [31,32]. This result aligns with observations made on insects sterilization under nitrogen environment as compared to air [33]. A study on *Glossina tachnoides* species irradiated as pupae under nitrogen revealed an improvement of the adult emergence rate [34]. Concerning the cold treatment, our results align with analogous study on *G. p. gambiensis*, which showed that the cold treatment exhibited no discernible effect on the emergence rate [23,35]. The emergence rate ranged from 89.1% to 96.9% and was higher than the 83–91% and the 64% obtained for *G. p. gambiensis* and *G. m. morsitans* respectively, stored at 9.2 ± 0.4°C and 10 ± 1°C [23,36]. These observations imply that the potential impact of cold, whether protective or not, appeared to be exposure-time-dependent [27]. However, additional stress factors, such as transportation and handling, may have contributed to the observed previous outcomes [37]. Our investigation into the radiation treatment of male survival time under a feeding regime showcased a remarkable radioprotective effect of a nitrogen atmosphere, evidenced by longer survival times even at a higher dose of 150 Gy. This aligns with previous studies on *G. morsitans* and *Glossina tachinoides* [33,34] as well as other insect species [27,38,39]. Additionally, our research revealed that in both chilled and non-chilled conditions, the adult survived longer as compared to the pupae. This observation aligns with the existing body of knowledge in this domain, which indicates that storage of the pupae in low temperature followed by irradiation may result in a decrease of the survival time in *G. p. gambiensis* and *G. morsitans* [23,33]. Turning to the assessment of sterility induced in females mated with irradiated males, nitrogen emerged as a clear radioprotective agent. Higher radiation doses were required to induce the same level of sterility in a nitrogen-enriched environment compared to air which is consistent with previous studies [24,27,38]. Specifically, doses of at least 115 Gy and 135 Gy were necessary in nitrogen radiation, with X-rays and gamma rays, respectively, compared to the 90 Gy and 110 Gy required in an air environment [40] . This emphasizes the potential of nitrogen and related

hypoxic treatments as viable alternatives for enhancing the quality parameters such as the survival of male *G. p. gambiensis*. Low oxygen has been known, for the last 45 years, to be the leading stressor to confer hormetic effects [41]. This protection may rely on limited somatic damage occurred during the radiation process by the reduction of the production of oxygen free radicals which are a threat to aerobic organisms and crucial factor to longevity and reproduction [42,43]. Indeed, it has been shown that irradiation in air creates free radicals that are detrimental to the quality of insects which could solved by excluding oxygen by flooding the containers with nitrogen or exhausting the oxygen through the pupal metabolism [44]. In addition, hypoxic conditions in tissues can induce an elevation in the overall antioxidant response and a reduction in oxidative damage [45]. In contrast, chilling of both pupae and adults demonstrated no radioprotective effect. On the contrary, the pupae displayed a slight higher sensitivity in both chilled and non-chilled conditions and corroborate with the results on *G. morsitans* when pupae were exposed to 2 °C prior to radiation [33]. This is in line with the role of the life stage in the insect radiosensitivity. Indeed, in most of insects species, the earlier stage being more radiosensitive even if the pupae aged of 25 days were biologically closed to the teneral flies emerged around 33 days post-larviposition [46] spermatogenesis occurs during the larval/pupal stage (Helinski et al., 2009; Itard, 1971). In addition, the study highlighted the efficacy of the X-Rad 320, which necessitated lower doses to induce an acceptable level of sterility for the SIT as indicated for previously assessed X-ray models ([40,47]. The effectiveness of 90 Gy aligns with earlier findings on this species [40] and emphasizes the suitability of X-ray sources for *G. p. gambiensis* sterilization. Above the effects of all treatments on the parameters, considering the insemination rate in females mated with irradiated males under both cold and nitrogen conditions indicates the males' ability to transfer sperm, a crucial aspect for the successful application of the Sterile Insect Technique.

In summary, our study sheds light on the radioprotective effects of a nitrogen environment irradiation, suggesting optimal doses of 115 Gy and 135 Gy for X- and gamma-rays, respectively. While emphasizing the need for further assessments through flight quality parameters, our findings underscore the potential of these adaptations for operational programs. Furthermore, standardization of protocols and the acquisition of necessary tools are imperative for comprehending the radiation biology and ensuring the production of high-quality sterile males in pest management initiatives [26]. The present findings demonstrate the effectiveness of the integrated cooling system in the X-Rad 320. Moreover, the prolonged survival of male adults irradiated under cold treatment suggests a potential alternative for selecting the

biological stage for irradiation, particularly in SIT campaigns with established mass production facilities. However, further investigations into the mating competitiveness of these males could significantly improve decision-making processes.

Conclusion

Our results not only affirm the protective effects of radiation in a nitrogen environment on *G. p. gambiensis* but also demonstrate the effectiveness of X-rays and gamma rays in achieving an acceptable level of sterility. The enhancements observed in the survival time hint at potential improvements in flight quality parameters and mating competitiveness. It is imperative to delve deeper into these aspects through additional investigations, while also emphasizing the importance of standardizing protocols. This is vital for seamlessly incorporating our findings into tsetse SIT programs. The insights derived from the effects of cold treatment emphasize the critical importance of carefully selecting the life stage for irradiation, considering both technical and logistical considerations. Additionally, the emergence of new technologies like the X-Rad 320, with its integrated cooling system, holds promise as an effective tool for sterilizing *G. p. gambiensis* adults.

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Chapter 6

General discussion, conclusion and perspectives

1. General discussion

The Sterile Insect Technique (SIT) is widely recognized as an environmentally acceptable method that, when integrated with other approaches in Area-Wide Integrated Pest Management (AW-IPM), contributes to the effective control of livestock and plant pests, and vectors of human diseases [147]. The success of SIT hinges on several key factors, with insect sterilization being a pivotal element in its implementation [74]. While the principles of this technique have evolved since its initial success in eradication efforts, the full potential of this technology, especially in its application to tsetse species, is yet to be realized. Research and Development (R&D) play a crucial role in enhancing the cost-effectiveness and the efficiency of all facets of SIT, including advancements in radiation biology [147,148]. The focus lies on perfecting sterilization techniques and management practices to achieve high-quality sterile males and continuous R&D efforts are dedicated to improving the output products released into the field. Ongoing investigations explore various aspects, such as the feasibility of employing SIT as a novel pest management method [149,150], the exploration of X-rays as an alternative to gamma-rays [102,104,105,110,151,152], the impact of radiation dose rate on biological response [129,153,154], the effect of radiation on the insect symbionts [155–159], the sensitivity of different life stages of insects to radiation [104,160], and the potential hormetic effects of radiation dose fractionation [128].

Additionally, research delves into understanding the effects of different atmospheric and environmental conditions on radiation sensitivity and somatic damage [161,162]. Despite considerable progress in research aimed at enhancing the SIT, the ongoing challenges lie in the technical complexity and cost-effectiveness of the technology [147]. The potential impact of global warming associated with climate change, coupled with the effects of globalization on insect pest dynamics, remains a serious concern [163–166]. Therefore, there are still research gaps and global considerations that require elucidation to firmly position SIT among the most effective biotechnologies available. In this section we discussed the key findings of the thesis in terms of their implications for current and potential SIT operational programs.

In Chapter 2, we investigated the suitability of using an X-ray Blood irradiator model Raycell Mk2 for sterilizing *G. p. gambiensis*, fruit flies and mosquito species. The results indicate that this model induces an acceptable level of sterility required for SIT implementation (>95%) in *G. p. gambiensis* males irradiated as pupae. The level of sterility obtained was comparable to that obtained in fruit flies (*Ceratitis capitata, Anastrepha ludens*) irradiated as

pupae, and mosquitoes (*Aedes aegypti, Anopheles arabiensis*) irradiated as pupae and adults. It was shown that the X-ray Blood irradiator has a wide range of applicability in insects as consistent outcomes were observed when evaluating the efficacy of X-rays using an alternate X-ray machine, Rad Source 2400 on the fruit flies species mentioned above [106] as well as on *Aedes albopictus* [104,108,109] and when applied to species within the Lepidoptera group, such as *Ephestia elutella* [110], *Spodoptera frugiperda* [151]. In the context of Navel Orangeworm (*Amyelois transitella*), X-rays emerge as a promising option for SIT, particularly in the irradiation of the adult stage [167]. However, it is noteworthy that employing X-rays during the larval stage of the same specie resulted in lethality, despite inducing an acceptable level of sterility at high doses [168]. Hence, X-ray sources emerge as a viable recommendation due to their cost-effectiveness and the avoidance of stringent regulations associated with gamma-ray sources. Nonetheless, given that this study presents the first report of X-ray Raycell Mk2 radiation dose response and suitability for sterilizing the *G. p. gambiensis* species, there were crucial gaps that necessitate exploration before extending a wide-scale recommendation in this and other tsetse species. Specifically, it is imperative to determine the optimal dose that induces an acceptable level of sterility while preserving flight quality and mating competitiveness parameters [74,130,131].

To fulfill these requirements, a thorough comparison was undertaken, whereby we evaluate the impacts of a gamma-ray source and two X-ray sources on sterility, adult emergence rate, flight propensity, survival rate, and mating competitiveness in semi field conditions. From this comparison, it was evident that X-rays are equally effective as gamma-rays regarding the parameters mentioned (Chapter 3). Both the Raycell MK2 and the Rad Source 2400 models achieved over 95% sterility, comparable to the Gamma-ray model Foss 812, when utilizing the optimal radiation dose for the *G. p. gambiensis* species [141]. In addition, a lower irradiation dose from X-ray sources, including Raycell MK2 and Rad Source 2400, was required to achieve the same level of sterility compared to gamma rays, as observed in the suitability study (Chapter 2). A similar trend was observed in the dose response of *Ceratitis capitata* to X-ray irradiation compared to gamma irradiation [106]. In contrast, these observations contradict the findings of Wang et al. [108], who noted that an equivalent dose of gamma-rays induced a more pronounced sterility effect in *Aedes albopictus* males as compared to X-rays. These inconsistencies might be related to the radiation dose rate at the exposure times or additional stress factors [169], as the relative biological effectiveness did not differ significantly between the X- and gamma-ray radiation [94,106,168]. Concerning the dose rate,

few studies have been done on insects and were mainly on gamma-rays [128,170–172] and their results have been divergent.

In addition to the dose rate, another crucial parameter that could account for this discrepancy may be the dosimetry system. This system is essential for elucidating the varying levels of sterility obtained with the same irradiation dose in different facilities [148]. In most studies, there was no system that measure the dose-rate or to ensure the delivered dose corresponds to the targeted dose [173]. This emphasizes the crucial role of the dosimetry system employed during our investigations, which measurements and reporting is often neglected [169,174]. Indeed, dosimetry, considered as a key component of quality assurance for radiation is important in all stages of the SIT implementation, from the initial research to operational programs [175]. However, considering the diversity of the dosimetry systems [173,176], an accurate, reliable dosimetry system [177] that is simple enough to be operated without special facilities requirements, but provides adequate precision and affordable to be used routinely, is needed. This ensures that irradiated insects are adequately sterilized [144] and fully competitive, thereby avoiding potential negative side effects of releasing under-sterilized insects, such as an accidental introductions of insects that can establish and persist over generations [80] . The ISO/ASTM 51940:2022 provides standard guidance for dosimetry for SIT programs (https://compass.astm.org/document/?contentCode=ASTM%7CE1940-22%7Cen-US&proxycl=https%3A%2F%2Fsecure.astm.org&fromLogin=true).

It is crucial that the optimal sterilization dose does not compromise the ability of irradiated and released males to compete with their wild counterparts and mate with wild females (Vreysen, 2005). Beyond induced sterility, it has been demonstrated that X-rays, when used at acceptable doses, do not compromise the quality of irradiated insects species, including parameters like flight quality and mating competitiveness, when compared to gamma-rays [106,108,152]. Our results align with these observations. No significant difference was found in the flight propensity, survival rate under starvation, and the relative mating index of males sterilized with either X- or gamma-ray sources. As gamma and X-rays exhibit similar relative biological effectiveness (RBE), previous studies have indicated no significant differences in the biological damage observed [106]; the somatic cells damage being expressed as the development of abnormalities, a reduction in lifespan, flight ability, mating propensity, etc. [74].

In summary, the results demonstrate the relevance and suitability of the Raycell Mk2 and Rad Source 2400 X-ray irradiators based on the discussed parameters above. However, concerns may arise during the popularization of these devices, particularly regarding their

processing capabilities for large-scale programs [98]. The processing capacity of Raycell Mk2 and Rad Source 2400 has been simulated, particularly for medium-sized and larger programs. Notably, the Raycell Mk2 source exhibited lower processing capacities in relation with its canister volume and low dose-rate, especially for treating blood in large-scale mass rearing facilities, such as the former insectarium of Bobo-Dioulasso, which exceeded a colony size of 500,000 females in 2022. To address these limitations, the use of multiple sources could be considered, potentially reducing transport and regulatory constraints, although the associated costs may not see a significant reduction. Additionally, it is important to note that X-ray sources require a stable electric power supply [102]. Indeed, X-ray systems are more prone to failures, often attributed to the fragility of the tubes and the complexity of the electronics, high-voltage systems, and external cooling units. However, this challenge could be addressed by deploying well-trained local technicians for maintenance as part of the tsetse eradication team [178].

The implications of these observations are essential for exploring new methods of collecting and processing tsetse blood meals to adapt to the lower processing capacity of X-ray sources. Collaborating with manufacturers to enhance this capability may be the ultimate alternative. Despite the highlighted limitations, our results indicate that both the Raycell Mk2 and the Rad Source 2400 are recommended for small- and medium-scale tsetse fly irradiation programs. While awaiting clarification on these limitations to assess the cost-effectiveness of X-ray sources, optimizing current protocols in facilities equipped with gamma-ray sources could offer a pathway to reduce costs and enhance the efficacy of ongoing programs. Several studies, exploring irradiation environments modulated by oxygen depletion or specific gases and cold treatments, along with fractionation of the optimal dose, aim to induce a potential hormetic effect and improve the quality of sterile males for the SIT [125–128,162,179–182].

In pursuit of this goal, a radiation-dose fractionation protocol has been developed, aligning with the current optimal radiation dose of 110 Gy for *G. p. gambiensis*, as detailed in Chapter 4. Our findings did not reveal a significant positive impact on either induced sterility or flight quality parameters. Only a slight improvement in flight propensity and survival under feeding regime was observed when the optimal dose was divided into 50 Gy and 60 Gy, administered with a 1-day interval. Interestingly, a prior study involving X-rays on the boll weevil, *Anthonomus grandis,* reached a similar conclusion, stating that radiation fractionation is not promising, as it led to a reduction in the lethal and sterilizing effects of X-rays [183]. Correlating these observations with induced mutations, it has been demonstrated that fractionation does not decrease the frequency of lethal mutations [184]. Contrastingly,

divergent results have been reported, indicating a potential enhancement in the quality of irradiated males with the use of fractionated radiation doses [128,185]. This variability might be attributed to species specificity, life stage-related factors, or additional treatments, as observed with *G. tachinoides* [186]. The hormetic effects of dose splitting as part of the continuous improvement of irradiation protocols, need further investigation considering the associated constraints. While marginal benefits have been observed, the increased workload due to dose splitting, repeated operator exposure, and constraints in sorting and transport [123,134,187] suggest that further study, possibly in combination with other potential hormetic treatments, is necessary before contemplating its implementation in operational programs. This raises the question: what would be the effect of modifying the atmosphere or temperature of the irradiation chamber on *G. p. gambiensis* in terms of improving the performance of sterilized males?

Chapter 5 focused on assessing the impact of gamma rays and X-rays on male *G. p. gambiensis* when irradiated as pupae and adults under a nitrogen-enriched atmosphere or exposed to low temperature. Irradiating *G. p. gambiensis* pupae under a nitrogen-enriched environment using gamma- and X-ray sources, revealed an increased radiation dose requirement to induce 95% sterility compared to the exposure in air (Chapter 3). Importantly, these findings are consistent with the observations in *Glossina pallidipes* species [188]. Remarkably, the nitrogen treatment demonstrated a radioprotective effect, echoing similar outcomes observed in various insect species such as, *Glossina tachinoides*, *Ceratitis capitata*, *Culex quinquefasciatus*, *Aedes aegypti* and *albopictus*, as documented in previous studies [162,180,182,186,189]. The increase in radio-tolerance is attributed to a reduction in irradiation-induced oxidative damage, as evidenced previously [180,190]. Furthermore, the higher radiation dose required for achieving sterility may significantly impact the regulation of antioxidant enzymes [191]. Noteworthy is the observation that a radiation dose of 115 Gy was sufficient with Rad Source 2400, in contrast to 135 Gy for Foss Model 812. This indicates a similar increase of 25 Gy for both sources between air and nitrogen radiation. This distinction reinforces and validates the findings regarding the differing effects of X- and gamma-rays, as elucidated in Chapter 3. Significantly, there is a discernible increase in the fly's longevity, implying potential enhancements in their performance in the field. Examining the potential performance of sterilized males under hypoxic conditions, ongoing additional experiments in both laboratory and semi-field conditions related to this chapter will play a crucial role in shaping the future of low oxygen-based irradiation of tsetse.

When modified environment effects during the irradiation process are considered, our results in Chapter 5, related to the impact of the cold treatment on male *G. p. gambiensis* either irradiated as pupae or adults, did not show any protective effect on induced sterility when using the new X-RAD 320 Precision technology that incorporate a cooling system. However, displaying a dose-response curves using a range of doses data revealed the suitability of the new X-ray device, with approximately 75 Gy and 90 Gy for chilled and non-chilled pupae and 90 Gy for both treatment in adults. Under both chilling and room temperature conditions, no significant difference in radiosensitivity was observed between pupae and adults, despite the pupae exhibiting a slight sensitivity at lower doses. This similarity in sensitivity could be attributed to the closer age of the irradiated life stages; indeed, the pupae, at 25 days old, may be biologically similar than the teneral flies used as adults. In addition, a similar lower radiation dose was required to induce the acceptable level of sterility as revealed in Chapters 2 and 3 with X-ray sources. These findings stand in contrast to those of a previous study, which uncovered that temperature significantly influences the dosage of radiation necessary to sterilize certain stored product pests, including *Sitophilus zeamais* in corn, *Sitophilus oryzae* in rice, *Araecerus fasciculatus* in coffee, and *Zabrotes subfasciatus* in beans [192]. In the case of tsetse, most studies involving low-temperature environments have focused on assessing their effects on male performance, often using a single dose, which does not allow for direct comparison [125,127]. However, the study on induced sterility in *G. p. gambiensis*, chilled at 8 °C prior to radiation, resulted in a higher required dose, 120 Gy for adequate sterilization compared to the 110 Gy needed for irradiation under room temperature [142]. It is worth noting that no dosimetry system was applied in this study. Once again, the dosimetry system revealing a lower absorbed dose in chilled conditions compared to non-chilled conditions in our study suggest potential errors in the actual absorbed doses due to the cold treatment. Nevertheless, a study has demonstrated that gafchromic films are now considerably less sensitive to temperature changes during exposure [193]. Despite this improvement, it is important to acknowledge that gafchromic radiation films may still exhibit sensitivity to variations in both temperature and humidity during irradiation and a careful consideration and specific corrections are needed [194]. The results indicating a longer survival of adults compared to pupae under different irradiation temperatures raise questions about the selection of the appropriate biological stage for irradiation in SIT programs aimed at tsetse eradication. Both life stages have been already used in successful campaigns. During the implementation of the project that led to the eradication of *G. austeni* on the island of Unguja (Zanzibar), adult stage were irradiated locally and released [83,195] whereas for Senegal tsetse eradication project,

pupae were irradiated [123,134]. Indeed, technical limitations complicate the feasibility of choosing the appropriate life stage for irradiation. One of these constraints is the geographical distance between the mass rearing facilities and the sites where sterile insects are released, as observed in numerous SIT projects, notably the *G. p. gambiensis* eradication project in Senegal. In this instance, pupae were provided by three external mass rearing centers situated in Burkina Faso, Austria, and Slovakia. This setup underscores the necessity to harmonize technical/scientific requirements with logistical considerations during the planning and execution of SIT programs.

Considering the experimental design in Chapters 3, 4 and 5, combining induced sterility and flight quality and/or mating competitiveness, would be interesting to be standardized, for dose-response studies. This seems to be commonly used in tsetse as indicated in previous studies [119,135,142,144] but is also implemented on other insect species [104,110,167]. However, feasibility studies for the use of the SIT or the suitability of irradiators have been limited, for various reasons. These studies often focus solely on the potential of inducing sterility without considering flight quality and mating parameters [196–198]. The significance of flight quality and mating parameters indicates that investigating induced sterility separately could be inefficient in terms of time and resources. In summary, the results underscore the importance of automatically incorporating specific product quality parameters, such as flight propensity, survival rate, and mating competitiveness, into dose-response studies. This practice is crucial for establishing radiation protocols, assessing the suitability of irradiation sources, and investigating the feasibility of implementing the SIT against emerging pest candidates [199]. Adopting this approach not only streamlines resource utilization and saves time but also expedites decision-making processes.

In the broader context of our discussion, the choice of irradiation source or radiation protocol in alignment with the characteristics of the sources or the radiation chamber environment, may be deemed acceptable solely from a scientific perspective, aiming to achieve the desired sterility for SIT. However, it becomes imperative to also consider regulatory concerns and the cost-effectiveness of the program. In the realm of SIT, success and challenges are intertwined, and primary constraints extend beyond scientific considerations. Factors such as the commitment of policy/decision makers or governments to the programs, the methods associated with the AW-IPM strategy, and broader implications for stakeholders play pivotal roles. A case in point is Burkina Faso, where the PATTEC project sought to eradicate the *G. p. gambiensis* species in the Mouhoun region by integrating bait and aerial insecticide spraying

technologies with SIT in an AW-IPM approach with the assistance of the beneficiary communities [57]. Despite achieving a significant 95% reduction in tsetse density, the project fell short of complete eradication. A major contributing factor was a shortage of financial resources towards the project's conclusion, originally funded by the African Union. Local funds mobilized were predominantly allocated to building and maintaining the insectary, a task completed just as the suppressed zone faced reinfestation. Addressing the lack of financial resources involves political engagement, but garnering support from decision-makers hinges on reducing the cost and complexity of SIT implementation. Among the drivers influencing government commitments, cost-effectiveness, and complexity in terms of feasibility studies and implementation are paramount. Therefore, prioritizing the availability of suitable X-ray sources based on their cost, safety, and efficiency becomes crucial. From a scientific perspective, a primary concern in the implementation of SIT revolves around generating a substantial quantity of high-quality sterile males. Knippling [77] emphasized the necessity of economically mass-rearing pest species at a low cost as a crucial prerequisite for successful SIT programs. The quality of sterilized males is influenced by the entire system, spanning from the colonization of the species, through the sterilization process, to the release system. Therefore, it is crucial to incorporate quality control tests at critical points throughout the entire process. Classical quality tests, especially focusing on emergence, flight, survival, and mating competitiveness, are essential but may be insufficient. In the context of irradiation, establishing a dosimetry system is crucial to ensure that the radiation doses absorbed by the exposed insect life stage correspond to the targeted doses, whether for research purposes or in operational programs. Ultimately, comprehensive capacity building among committed human resources, simultaneously addressing both research and operational domains, emerges as a pivotal catalyst [200]. This approach not only effectively addresses prevailing challenges but also drives continuous advancements in the integrated management of insect pests.

Above all, when considering the various aspects associated with the implementation of the SIT, it is crucial to emphasize that this technology cannot be implemented in isolation. Instead, it should be integrated with other control methods, such as the use of insecticides through ground and aerial spraying or insecticide bait technology, especially in the context of combating tsetse. It is however crucial that the technical and economic feasibility to integrate the SIT as a component for AW-IPM should be assessed before SIT is considered for a tsetse species and intervention area. Furthermore, if SIT is found not to be feasible for a specific tsetse species and intervention area, it should not be considered a failure, it is just an indication

that it is not suitable for this specific situation. It is imperative to recognize that within the perspective of AW-IPM, especially against tsetse, it is crucial to assess whether the inclusion of an SIT component is appropriate. Acknowledging that SIT may not always be feasible enables the conservation of resources and time, redirecting them towards alternative control methods, particularly in the context of tsetse management. Achieving this goal necessitates not only securing commitment from policymakers but also convincing them through scientific and technical evidence derived from the collection and analysis of baseline data.

While implementing SIT for tsetse control may present challenges, adopting approaches such as the phased conditional approach (PCA) or the Progressive Control Pathways (PCP) provides systematic frameworks for planning and evaluating interventions [22,200,201]. This strategic approach proves to be a resilient solution, not only saving both time and money but also enhancing the visibility and effectiveness of the intervention.

2. Conclusion and perspectives

This thesis aimed to advance the SIT by exploring alternative radiation sources and improving existing radiation protocols. The results underscore the suitability of three X-ray sources—Raycell Mk2, Rad Source 2400, and X-Rad 320—for the sterilization of tsetse flies. These findings may also be applicable to other targeted species such as fruit flies and mosquitoes, particularly in the context of the sterile insect technique. In comparison with the widely used gamma-rays under the same laboratory and semi-field conditions, no significant differences were observed in terms of induced sterility and the radiation impact on the quality of sterile males. Consequently, this thesis serves as a proof of concept, supporting recommendations for the use of X-ray sources in in current and upcoming SIT programs, with considerations for further needs of refinement and constraints. Additionally, the results indicate that existing irradiation protocols based on gamma-rays could be optimized through radiation dose fractionation and irradiation under modified atmospheric and temperature conditions. However, given the current results, the workload, pupae sorting, and shipment constraints associated with dose fractionation, an immediate recommendation may not be warranted. Looking ahead, evaluating the impact of X-ray radiation under these conditions on the flight and mating competitiveness could definitively influence the decision-making process. The ongoing irradiation conducted within a nitrogen-enriched environment in the course of this thesis appears to hold promise for enhancing the quality of sterilized flies, presenting a clear avenue for further exploration and standardization. This perspective is also applicable to the cold treatment concerning the flight/mating quality parameters of the sterile males since a lower radiation dose is needed under chilling conditions to achieve the necessary level of sterility. In addition, there are additional perspectives that may support the suggested use of X-ray sources and evaluated irradiation protocols. Investigating the impact of radiation on protein expression, especially those associated with immunity or defence against radiation stress, could offer valuable insights, contributing to the validation of both current and future radiation protocols. Additionally, investigating the impact of these irradiation protocols on tsetse symbionts, which can influence insect vector competency or refractoriness, has the potential to anticipate negative side effects. This approach could expedite the validation and widespread adoption of these methods. Finally, it should be recognized and implemented, as it is currently ongoing, a reliable dosimetry system which is essential in both research and operational programs. In conclusion, in light of these significant findings and the ongoing implementation of dosimetry

systems, the integration of X-ray sources and refined protocols not only emphasizes their potential to advance current SIT programs but also underscores the imperative for further exploration. In addition, the utilization of E-beam radiation sources, which encounter fewer constraints compared to gamma radiation, may indeed serve as inspiration for further trials involving tsetse flies. These perspectives will ensure a robust and effective approach to insect population control strategies in the future, building on the knowledge gained in this thesis.

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Curriculum vitae

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EDUCATION

- ✓ **Ph.D. in Natural Sciences**, ***Vienna University of Technology*** [August 2022 – Now] / Vienna, Austria
- ✓ **Ph.D. in Tropical Animal Health,** ***Nazi Boni University*** [17/12/2020 – 27/10/2023] / Bobo-Dioulasso, Burkina Faso
- ✓ **MBA in Project Management,** ***Institute of Management*** [15/09/2015 – 22/05/2017] / Dakar, Senegal
- ✓ **MSc in Animal Production and Sustainable Development,** ***Inter-States School of Medicine and Veterinary Sciences of Dakar*** [01/10/2015 – 04/06/2017] / Dakar, Senegal
- ✓ **Doctorate in Veterinary Medicine,** ***Inter-States School of Medicine and Veterinary Sciences of Dakar*** [01/10/2010 – 04/06/2016] / Dakar, Senegal

PROFESSIONAL EXPERIENCE

Consultant

Joint FAO/IAEA Insect Pest Control Laboratory [02/08/2021 – Current] / Vienna, Austria

Director of Integrated Pest Management Department

Insectarium of Bobo-Dioulasso-Tsetse and trypanosomosis eradication campaign / National veterinary services [13/12/2019 – 30/07/2021] / Bobo-Dioulasso, Burkina Faso

Research Officer

Technical Secretariat of beekeeping / National veterinary services [30/12/2016 – 12/12/2019] / Ouagadougou, Burkina Faso

Consultancies

- ✓ ***Feasibility study for establishing an apiary at the school application farm***, *National School of Livestock and Animal Health* [04/01/2018 – 10/01/2018] / Ouagadougou, Burkina Faso

- ✓ ***Techniques for controlling African animal trypanosomosis and their vectors***, *Anti-tsetse school* [29/11/2020 – 04/12/2020] / *Bobo-Dioulasso, Burkina Faso*
- ✓ ***Training on beekeeping basics and techniques for sampling, conservation, and diagnosing bee pathologies***, *West Africa Competitiveness Program/ Technical Secretariat of Beekeeping [06/04/2020 – 10/104/2020] / Bobo-Dioulasso, Burkina Faso*

Temporary Lecturer

- ✓ **University of Ziniaré** and **University of Tenkodogo** [2019-2021], Ziniaré, Tenkodogo, Burkina Faso
- ✓ **National School of Livestock and Animal Health**, Ouagadougou, Burkina Faso
- ✓ **Private Schools of Livestock and Animal Health,** various locations, Burkina Faso
- ✓ **Inter-states School of Medicine and Veterinary Sciences of Dakar** [02/11/2015 – 31/07/2016] / Dakar, Senegal

TRAININGS

- ✓ **Current and Emerging Threats to Crops Innovation Lab Project Management Training Course**, Feed the Future - U.S. Government's global hunger & food security initiative [May 2022], Online.
- ✓ **Scientific writing in English course**, FAO/IAEA [September 2021] / Seibersdorf, Austria
- ✓ **Basics in epidemiology of animal and zoonotic diseases,** Centre International de Recherche Agronomique pour le Développement [04/11/2019-29/02/2020], Online.
- ✓ **Training in strategic planning and results-based project/program monitoring and evaluation**, New Vision Consulting SARL [12/12/2019-18/12/2019], Ouagadougou, Burkina Faso
- ✓ **Training on bee health: sampling and diagnosis techniques**, NGO Wendpuiré [17-23/11/2019], Koudougou, Burkina Faso.
- ✓ **Gender and rural development: tools, analysis and actions,** Joseph KI-ZERBO University of Ouagadougou [October 2018] / Ouagadougou, Burkina Faso0
- ✓ **Training on "Bees and environment": Bee biology, Bees and environment, Beekeeping practices, Bee health, Beekeeping sector**, National Veterinary, Food and Nutrition School (ONIRIS)/France Digital University [23/04/2019-04/06/2019] / Online.

SCIENTIFIC PUBLICATIONS AND COMMUNICATIONS

Articles

1. Pagabeleguem S, Somda BDE, Dera KM, Barro D, **Kaboré BA**, Toé AI Belem AMG & Ouedraog, GMS, 2024. Assessing the impact of COVID-19 on high-workload facilities: a case study of *Glossina palpalis gambiensis* productivity at the insectarium in Bobo-Dioulasso, Burkina Faso. *Sciences de la vie, de la terre et agronomie*, **11**(2).

2. **Kaboré BA**, Taqi SD, Mkinga A, Morales Zambrana AE, Mach RL, Vreysen MJB & de Beer CJ, 2024. Radiation dose fractionation and its potential hormetic effects on male *Glossina palpalis gambiensis* (Diptera: Glossinidae): a comparative study of reproductive and flight quality parameters. *Parasite* 31, 4. https://doi.org/10.1051/parasite/2024001

3. Minougou N, **Kaboré BA**, Savadogo M, Dahourou LD, Dera KM, Teko-Agbo A, 2023. Assessing the prevalence of aflatoxin b1 in poultry feed in Dakar, Senegal: implications for animal and public health. *Int. J. Biol. Chem. Sci.,* **17**(7): 2677-2688. DOI: https://dx.doi.org/10.4314/ijbcs.v17i7.7

4. **Kaboré BA**, Nawaj A, Maiga H, Soukia O, Pagabeleguem S, Ouédraogo/Sanon MSG, Vreysen MJB, Mach RL, de Beer CJ, 2023. X-rays are as effective as gamma-rays for the sterilization of *Glossina palpalis gambiensis* Vanderplank, 1911 (Diptera: Glossinidae) for use in the sterile insect technique. *Sci Rep*, 13:17633. https://doi.org/10.1038/s41598-023-44479-8

5. Dera KM, **Kaboré BA**, Pagabeleguem S, Ouedraogo AZ, Toé AI, Ira M, Belem AMG, Ouedraogo/Sanon GMS, 2023. Influence of environmental conditions on mass rearing parameters of tsetse flies at the Bobo-Dioulasso insectary (Burkina Faso): retrospective study. Int. J. Biol. Chem. Sci. 17(3): 1020-1032. https://doi.org/10.4314/ijbcs.v17i3.21

6. **Kaboré B. A., 2023.** Securing the Future of Beekeeping in West Africa: A Call for Robust Research and Sanitary Surveillance to Safeguard Bee Health and Sustainability. *Agri Res & Tech: Open Access J.,* **27**(4): 556382. DOI:10.19080/ARTOAJ.2023.27.556382

7. Dahourou LD, Bonkoungou L, Ouedraogo WA, Zangre H, **Kabore BA**., Tapsoba AS, & Traore A, 2023. Prevalence and risk factors associated with subclinical mastitis in peri-urban bovine dairy farms of Ouagadougou and Bobo Dioulasso in Burkina Faso, West Africa. *Iraqi Journal of Veterinary Sciences*, **37**(4), 831-837. https://doi.org/10.33899/ijvs.2023.135679.2501

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2. **Kaboré BA**, de Beer CJ, 2024. Advancing the Sterile Insect Technique for tsetse (Diptera: Glossinidae): Exploring alternative radiation sources and protocols, Joint FAO/IAEA Insect Pest Control Laboratory Semir, 29 February 2024, Vienna, Austria
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