DEVELOPING ALTERNATIVES TO GAMMA IRRADIATION FOR THE STERILE INSECT TECHNIQUE

I-1. Introduction

Almost all insect pest control programmes currently releasing sterile insects as part of the area-wide integrated application of the sterile insect technique (SIT) use radioisotope irradiators that are loaded either with cobalt-60 or caesium-137, which produce ionizing radiation consisting of gamma rays. Irradiators such as the Gammacell 220 have proven to be extremely reliable for the purpose of achieving sterilization of target insect pests. However, the growing logistical complexities of the transboundary shipment of radioisotopes and the fear of terrorism are making the reloading of existing sources, the acquisition of new ones and their shipment to Member States across international borders increasingly difficult.

The situation was exacerbated in 2006, when the production of the Gammacell 220, the source most commonly used for irradiating insects for sterilization purposes was discontinued. Agency requests already issued to procure Gammacell 220 units for various technical cooperation projects were no longer honoured, thereby jeopardizing the implementation of many large on-going and new SIT programmes in Member States which depend on these units. The purpose of this annex is to provide an overview of efforts to develop alternatives to the use of gamma irradiation for SIT applications.

I-2. Search for alternative non-gamma radiation sources

In response to these setbacks, the Agency initiated efforts in the mid-2000s to explore other options to sterilize insects for use in insect pest management programmes with an SIT component. Alternative technologies using low-energy X-ray irradiation had at this time already been under development. Low-energy X-ray irradiators emit X-rays only when the electrical power is turned on, and consist of an X-ray tube and a device to transport the insect canister through the X-ray beam. The X-ray tube consists of an electron source, generally a heated wire filament which acts as the cathode and emits electrons, and a high-atomic number target material as the anode from which X-rays are generated. The electrons emitted by the cathode are not additionally accelerated, which means that no large and costly accelerators are needed, and because the energy is in the range of a few hundred keV, this requires much less shielding than in the case of gamma irradiators and allows the unit to be self-contained.

In view of the high demand in Member States for sterilization hardware, technical advice and training, the Agency procured a low-energy X-ray machine from a company that has a patent on the design of the X-ray tube used. Over the last years, the Agency has adapted, improved and validated the machine for insect sterilization.

FIG. I-1. X-ray irradiator (foreground) and water cooling unit (background).

I-3. Self-contained low-energy X-ray irradiators

For the past several years, self-contained low-energy X-ray irradiators have been marketed for the specific purpose of blood irradiation (which requires a dose of about 25 Gy), and between 50 and 100 units are operating successfully at hospitals and medical institutes across North America. Following this success, a research irradiator with a radically different tube was developed, with a canister volume of about 1.5 litres and a dose rate of about 5 Gy/min, which is relatively low for insect irradiation (requiring a dose of about 100 Gy) on a commercial basis. Such irradiators have been upgraded in the last five years to yield dose rates of up to 100 Gy/min. This was achieved by changes in the design of the X-ray tube, allowing a much higher power dissipation and improved dose uniformity.

These irradiators can be further configured (at additional cost) to address the requirements of the programme/customer in terms of dose and throughput. However, the X-ray irradiator that was procured for the Insect Pest Control Laboratory of the FAO/IAEA Agriculture and Biotechnology Laboratories in Seibersdorf, Austria, was an off-the-shelf product. It was delivered with a maximum energy of 150 keV and a variable current of 0–35 mA for a maximum total power requirement of just over 5 kW. It was supplied with its own external passive water cooling unit to remove the waste heat from the X-ray tube.

This unit had one horizontal X-ray tube and five horizontally aligned irradiation canisters made of cardboard that rotate around the irradiation tube. Such a configuration consumes 5.3 kW during operation and has a capacity to sterilize up to 20 litres of insects with 150 Gy in five minutes, or to decontaminate 10 litres of blood as diet for tsetse flies with 1 kGy in 30 minutes.

I-4. Development and validation

The two main issues arising initially with the above X-ray irradiator were related to reliability and dose rate and distribution. The X-ray tube supplied with the machine failed repeatedly when the high voltage arced across the face of the insulator in the base of the tube. This caused the voltage from the high voltage power supply to collapse, without the possibility of recovery due to the conducting path formed by the arc. The only solution was to replace the sealing washer and silicone grease and remake the connection. This took approximately 30 minutes. However, each time an arc occurred, it caused further damage to the insulator, making it more likely that a new arc would form. After several such instances it became impossible to use the tube.

Consequently, the first X-ray tube was replaced with a modified tube which had better cooling water flow around the tube, as well as an improved process for easier tube installation and exchange. This tube can now be operated at up to 45 mA rather than 35 mA. This increased the power output of the tube by about 28% with a corresponding increase in the dose rate. This tube has been in operation since 2009 without any problems, thereby confirming its greater reliability.

FIG. I-2. Detail of X-ray tube end cap insulator showing damage caused by high voltage arcing.

The second issue was related to dose distribution and dose rate. The machine was delivered with an original specification of a dose rate of 45 Gy/min, but the measured dose rate at the centre of a 180 mm diameter canister was only about 16 Gy/min. Further, and more importantly, the dose distribution within this volume was very poor, with the ratio of maximum to minimum dose (dose uniformity ratio (DUR)) being about 6. In a normal production environment, a DUR of 1.4 would be considered as acceptable.

The dose rate and DUR will vary with the diameter of the canister, the density of the material being irradiated and its distance from the tube. The density of the material cannot be changed, but the other two factors can. Dose rate and DUR will both improve (dose rate increase, DUR decrease) in a smaller diameter canister, whilst dose rate will decrease but the DUR will improve when the canister is further from the X-ray tube. A 120 mm diameter canister was placed as far from the X-ray tube as the machine permitted, giving a spacing of about 70 mm. In this configuration the central dose rate was about 20 Gy/min and the dose uniformity was greatly improved, with a DUR of about 1.6. The central region had a very good dose distribution (±5% in the central region of diameter 7 cm and length 10 cm) and the dose increased only slowly towards the outside of the canister (+15%), but the fall in dose towards the ends was much greater (-25–30%). To obtain the desirable DUR of 1.4 or better would require blocking off about 2 cm from each end of the canister to avoid the lowest dose areas, leaving a volume of just over 1.5 litres.

The smaller canister size (down from the original volume of four litres) is an important issue. It would increase the number of handling operations because it would require many smaller batches of insect pupae. As the dose only rises slowly around the centre line of the cylindrical canister, the working volume may be increased by increasing the diameter whilst reducing the length still further to obtain the desired DUR. Therefore a 160 mm diameter canister was tested, with thicker end walls to increase scatter. Based on the above it was estimated that a volume of three litres or more may be possible, and with the increased spacing from the X-ray tube it should be possible to accommodate six or eight canisters per irradiation.

I-5. Improving dose uniformity

It was realized that one of the causes of the poor dose uniformity was that X-rays, unlike gamma radiation from a cobalt-60 source, contain a wide energy spread, from about 30 to 150 keV. In the case of an X-ray irradiator, the low-energy X-rays (photons) deliver a high dose at the surface near the entrance of the canister, resulting in a high DUR. Thus, the DUR could be decreased by 'hardening' the X-ray energy spectrum by removing the low-energy photons before they reach the canister containing the pupae. A metal jacket around the canister can easily absorb these photons. The DUR for the bare canister (without metal surround) was 1.21 (considering only the direction along the diameter). Surrounding the canister with a jacket of brass 1 mm thick made this ratio almost unity. However, it also reduced the dose rate in the central region by about 70%. On the other hand, surrounding the canister with a 0.5 mm steel jacket resulted in a DUR of about 1.06, and the dose rate reduction was only 40% as compared to that for the bare canister. Thus, for hardening the X-ray spectrum in the irradiator, it is recommended to use canisters with a 0.5 mm steel jacket.

The axial and radial dose distributions for the final geometry of the canister were also determined. These measurements were made with all five canisters full of instant rice (which behaves very similarly to insect pupae under irradiation). Two separate runs were made; one for axial dose distribution and the other for radial dose distribution. For the axial run, three 20 cm-long Gafchromic® films were placed within the canister along its length: one in the centre (along the axis) and two near the periphery (laid on the curved surface). For the radial run, two 18 cm-long Gafchromic® films were placed perpendicular to each other and both along the diameter going through the centre of the canister. For both runs, the irradiator was operated at 150 kV and 17.5 mA for 20 min, with a rotation speed of 5 rpm. Low current was selected so that there would be several revolutions of the canisters, meaning that the dose distribution is not significantly affected by the last, incomplete revolution. For these measurements, the canister was 20 cm long. To achieve a DUR of 1.3, the canister was shortened to 15 cm, yielding a volume of just over 3.7 litres. Thus 18 litres of pupae can be irradiated per batch.

FIG. I-3. Improved canister design with internal 0.5 mm steel filter.

The dose rate in the centre of the canister filled with instant rice was about 14 Gy/min. When the canister was filled with fruit fly pupae the dose rate decreased by 6%. This dose rate was measured by a Farmer type (0.18 cm^3) ionization chamber which was calibrated in the energy range of 40 keV to 1.33 MeV with traceability to the National Institute of Standards and Technology (NIST) in the USA. Such a reference dosimetry system is essential for the calibration of the routine Gafchromic® dosimetry system.

The machine also had to be significantly modified in terms of an improved carousel system and new canisters, as well as some other changes. The new carousel system allowed more precise alignment of the canisters with the X-ray tube and has made the canisters much more secure so that they should now not be able to become dislodged. The canisters themselves are now made of carbon fibre reinforced resin, which is lightweight,

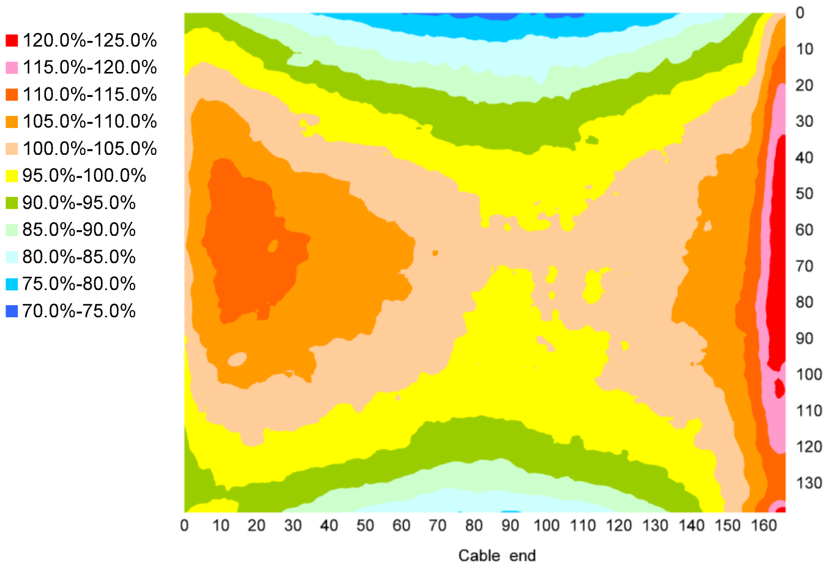

FIG. I-4. Dose distribution map in a canister with 0.5 mm steel filter.

waterproof and almost transparent to X-rays, and have the steel filtration incorporated inside. The length and longitudinal positioning of the canisters has been adjusted to give a good DUR of less than 1.3. Various inserts have been developed such as for the irradiation of mosquito pupae.

In addition, the software used to operate the X-ray machine had to be revised to permit the selection of a predetermined energy rather than a fixed power and time, and to protect specific sections of the program with a password to prevent unintended modification.

FIG. I-5. Canister insert for irradiation of mosquito pupae showing the nested trays used to hold the pupae in a minimum quantity of water.

I-6. Development of dosimetry

During this development work it became apparent that there were also problems with the Gafchromic® routine dosimetry system. The standard procedure used during cobalt-60 irradiation does not work for X-ray irradiation, as the Gafchromic® film is much more sensitive at these low energies to the material surrounding it, in the range of a few hundred micrometres. Extensive tests have clarified the necessary conditions for using Gafchromic® film dosimeters with the X-ray machine, the principal one being that the film must always be enclosed in the same material. The recommendation is to use standard dosimeter envelopes made out of paper. A separate calibration from that used with gamma irradiation is also required.

During this improvement/validation process it turned out that the existing Standard Operating Procedure for Gafchromic® Dosimetry needs revision for low energy X-radiation. Consequently a new SOP was prepared and a new version of the original SOP specifically for gamma radiation using cobalt-60 or caesium-137 has also been prepared. In order to further enhance the dosimetry and to simplify the calibration of X-ray sources, the Agency, in collaboration with Centro Estrategico de Pesquisa, Tecnologia e Inovacao – CETECBR in Pernambuco, Brazil, will continue to work on developing and characterizing an alanine/electron spin resonance (ESR) dosimetry system to use as a transfer standard dosimeter, and to establish a dosimetry service in Member States.

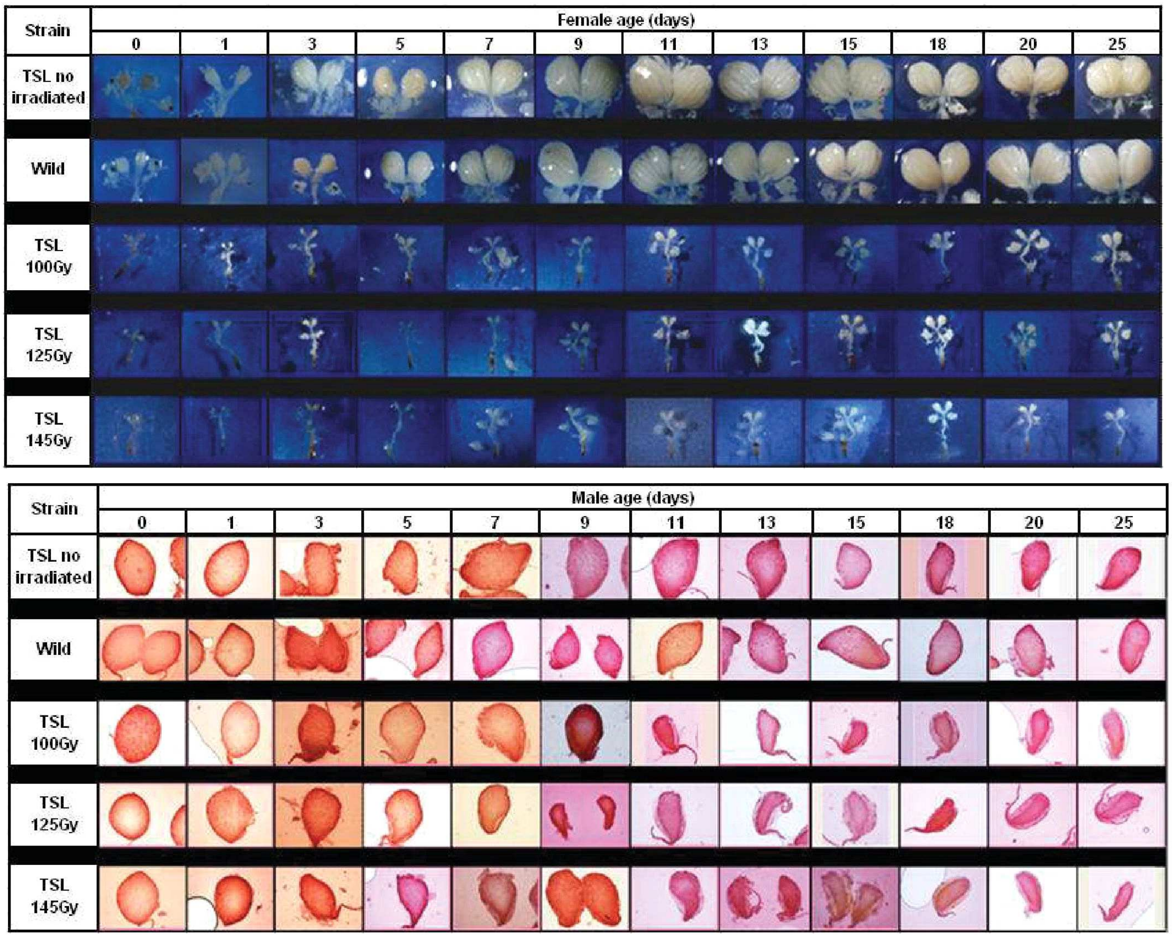

FIG. I-6. Effect of gamma irradiation (100, 125 and 145 Gy) and no irradiation on female (upper) and male (lower) reproductive systems of the Mediterranean fruit fly (comparing wild flies and temperature sensitive lethal (tsl) genetic sexing strain developed by the Agency).

I-7. Biological efficacy

Trials have been carried out to determine the differing effects, if any, between sterilization of insects with ionizing energy sourced from cobalt-60 (gamma irradiation) or from an X-ray source. As the FAO/IAEA Agriculture and Biotechnology Laboratories in Seibersdorf have both types of irradiators on-site it is in a unique position to test for any differences in the effectiveness of the two systems with regard to their use in SIT programmes. Three fruit fly pest species were assessed: the South American fruit fly *Anastrepha fraterculus,* the melon fly *Bactrocera cucurbitae*, and the Mediterranean fruit fly *Ceratitis capitata*. Pupae of the same age were irradiated at the same nominal doses in either the X-ray machine or a gamma irradiator and their quality assessed under the same conditions. Dosimetric procedures conducted after treatment determined actual doses received by the pupae. Figure I-6 shows the effects of radiation which can be seen when dissecting the male and female reproductive organs of irradiated fruit flies, in particular the deterioration of ovaries in females and of spermatogenesis in males. Tests on the treated and untreated insects included standard quality control procedures for sterile and fertile males and females. Eggs from pairings of irradiated males with non-irradiated female flies gave a measure of the residual fertility of the insects treated with either gamma rays or X-rays (see Table 1). For each sterility level calculated (50%, 90% and 99%), the relative biological effectiveness (RBE) of gamma radiation did not differ significantly from that of X-rays, and adult emergence rates and mating competitiveness between gamma ray and X-ray treated males competing for fertile females in field cages have so far revealed no significant differences.

Table 1. Comparison of gamma and X-radiation doses (Gy) calculated from the linear regression equations of Probit sterility on log dose for selected sterility levels and their estimated RBE values

Pest species	Treatment	D_{50}[a]	RBE[b]	D_{90}	RBE	D_{99}	RBE[b]
C. capitata	X-rays	20.4 (17.9; 23.2)[c]	1 (0.8; 1.1)	46.8 (41.5; 54.1)	1 (0.9; 1.1)	91.2 (83.6; 101.3)	1 (0.9; 1.1)
	γ-rays	27.9 (22.9; 33.9)	0.7[ns,d] (0.5; 1.0)	63.8 (53.9; 75.8)	0.7[ns] (0.4; 1.0)	124.9 (94.9; 160.9)	0.7[ns] (0.5; 1.0)
A. fraterculus males	X-rays	13.0 (9.02; 18.8)	1 (0.8; 1.3)	23.5 (18.8; 29.8)	1 (0.9; 1.2)	37.8 (27.7; 52.1)	1 (0.8; 1.2)
	γ-rays	7.6 (5.3; 11.1)	1.7[ns] (0.4; 6.9)	18.2 (15.3; 21.9)	1.2[ns] (0.3; 5.1)	36.3 (27.8; 49.4)	1.04[ns] (0.3; 4.1)
A. fraterculus females	X-rays	27.1 (17.7; 41.4)	1 (0.7; 1.4)	41.2 (25.4; 68.3)	1 (0.7; 1.4)	57.8 (30.1; 109.7)	1 (0.6; 1.6)
	γ-rays	23.8 (15.1; 36.7)	1.1[ns] (0.3; 4.4)	38.6 (21.3; 71.2)	1.1[ns] (0.3; 4.2)	57.3 (25.7; 125.4)	1.01[ns] (0.2; 4.4)

[a] D = dose (Gy) that induces 50%, 90% or 99% sterility.
[b] RBE = relative biological effectiveness (relative to X-rays).
[c] Confidence interval stated at 95% confidence level.
[d] When the confidence interval includes the value 1 for D50, D90, or D99 RBE, then the D50, D90, or D99 values are not significantly different ($P > 0.05$; ns, not significant).

I-8. Conclusion

Agency efforts to develop alternatives to the use of gamma irradiation in SIT applications have succeeded, as shown by the similar radiation effects obtained for insects irradiated with gamma rays and with X-rays. It is hoped that these results will stimulate the development of more X-ray based irradiation systems in order to increase competition and bring down the prices of equipment used in Member States. After completing the characterization of the X-ray irradiator, the Agency ordered several units for use in Member States. These new machines incorporate all the changes and modifications that were identified during the testing phase at the FAO/IAEA Insect Pest Control Laboratory in Seibersdorf, Austria. One unit has now been operating without any problems for several months in a fruit fly SIT programme in Brazil, another was installed in Costa Rica in late 2011, and additional units will shortly be installed in Burkina Faso and Pakistan. Nevertheless, it will take several years to collect sufficient data to assess whether this really is a viable alternative to the sterilization of insects at mass rearing factories under routine large-scale operational conditions.

REFERENCES

MASTRANGELO, T., PARKER, A.G., JESSUP, A., PEREIRA, R., OROZCO-DÁVILA, D., ISLAM, A., DAMMALAGE, T., WALDER, J.M.M., A new generation of X ray irradiators for insect sterilization, Journal of Economic Entomology **103** 1 (2009) 85–94.

MEHTA, K., Radiation sources supporting the use of natural enemies for biological control of agricultural pests, Biocontrol Science and Technology **19** S1 (2009) 335–362.

MEHTA, K., PARKER, A., Characterization and dosimetry of a practical x-ray alternative to self-shielded gamma irradiators, Radiation Physics and Chemistry **80** (2011) 107–13.