CHAPTER 3.3.

STERILIZING INSECTS WITH IONIZING RADIATION

A. BAKRI[1,2], K. MEHTA[3] AND D. R. LANCE[4]

[1]Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Wagramer Strasse 5, A-1400 Vienna, Austria
[2]Current Address: Unit of Biological Control of Insects, Faculty of Science Semlalia, University Cadi Ayyad, Marrakech 40000, Morocco
[3]Brünner Strasse 133-3-29, A-1210 Vienna, Austria
[4]USDA/APHIS/PPQ, Bldg. 1398, Otis ANGB, MA 02542, USA

TABLE OF CONTENTS

SUMMARY

Exposure to ionizing radiation is currently the method of choice for rendering insects reproductively sterile for area-wide integrated pest management (AW-IPM) programmes that integrate the sterile insect technique (SIT). Gamma radiation from isotopic sources (cobalt-60 or caesium-137) is most often used, but high-energy electrons and X-rays are other practical options. Insect irradiation is safe and reliable when established safety and quality-assurance guidelines are followed. The key processing parameter is absorbed dose, which must be tightly controlled to ensure that treated insects are sufficiently sterile in their reproductive cells and yet able to compete for mates with wild insects. To that end, accurate dosimetry (measurement of absorbed dose) is critical. Irradiation data generated since the 1950s, covering over 300 arthropod species, indicate that the dose needed for sterilization of arthropods varies from less than 5 Gy for blaberid cockroaches to 300 Gy or more for some arctiid and pyralid moths. Factors such as oxygen level, and insect age and stage during irradiation, and many others, influence both the absorbed dose required for sterilization and the viability of irradiated insects. Consideration of these factors in the design of irradiation protocols can help to find a balance between the sterility and competitiveness of insects produced for programmes that release sterile insects. Many programmes apply “precautionary” radiation doses to increase the security margin of sterilization, but this overdosing often lowers competitiveness to the point where the overall induced sterility in the wild population is reduced significantly.

1. INTRODUCTION

The potential of ionizing radiation to interact with materials has numerous applications in industry, medicine, and agriculture. Ionizing radiation breaks down molecules, causing various effects in irradiated material. Radiation can cause polymerization of plastics, and can kill pathogens and microorganisms, leading to applications in food processing and the sterilization of health-care products. In organisms, composed of differentiated and undifferentiated cells, mitotically active cells, such as stem and germ cells, are the most radiation-sensitive cells. In the case of the sterile insect technique (SIT), radiation can make an insect reproductively sterile by damaging the chromosomes of gonial cells, specifically causing germ-cell chromosome fragmentation (dominant lethal mutations, translocations, and other chromosomal aberrations), that lead to the production of imbalanced gametes and subsequently the inhibition of mitosis and death of fertilized eggs or embryos (Klassen, this volume; Robinson, this volume). In adult insects, midgut stem cells, which undergo continuing mitotic divisions, are particularly sensitive to ionizing irradiation, and the irradiation of certain species may cause a significant reduction in lifespan and increased mortality (Sakurai et al. 2000). Nevertheless, the successful sterilization of certain insect species, without a reduction in their lifespan, may indicate that cell replacement in the midgut is either not affected or is not of major importance to viability (Riemann and Flint 1967). Somatic cells, generally differentiated cells that have lost their ability to divide, are less sensitive to radiation than stem cells. Thus a lethal effect requires a higher radiation dose than a reproductive sterilization effect. The impact of radiation on somatic cells is expressed as the development of abnormalities, a reduction in lifespan, flight ability, mating propensity, and nutrition, and ultimately the death of the insect.

Radiation sterilization of insects is a relatively straightforward process, with reliable quality control procedures. The key parameter is the absorbed dose of radiation, which is expressed in Système International d'Unités (SI) units as gray (Gy), where 1 Gy is equivalent to 1 joule (J) of absorbed energy in 1 kg of a specified material (1 Gy = 100 rad). As long as the dose is delivered correctly, efficacy of the irradiation process is guaranteed. Other advantages of using radiation to sterilize insects include: (1) temperature rise during the process is insignificant, (2) sterile insects can be released immediately after processing, (3) irradiation does not add residues that could be harmful to human health or the environment, and (4) radiation can pass through packaging material, allowing insects to be irradiated after having been packaged.

In the 1950s and 1960s, numerous mutagenic chemicals were tested as alternatives to radiation to induce sterilization in insects (Knipling 1979; Klassen, this volume; Lance and McInnis, this volume). Chemosterilants were added to rearing diets, applied topically to insects, or even deployed in attractant-baited devices in the field. The efficacies of irradiating and chemosterilizing insects for population control were, in general, similar (Guerra et al. 1972, Flint et al. 1975, Moursy et al. 1988). However, today, chemosterilants are not used for sterilizing mass-reared insects. Most chemosterilants are carcinogenic, mutagenic, and/or teratogenic, leading to environmental and human-health issues such as the integrity of ecological food chains, waste disposal, e.g. spent insect diet, and worker safety

(Hayes 1968, Bracken and Dondale 1972, Bartlett and Staten 1996). Insect resistance to chemosterilants is an additional concern (Klassen and Matsumura 1966). Exposure to ionizing radiation is now the principal method of inducing sterility in area-wide integrated pest management (AW-IPM) programmes that release sterile insects.

2. RADIATION SOURCES

The suitability of a radiation type for the SIT depends on properties, such as relative biological effectiveness (RBE), penetrability, availability, safety, and cost. The RBE of radiation is defined as the ratio of the dose of 200–250 kV X-rays required to produce a specific biological effect to the dose of radiation required to produce the same effect. The RBE of radiation for the induction of chromosome aberrations depends on its linear energy transfer (LET — the energy imparted to a medium by a charged particle of a specified energy, per unit distance). Radiation with a higher LET is more effective in inducing sterility, and most likely would yield insects that are more competitive (North 1975). However, a higher LET also means that penetration is limited. For example, alpha particles have a high value of LET, but can penetrate only a fraction of a millimetre into a container of insects, which makes them unsuitable for sterilization. Neutrons are more effective than gamma rays or X-rays in sterilizing insects (Hooper 1971, North 1975, Offori and Czock 1975). However, neutrons can induce radioactivity in irradiated materials, which, along with the immobility of nuclear reactors (the usual source of neutrons), makes their use impractical for most programmes.

Considering this, the types of radiation that can be used practically in programmes that release sterile insects include gamma rays, high-energy electrons, and X-rays (Bushland and Hopkins 1951, 1953; Baumhover et al. 1955; Lindquist 1955). All have similar effects on materials (since they have a similar RBE), and in particular on insects. For certain insect life stages and radiation doses, several studies found no significant difference between electrons and gamma rays in their lethal effects (Hooper 1971, Adem et al. 1978, Watters 1979, Dohino et al. 1994).

To maintain the fitness of irradiated insects, and for the safety of workers, the induction of radioactivity in irradiated materials, such as canisters and insects, must be avoided. This is achieved by ensuring that energy used for the SIT is less than 5 million electron volts (MeV) for photons (gamma rays or X-rays), and 10 MeV for electrons (Elias and Cohen 1977, Codex Alimentarius 1983, FAO/IAEA/WHO 1999, IAEA 2002a). Thus, gamma rays from cobalt-60 (^{60}Co) (photon energies are 1.17 and 1.33 MeV) and caesium-137 (^{137}Cs) (0.66 MeV), electrons generated by accelerators with energy less than 10 MeV, and X-rays generated from electron beams with energy below 5 MeV, are acceptable for sterilizing insects.

2.1. Radioisotopes

Currently, the most commonly used radiation for the SIT is gamma radiation from the radioisotopes ^{60}Co and ^{137}Cs. These isotopes have long half-lives, and the energy of their gamma rays is relatively high (Table 1). To provide the same throughput,

caesium sources, because of the difference in photon energy, require about four times more activity than cobalt sources. Cobalt-60 is produced by placing small cylinders of natural cobalt (which is 100% ^{59}Co) into a nuclear reactor, where the ^{59}Co atoms absorb neutrons and are converted into ^{60}Co. These cylinders are removed from the reactor after 1 or 2 years, and are further encapsulated in corrosion-resistant stainless steel to produce source pencils. Caesium-137 is produced from the fission of uranium and plutonium, and must be chemically separated from other fission products and actinides present in used nuclear fuel. This process is very elaborate, and thus the use of caesium is declining for radiation processing, including in AW-IPM programmes that apply the SIT.

Table 1. Comparison of properties of Co-60 and Cs-137

Property	Co-60	Cs-137
Production mode	Neutron absorption in nuclear reactors	Chemical separation from spent nuclear fuel, e.g. uranium
Half-life	5.271 years	30.07 years
Photon energy	1.17 and 1.33 MeV (in equal proportions)	0.66 MeV
50% dose-decrease (depth in water)	23 cm	21 cm

2.2. Electron Beam

In the near future, the use of high-energy (5–10 MeV) electrons to sterilize insects will likely increase. Such electrons are generated by an electron accelerator, which does not involve any radioactive materials. Electrons are introduced into an accelerating structure from an injector, where they are accelerated to the designed high energy that can be derived from a variety of sources depending on the type of accelerator. An electron accelerator yields a narrow and intense electron beam, and thus the dose rate can be up to 1000 times greater than from a gamma irradiator.

2.3. X-Rays

When a beam of electrons strikes material with a high atomic number, e.g. tungsten, X-rays are generated. X-rays, like gamma rays, are electromagnetic radiation. Radiation generated in this manner (by the rapid deceleration of a charged particle) is also known as “Bremsstrahlung” (literally “braking radiation”). While gamma rays from radioisotopes have discrete energies, “Bremsstrahlung” has a broad energy spectrum with a maximum equal to the energy of the incident electrons. Gamma rays from ^{60}Co or ^{137}Cs, and X-rays, penetrate irradiated materials more deeply than

electrons. For example, for ^{60}Co gamma rays, dose decreases to half at a depth of about 20 cm in water, but for 10-MeV electrons, the useful depth is only about 4 cm.

3. RADIATION TECHNOLOGY AND STERILIZATION PROCESS

3.1. Irradiation Units

The design of an irradiation unit affects the dose distribution and the attainable dose range. A unit may be designed either for a specific application (or product) or for multiple applications, depending on local considerations and user requirements. The basic components of an irradiation unit (gamma-ray or electron) include:

- Radiation source (radioisotope gamma source or accelerator) and the associated control systems, sometimes referred to as an "irradiator"
- System for transporting the product, e.g. insects, or in some cases the source, to and from the position at which irradiation occurs
- Shielding to protect workers and the surrounding environment from radiation

The irradiation unit should include a dosimetry laboratory, and a product-handling system with areas designated for receiving and for segregated pre- and post-irradiation storage.

3.1.1. Gamma Irradiators

The radiation source consists typically of several source pencils of either cobalt or caesium. The dose rate is predetermined by the current activity of the source, and the operator controls the absorbed dose delivered to the insects by adjusting the time that they are exposed to radiation (an exception — in some large-scale irradiators, several dose rates can be obtained by raising different subsets of the source pencils into the irradiation room). The only variation in the source output is the known reduction in activity (strength) caused by radioactive decay, which can have a significant impact on the programme (financial as well as scheduling) if not taken into account. The activity of a cobalt source, for example, decreases about 12% annually. The irradiator operator compensates for this loss of activity by incrementally increasing irradiation time (approximately 1% each month) to maintain the same dose to the insects. Since irradiation times eventually become impractically long, sources need to be replenished at regular intervals, depending on the initial activity of the source and the operational requirements.

Typically there are two types of gamma irradiators used in programmes that release sterile insects — self-contained dry-storage irradiators, and large-scale panoramic irradiators.

Self-Contained Dry-Storage Irradiators. At present, most sterilization of insects is accomplished using gamma rays from self-contained irradiators (Fig. 1). These devices house the radiation source within a protective shield of lead, or other appropriate high-atomic number material, and they usually have a mechanism to rotate or lower the canister of insects from the loading position to the irradiation

position. These canisters, which are reusable and generally made of steel, aluminium, or plastic, hold packaging containers of insects during irradiation. To irradiate, a canister is placed in the irradiation chamber while it is in the loading (shielded) position, and the timer is set to deliver the pre-selected dose. On the push of a button, the chamber is automatically moved to the irradiation position. In most self-contained irradiators, the irradiation position is in the centre of an annular (circular) array of long parallel pencils that contain the encapsulated radiation source. With this design, the dose is relatively uniform within the irradiation chamber (section 3.3.2.). An alternate method of achieving a relatively uniform dose is to rotate the canister of insects on a turntable. The axis of rotation is parallel to the source pencils, which are usually vertical. The canister stays in the irradiation position for the set time interval, and then automatically returns to the loading position at the end of the treatment. Self-contained dry-storage irradiators provide a high-dose rate but a small irradiation volume (1 to 4 litres), and are suitable for research as well as small-scale programmes that apply the SIT.

Figure 1. In preparation for irradiation, a canister of insects is placed in the irradiation chamber (while it is in the shielded position) of a self-contained gamma irradiator. Depending on the dose rate of the day, the timer on the control panel (bottom right) is set to give the desired dose.

Large-Scale Panoramic Irradiators. For large-volume irradiation, panoramic irradiators are more suitable. The source consists of either several ^{60}Co rods (pencils) arranged in a plane or a single rod that can be raised/lowered into a large irradiation room. When retracted from this room, the source is shielded either by water (wet storage), lead or other appropriate high-atomic number material (dry storage). Since isotopic sources emit gamma rays isotropically (in all directions), they may be surrounded by canisters of insects to increase the energy utilization efficiency, and several canisters can be irradiated simultaneously.

Many large-scale irradiators run in a continuous-operation mode, in which canisters of insects are carried on a conveyor around a central source. The canisters may pass by the source several times to increase dose uniformity in the canisters as well as energy utilization. The speed of the conveyor is selected so that the insects receive the intended dose. The source is moved to the storage position only when the irradiator is not in use. An alternate method is batch operation, where several canisters of insects are placed in the irradiation room while the source is in its storage position. The source is then moved into the irradiation room for the length of time required to achieve the desired absorbed dose. To improve dose uniformity, each canister may be rotated on its own axis during irradiation using turntables.

3.1.2. Electron and X-Ray Irradiators

Accelerator-generated radiation has two modes, electrons and X-rays produced from these electrons. The two principal electron-beam characteristics are beam (particle) energy, in MeV, and the average current, in milliamperes (mA). The beam energy affects the penetration of electrons in a material (thus dictating the useful size of the canister for irradiation), and the average beam current affects absorbed-dose rate (thus determining throughput, e.g. the number of canisters treated per hour). Unlike gamma radiation, electron beams are rather focused (for both modes), and typically conveyors are used to move canisters of insects continuously through the beam. Since X-rays penetrate deeper than the electrons, from which they are generated, larger canisters of insects can be used when using the X-ray mode.

3.1.3. Selection of Irradiator

Since gamma rays and electrons have similar sterilizing effects, the choice of source for SIT irradiation is based on other considerations, such as penetration, cost, product throughput (DIR-SIT in IDIDAS (2004)), expertise available at the site, and environmental and safety factors. The shallow penetration of electrons restricts the size of the canister used for irradiation. In addition, gamma irradiators are usually simpler to operate, and less expensive, than accelerators, at least within the range of power required for SIT applications. Electron accelerators, however, may have more public acceptance because they produce no radiation when switched off, and there are no transportation or radioactive waste issues (Cavalloro and Delrio 1974, Piedade-Guerreiro and Gomes da Silva 1983, Cleland and Pageau 1985, Smittle 1993, EBFRF 2004, FDACS 2004, LAF 2004). The power emitted by a gamma-ray source containing 100 kCi of ^{60}Co is roughly equivalent to that of a 1.5 kW electron accelerator. The power capacity of currently available commercial accelerators with

5–10 MeV electrons is usually much greater than this, making them unsuitable for dedicated SIT use. X-ray irradiators have the advantages of both gamma irradiators (high penetrability) and accelerators (no radioactivity when switched off). However the efficiency of converting electrons into X-rays is about 7% for 5 MeV electrons; thus 93% of the electron beam power is "wasted" in heating the converter target material (Farrell et al. 1983). Based on all of these factors, almost all current insect sterilization programmes have chosen to use gamma irradiators (Table 2).

3.2. Radiation Safety

It is essential that written descriptions of specific safety procedures, for all activities at an irradiation unit, are prepared. Before using a radiation source, workers must be given detailed training on relevant national legislation and regulations, and on safety procedures for the manufacture, transport, installation and use of a radiation source (IAEA 1992, IAEA 1996a, IAEA 2003).

Irradiators are designed to keep the radiation exposure and dose to workers "as low as reasonably achievable" (ALARA), and within predetermined levels. These dose limits are based on the recommendations of several agencies of the United Nations (UN), including the International Atomic Energy Agency (IAEA), Food and Agriculture Organization of the United Nations (FAO), and World Health Organization (WHO) (IAEA 1996a). Appropriate safety methods and procedures have been developed for each type of irradiator, and when operated correctly with the appropriate safeguards, they are safe and easy to use. Irradiators are usually licensed by national atomic energy authorities, which set certain requirements such as restricting access to certain areas and authorized persons, a periodic survey of the radiation field in the vicinity where workers could be present, the use of personal radiation dosimeters, and the availability of radiation survey meters. These requirements are specifically aimed at protecting all workers from radiation. In addition, irradiators incorporate interlocks that prevent unintentional access to areas with high-radiation fields. Cases of accidental exposure to ^{60}Co gamma rays are usually reported by the IAEA (IAEA 1996b, Gonzalez 1999), and data from such historic cases are useful for probabilistic risk assessment. When the useful life of a gamma source is over, the irradiator or the source pencils are usually returned to the supplier for storage, reuse, recycling, or disposal. This is now becoming a costly procedure.

During handling of insects, especially adult Lepidoptera, irradiator operators may be exposed to insect allergens, and additional safety measures may be required to minimize the risk of allergy and health hazards (Parker, this volume).

Table 2. Examples of insect mass-rearing facilities, and the types of irradiators used for reproductive sterilization (more extensive list found in IDIDAS (2004))

Location of facility	Insect reared	Dose (Gy)[1]	Initial activity (kCi)	Irradiator model (MANUFACTURER)	Source
Argentina	*Ceratitis capitata*[4]	110	20	IMCO-20[2]	Co-60
Canada	*Cydia pomonella*[5]	150	24	Gammacell® 220[2] (NORDION)	Co-60
Chile	*Ceratitis capitata*	120	160	Gammacell® 220[2] (NORDION)	Co-60
Guatemala	*Ceratitis capitata*	100–145	11	Gammacell® 220 E[2] (2 units) (NORDION)	Co-60
			12	Gammacell® 220 R[2] (J. L. SHEPHERD)	Co-60
			42	Husman 521A[2] (ISOMEDIX)	Cs-137
			46	Husman 521[2] (ISOMEDIX)	Cs-137
Mexico	*Anastrepha ludens*[6]	80	35	JS-7400[3] (NORDION)	Co-60
	Anastrepha obliqua[7]	80			
	Ceratitis capitata	100			
Mexico	*Cochliomyia hominivorax*[8]	80	47	Husman 520[2] (3 units) (ISOMEDIX)	Cs-137
Philippines	*Bactrocera philippinensis*[9]	64–104	30	GB 651 PT[3] (NORDION)	Co-60
Portugal	*Ceratitis capitata*	100	20	Gammacell® 220[2] (NORDION)	Co-60
South Africa	*Ceratitis capitata*	90	10	(LOCAL MANUFACTURER)[3]	Co-60
Thailand	*Bactrocera dorsalis*[10]	90	24	Gammacell® 220[2] (NORDION)	Co-60
USA (Hawaii) CDFA[11]/USDA	*Ceratitis capitata*	140	47	Husman 521[2] (2 units) (ISOMEDIX)	Cs-137
USA (Hawaii) ARS/USDA[12]	*Ceratitis capitata*	120	24	Gammacell® 220[2] (NORDION)	Co-60
USA (Texas)	*Anastrepha ludens*	70	38	Husman 521[2] (ISOMEDIX)	Cs-137

[1] Sterility-inducing dose in hypoxia (except *Cydia pomonella*)
[2] Self-contained dry-storage irradiator
[3] Panoramic irradiator
[4] *C. capitata* (Wiedemann)
[5] *C. pomonella* (L.)
[6] *A. ludens* (Loew)
[7] *A. obliqua* (Macquart)
[8] *C. hominivorax* (Coquerel)
[9] *B. philippinensis* Drew and Hancock
[10] *B. dorsalis* Hendel
[11] California Department of Food and Agriculture
[12] Agricultural Research Service, United States Department of Agriculture

3.3. Measurement and Distribution of Absorbed Dose

3.3.1. Radiation Dosimetry

For the success of a programme using the SIT, the absorbed dose delivered to the insects needs to be accurately quantified and controlled. Also, if contractual arrangements or national regulations prescribe specific doses, the programme will require adequate means to demonstrate compliance. Therefore the programmes need to have an established dosimetry system to accurately measure absorbed dose and estimate the associated confidence interval, a process known as dosimetry (ISO/ASTM 2004a). Dosimetry is performed using dosimeters — devices that, when irradiated, exhibit a quantifiable change in some property, e.g. colour, that can be related to the absorbed dose. A dosimetry system includes dosimeters (that are placed into the canister), measuring instruments (to read the change in the dosimeters) along with their associated reference standards, and procedures for using them (ISO/ASTM 2004b).

Dosimeters are commonly used in sterile insect production for such tasks as absorbed-dose mapping (section 3.3.2.), process control (section 3.5.3.), and qualification of the irradiator (section 3.5.2.). Several dosimeters are suitable for routine dosimetry at SIT facilities (ISO/ASTM 2004a). Many sterile insect production facilities use radiochromic film systems because they are relatively affordable and are simple to use (avoiding extensive training) (IAEA 2004). Procedures for calibrating routine dosimetry systems, and for determining radiation fields in irradiators used for insect sterilization, are described in ISO/ASTM standards (section 3.5.1.) (ISO/ASTM 2004a, 2004b, 2004c, 2004d), which are updated periodically, and in IAEA technical reports (IAEA 2002b). Reference-standard dosimeters are used to calibrate the routine dosimetry system and radiation fields, e.g. determining the dose rate at a reference position in a self-contained gamma irradiator. Sterile insect production facilities use reference-standard dosimeters for both of these purposes. Externally accredited dosimetry laboratories typically provide these dosimeters and make the readings, resulting in measurements that are "traceable" to national or international standards.

3.3.2. Absorbed-Dose Mapping

Ideally, it would be desirable to irradiate all insects in a container (or a canister) at the same dose. In practice, because of the characteristic of radiation interaction with matter, there is a systematic pattern of dose variation within the canister, and therefore not all insects receive the same dose. Dose distribution within the canister is determined by "dose mapping", which typically is conducted by placing several dosimeters at known locations throughout the canister. Dose mapping provides operators of SIT irradiators with information on the dose within the canister, including areas of maximum and minimum dose, the dose-uniformity ratio (maximum dose/minimum dose), and areas where the dose rate is relatively uniform (Fig. 2). Techniques for dose mapping are described in detail in ISO/ASTM (2004a) and Walker et al. (1997).

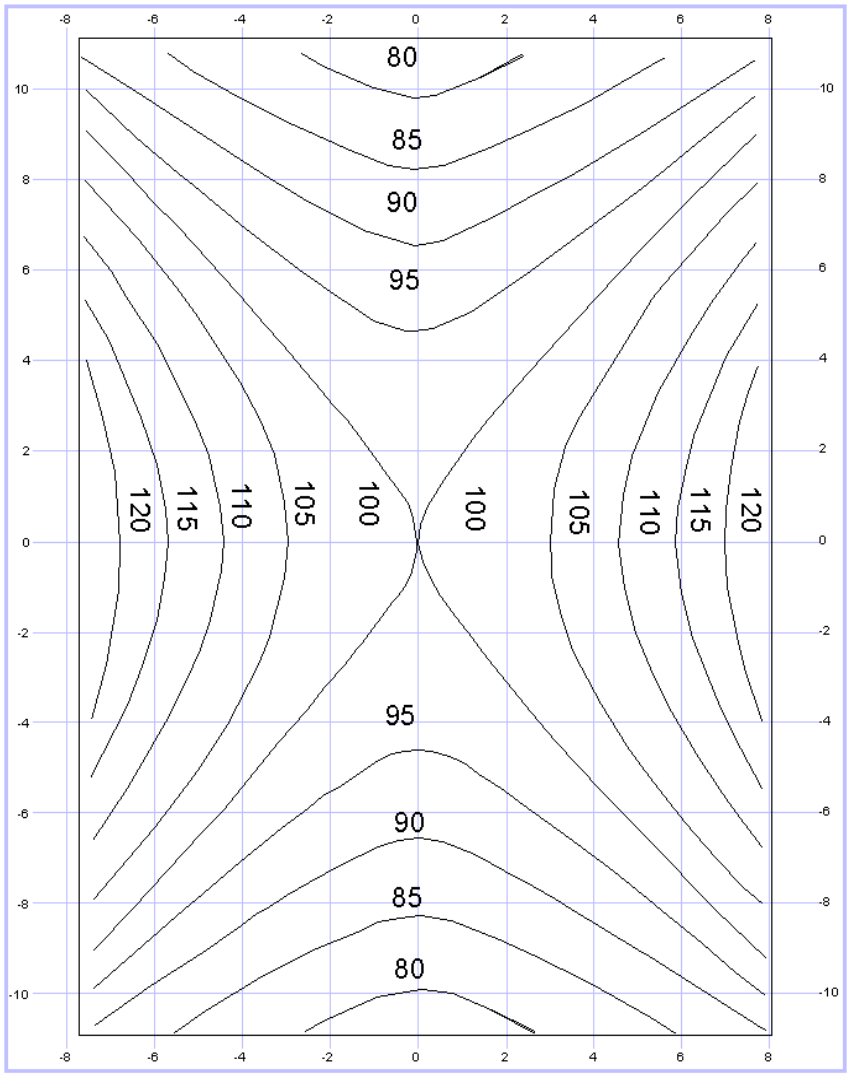

Figure 2. Example of isodose curves in the irradiation chamber of a Gammacell® 220. Values are normalized to 100 in the centre of the gamma field. The field is most uniform in the centre. Grid is at 2-cm intervals from the centre of the chamber. (Figure from MDS Nordion, reproduced with permission)

3.4. Radiation Sterilization of Insects

3.4.1. Selecting Sterilizing Dose

The absorbed dose that is used to induce sterility is of prime importance to programmes that release sterile insects. As it increases, sterility increases, but insect quality and competitiveness will decrease (Calkins and Parker, this volume; Lance and McInnis, this volume). Insects that receive too low a dose are not sufficiently sterile, and those that receive too high a dose will be non-competitive, reducing the effectiveness of the programme. Quite often, full (100%) sterility may not be the most favourable condition for a programme, and thus process optimization is necessary to balance sterility level and competitiveness, taking into consideration factors that could affect the radiation sensitivity of insects (section 4) and programme requirements. If quarantine security is a consideration, 100% sterility may be required for any released females. Males, however, tend to be less radiosensitive, and, in many species, eliminating a residual egg hatch of 1% (or less) from fertile females mated to irradiated males (even though many of these eggs do not survive other stages) requires doses that substantially reduce the ability of males to compete with, and thus induce sterility into, wild populations (Fisher 1997, Toledo et al. 2004).

In reality, because of the unavoidable dose variability within a canister (as mentioned above), sterile insect production facilities define an acceptable range of doses given to the insects. Most often, programmes or regulatory officials specify a minimum dose that all insects must receive to ensure sufficient sterility. Due to dose variability, most insects actually receive a dose that is somewhat higher than that minimum. An alternate approach is to specify an optimum (or central target) dose, and set this as the average or median dose within the irradiated volume of insects. In either case, the dose uniformity ratio should be small; the goal is to sterilize all insects sufficiently without treating large proportions with doses that are high enough to substantially reduce competitiveness. Unit operators can often adjust process parameters to achieve a more uniform dose distribution (section 3.5.2.).

Induced lethal mutations may exert lethality at any stage of development. Quite often, for reasons of simplicity and convenience, the induction of detrimental lethal mutations is made based solely on egg hatchability. However lethal mutations occur at all developmental stages. Therefore researchers should measure dose effects all along this developmental continuum, or the actual survivorship from egg to adult, to give a true picture of induced sterility. As a result, 99 or 100% sterility in the egg stage is not essential, nor desirable, if it drastically reduces the competitiveness and vigour of the sterile insect.

An informed decision on treatment dose requires accurate data on how factors such as dose, insect stage and age, and various process parameters affect levels of sterility and insect quality. For programmes that apply the SIT, the accuracy and value of such data depend on the use of standardized dosimetry systems, procedures, and reporting methods (ISO/ASTM 2004c). Published data on the radiation biology of the same or similar species can provide guidance, but, in many cases, are of limited value because dosimetric procedures, dose-measurement traceability, dose distribution, and other pertinent information are often not reported. In addition, the

details of insect-handling procedures, and, perhaps, strain-related differences, can influence radiation sensitivity (section 4.).

3.4.2. Preparing Insects for Irradiation

Stage/Age of Insects. The selection of the insect development stage and age that will be irradiated is based on knowledge of the timing of maturity of insect reproductive organs (section 4.2.), handling suitability during irradiation and subsequent shipping, and sensitivity to somatic damage. For many holometabolous species (having complete metamorphosis), a good time for irradiation is late in the pupal stage, or early in the adult stage, when germ tissues have formed (Anwar et al. 1971, Ohinata et al. 1971, 1977, 1978). For example, tephritid flies are usually irradiated 1 or 2 days prior to adult emergence (pupae kept at about 25°C). Flies that are irradiated earlier in the pupal stage will tend to be of lower quality (in terms of mating propensity, flight, and sex pheromone production), an indication that somatic tissues were adversely affected (Fletcher and Giannakakis 1973). However, when tephritid pupae are irradiated too close to adult emergence, females can already have some developed oocytes that, in spite of having been irradiated, can become viable eggs (Williamson et al. 1985). Ideally, the development and maturity stage should show an external physical indicator that acts as a quick and reliable identification tool, such as pupal eye-pigment colour in the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Ruhm and Calkins 1981, Seo et al. 1987).

In the pentatomid bug *Nezara viridula* (L.), sexual maturity and mating occur 5–17 days after adult emergence. Fourth- and fifth-instar nymphs are most frequently selected for irradiation because they are less radioresistant than adults, and male and female reproductive systems are already well developed at this stage (Kiritani 1963, Mitchell and Mau 1969). However Williamson et al. (1985) found in the Mediterranean fruit fly that, due to advanced egg development in the ovaries, irradiation at 1 day before emergence or later resulted in some fertility.

Packaging for Irradiation. Insects for AW-IPM programmes that integrate the SIT are usually irradiated within primary packaging containers that are subsequently transferred, unopened, to an emergence facility where the adult insects are prepared for release. These containers provide protection to the sterile insects, and guard against their escape. They also prevent tampering. A variety of packaging containers has been used, e.g. 2- and 4-litre polyethylene bags, unwaxed paper cups (with lids), paper boxes, and plastic bottles of up to 15-litre capacity. SIT irradiation protocols may incorporate reusable canisters (typically steel, aluminium or plastic) that hold the primary containers during irradiation. The size and shape of these canisters are usually a function of the size and shape of the irradiation chamber, especially in the case of self-contained irradiators (section 3.5.2.).

If insects are irradiated in a reduced-oxygen atmosphere, as a means of reducing the formation of free radicals (section 4.1.), the packaging container must be airtight. For example, tephritid pupae are sealed, with as little air space as possible, in plastic bags or bottles and then held at cool temperatures (12–20°C) for at least 1 hour

before irradiation. During this period the insects exhaust most of the oxygen remaining within the container. Hypoxia (a deficiency of oxygen reaching the tissues of the body) can also be achieved by saturating the atmosphere within the container with helium or, more commonly, nitrogen, prior to and during irradiation (Ashraf et al. 1975; Ohinata et al. 1977, 1978; Hooper 1989).

3.5. Quality Assurance

3.5.1. Quality Assurance Programmes

Quality assurance (QA) is an important part of any successful AW-IPM programme using the SIT. A QA programme provides various benefits with respect to irradiation procedures, including:

- Success of the process — adequately sterilized insects of good quality can be produced consistently
- Compliance with regulations — a QA programme makes it convenient to audit the process against established standards
- Harmonization — as international trade is growing, it has become more important to ensure dependable uniformity across geographical and political regions
- Public acceptance — when the public realizes that SIT facilities strictly follow set procedures and document the process, it has more confidence in the programme

An effective QA programme includes standard operating procedures (SOPs) for all activities related to packaging, sterilization, and dosimetry. Also, processing equipment that controls key operating parameters (those affecting dose) is periodically tested and/or calibrated to verify that the irradiator is operating properly. This is then documented as part of the record for the QA programme.

For many processes related to insect sterilization using radiation, standards and guidelines are available that can be incorporated into a facility's QA programme. These include dosimetry standards developed by the International Organization of Standardization and the American Society for Testing and Materials (ISO/ASTM 2004a, 2004b, 2004c, 2004d, 2004e), an SOP for using the Gafchromic® film dosimetry system for the SIT (IAEA 2004), and a comprehensive quality-control manual for applying the SIT against fruit flies (FAO/IAEA/USDA 2003).

3.5.2. Irradiator Operation and Configuration

When an irradiator is installed, it is evaluated to ensure that it is working according to the manufacturer's specifications, and to develop baseline data on its performance. These two activities are known as installation qualification and operational qualification, respectively (ISO/ASTM 2004a). Operational qualification includes, among other things, initial dose mapping (section 3.3.2.), and measurement of the dose rate at a reference position, e.g. at the centre of a fully filled canister (section 3.3.1.). The reference dose rate is then used to establish the basic relationship between key operating parameters, such as timer setting or conveyor speed, and absorbed dose. Dosimetry standards recommend repeating periodically

the reference dose-rate measurement, e.g. every 3 years for gamma irradiators (ISO/ASTM 2004a). A caesium-137 (^{137}Cs) source, in particular, may contain impurities (^{134}Cs) that affect the decay rate, and thus, over time, the dose rate. Reference-standard dosimetry and dose mapping are repeated as appropriate following any changes in the irradiator, such as source renewal in gamma irradiators, that could affect the dose rate or dose distribution.

Before insects are sterilized, key process parameters are established as part of performance qualification. For most insect irradiators, the absorbed dose delivered to the insects is controlled by adjusting a single parameter, such as timer setting (irradiation time) or conveyor speed. Values of these parameters depend on the dose specifications (section 3.4.1.) and the reference dose rate on the day of irradiation. Dose mapping is again performed to ensure that all insects within a given canister will receive an appropriate dose. If necessary, process parameters can often be adjusted to improve dose uniformity; common alterations include optimising the size or shape of the canister, rotating the canister on a turntable during irradiation, using dose attenuators, and using plugs of simulated product, e.g. styrofoam, in the canister or irradiation chamber to exclude insects from areas with unacceptably low or high dose rates. This procedure establishes a canister design and a loading configuration of insects that result in an acceptably uniform dose distribution. The results of this mapping may also be used to establish a reference location for performing routine dosimetry as part of process control (section 3.5.3.).

3.5.3. Process Control

The accidental release of insects that are not irradiated properly could potentially be disastrous (Knipling 1982), especially in programmes like those in California and Florida, USA, where the SIT is used to eradicate extremely small pest populations and/or as a prophylactic measure to prevent the establishment of newly introduced pests. To avoid this problem, programmes that release sterile insects implement various process-control elements to help ensure that all insects are irradiated according to specifications (FAO/IAEA/USDA 2003). In addition to the elements listed below, programmes applying the SIT often monitor relevant process parameters such as information on the preparation and packaging of insects, setting of the irradiator timer, conveyor speed, canister specifications, and position and loading of the canisters. The results of process monitoring are routinely documented as part of the record of the QA programme.

Sterility Testing. Most AW-IPM programmes that integrate the SIT test samples of irradiated insects on a regularly scheduled basis to confirm that specified levels of sterility are being achieved. The quality-control manual for using the SIT against fruit flies suggests that this could be done for every shipment (FAO/IAEA/USDA 2003), comparing the egg hatch from pairings of irradiated and non-irradiated insects with that from crosses of non-irradiated insects. Besides making regularly scheduled sterility tests, unscheduled tests should also be conducted whenever changes are made to any equipment or procedures and before any insects are shipped. However, it takes time to obtain the results of a sterility test, and the results

may be known too late to prevent the release of incorrectly treated insects. Therefore sterility testing must be supplemented with other methods, such as routine dosimetry.

In addition, the competitiveness of the sterile insects needs to be checked with insects of the target population to ensure the efficacy of the programme. Such testing helps to ensure that all procedures are being followed correctly, including rearing, pre-irradiation preparation (e.g. age-based selection of insects), packaging for hypoxia or nitrogen (if used), temperature control, irradiation-dose control, and post-irradiation handling.

Routine Dosimetry. The regular use of routine dosimetry can help to confirm that insects are being irradiated according to programme specifications, and may be required for every shipment (FAO/IAEA/USDA 2003). This is usually done by placing dosimeters on packaging containers or canisters at a specific location (which may be the reference location identified through dose mapping performed during performance qualification (section 3.5.2.)), where the dose rate has a known and predictable relationship to the minimum and maximum dose rate within those canisters. Unlike sterility testing, routine dosimetry can identify problems in the irradiation process quickly enough so that improperly sterilized batches of insects can be intercepted prior to release in the field. Although standard dosimetry is faster than sterility testing, a third control described below provides an immediate visual confirmation check that a given container has gone through the irradiation process.

Radiation-Sensitive Indicators. A radiation-sensitive indicator is a material, such as a coated or impregnated adhesive-backed (or adhesive-fronted) substrate, ink, or coating, that undergoes a qualitative visual change when exposed to a specified dose of radiation (ISO/ASTM 2004e). The dose at which the indicator changes should ideally be below, but near, the minimum dose required. Since the degree of colour change is not proportional to the dose, these indicators cannot substitute for dosimeters.

Indicators are used as aids in tracking whether or not specific containers have been irradiated. The quality-control manual (FAO/IAEA/USDA 2003) suggests that an indicator should be attached to each packaging container of insects to help ensure (along with product segregation protocols and other procedural methods) that non-irradiated insects are not unintentionally released in the field. Also, indicators could potentially be used to assist in tracking multiple passes of containers through an irradiator when the sterilizing dose is fractionated into several smaller doses (section 4.1.).

Indicators that are exposed to excessive humidity, high temperature or UV radiation, e.g. sunlight, before or after irradiation may give erroneous readings; hence they are useful only within an irradiation unit where these conditions are controlled.

Recommended dosimetric procedures, including routine dosimetry and the use of indicators for programmes releasing sterile insects, are described in published

standards and guides, e.g. ISO/ASTM 2004a, 2004d, 2004e; FAO/IAEA/USDA 2003.

4. FACTORS MODIFYING INSECT RADIATION SENSITIVITY

The sensitivity of arthropods to radiation depends on many parameters. Radiation sensitivity varies widely among species (section 6.), but environmental conditions, and the biological state of the organism at the time of irradiation, can also have significant influences. These latter factors, in many cases, can be combined to optimize the quality of sterilized insects.

4.1. Environmental and Physical Factors

4.1.1. Ambient Atmosphere

Oxygen levels affect the sensitivity of insects to radiation (Baldwin and Chant 1971, Economopoulos 1977, Ohinata et al. 1977, Rananavare et al. 1991, Fisher 1997). Damage induced by radiation is typically lower in an oxygen-reduced environment (hypoxia) than in air, so usually higher doses are needed to produce comparable reproductive sterility. However, because the magnitude of this protective effect tends to be greater for somatic damage than sterility, the use of oxygen-reduced atmospheres is a common strategy to improve sterile insect competitiveness without sacrificing sterility (Calkins and Parker, this volume; Lance and McInnis, this volume). Methods for inducing hypoxia are described in section 3.4.2.

The increased radiation damage in a high-oxygen environment is a general phenomenon in radiobiology. For the protective effect of low oxygen to be seen, the tissues must be anoxic or hypoxic during irradiation; exposure to oxygen before or after is without effect. Ionizing radiation initiates a chain of oxidative reactions, along the radiation path in the tissues, and the formation of free radicals, which in the absence of oxygen might be neutralized by combining with hydrogen radicals, resulting in no net damage. In the presence of oxygen, damaging peroxy-radicals may be formed, and the organic molecules, including the germ cell chromosomes, are irreversibly altered, e.g. dominant lethal mutations, leading to the production of imbalanced gametes as described above (Klassen, this volume; Robinson, this volume). It must be noted that high-LET radiation (e.g. alphas, neutrons) is less affected by the presence or absence of oxygen than low-LET radiation (X-rays and gamma radiation). This may be because high-LET radiation causes several ionizations within one macromolecule, damaging it beyond repair (Pizzarello and Witcofski 1967).

4.1.2. Dose Rate

The adverse effects of radiation appear, in general, to be lessened by reducing the rate at which the sterilizing dose is delivered to the insects. This can be done by using a lower dose rate, and longer irradiation time, for a single irradiation (Yanders 1959, Nair and Subramanyam 1963, Hooper 1975, Mayas 1975, Ilao 1977). An alternate approach to conserve insect quality is dose fractionation, where the

sterilizing dose is delivered over time in a series of smaller irradiations (North 1975, LaChance and Graham 1984, Haynes 1993, Tamhankar and Shantharam 2001). However, because of its impracticality, current AW-IPM programmes applying the SIT do not follow this procedure.

4.1.3. Temperature

There are some data to suggest that irradiation at reduced temperatures tends to increase the resistance of arthropods to radiation (Rananavare et al. 1991). Cool temperatures, to a certain limit, and hypoxia, also reduce the metabolic rate, and therefore the development rate, of insects during irradiation.

4.2. Biological Factors

4.2.1. Cell Stage and Characteristics

The most radiosensitive cells are those (1) with a high mitotic rate, (2) with a long mitotic future (i.e. under normal circumstances, they will undergo many divisions), and (3) which are of a primitive type. These generalizations, with some exceptions, have become known as the Law of Bergonie and Tribondeau (Casarett 1968). In this regard, germ cells are the most radiosensitive, and show different killing and sterilization susceptibility according to their development stage.

It is generally accepted that chromosomal damage (structural and numerical anomalies) is the cause of dominant lethal mutations. Dominant lethal mutations occurring in a germ cell do not cause dysfunction of the gamete, but are lethal to the fertilized egg or developing embryo (Robinson, this volume). The earlier stages of spermatogenesis (spermatocytes and spermatogonia) are generally more radiosensitive than later stages (spermatids and spermatozoa) (Proverbs 1969). Dey and Manna (1983) found that chromosomes in spermatogonial metaphase and anaphase I were more sensitive to X-rays than those in other stages. Germ-cell sensitivity in female insects is, however, complicated by the presence of nurse cells that are most susceptible to injury during mitosis (LaChance and Leverich 1962).

The dose required to inhibit mitosis is reported to be inversely proportional to the number of chromosomes, and correlates with the average interphase chromosome volume. The larger the nuclear volume, apparently the greater is the sensitivity. Similar relationships were determined in animals and plants, and used to predict their sensitivity to chronic irradiation (Sparrow et al. 1963, Sparrow et al. 1967, Casarett 1968, Whicker and Schultz 1982, Jacquet and Leonard 1983). Furthermore, radiosensitivity appears to be influenced by additional parameters including cell repopulation capacity, tissue and organ regeneration ability, and biological repair (Harrison and Anderson 1996).

Chromosome organization can also affect the response to radiation. Several insect orders (Hemiptera, Lepidoptera, Trichoptera, Odonata and Dermaptera) have holokinetic chromosomes, i.e. properties of the centromere are distributed over the entire chromosome (Kuznetsova and Chubareva 1979). LaChance and Riemann (1973) suggested that, in these taxa, most dominant lethal mutations cause death

after blastoderm formation. In other orders, dominant lethal mutations are expressed during the early cleavage divisions.

4.2.2. Developmental Stage and Age

Age and developmental stage are important parameters to be taken into consideration when deciding on radiation process parameters for the SIT. In general, adults are more radioresistant than pupae, which in turn are more resistant than larvae. Similarly, older pupae tend to be more resistant to radiation than younger pupae (Ismail et al. 1987, Ahmed et al. 1990, Hamed and Khattak 1991, Dongre et al. 1997). Also there is a negative relationship between the age of eggs and their sensitivity to treatment (Chand and Sehgal 1978).

4.2.3. Sex

Regarding sterilization or disinfestation, female arthropods are, on average, usually more radiosensitive than males (Cogburn et al. 1973, Hooper 1989, Hallman 1998), but there are numerous exceptions. For example, males were found to be more radiosensitive than females in the hemipteran families Pyrrhocoidae, Piesmidae, and Pentatomidae (Mau et al. 1967), the American cockroach *Periplaneta americana* (L.) (Wharton and Wharton 1959), certain Coleoptera (section 6.2.), and ixodid ticks (Purnell et al. 1972).

The wide variation reported among species in relative radiosensitivity of males versus females likely results in part from differences in the maturity of oocytes present when females are irradiated. For example, if Mediterranean fruit fly female pupae are irradiated two or more days before adult emergence, egg production is completely stopped by doses well below those needed to sterilize males. However, on the day before emergence and at later times, females contain increasing numbers of oocytes that mature into viable eggs even if irradiated at doses sufficient to sterilize males (Williamson et al. 1985).

4.2.4. Size and Weight

Early studies (Wharton and Wharton 1957, Willard and Cherry 1975) suggested that species with large adults would tend to be more radiosensitive than those with small adults. Experiments have shown that *Periplaneta americana* is killed or sterilized by radiation doses to which smaller insects in genera such as *Drosophila*, *Habrobracon*, and *Tribolium* are resistant. However, subsequently, the correlation between size, weight, and radiosensitivity has not proved to be very strong.

4.2.5. Diapause

The effects of diapause on insect sensitivity to radiation appear to vary. Mansour (2003) found that radiation-related reductions in adult emergence were greater following treatment of diapausing than that of non-diapausing larvae of the codling moth *Cydia pomonella*, but other authors reported that diapausing and non-diapausing larvae of other species were equally sensitive to irradiation (Ignatowicz 1997, Hallman 2000). Carpenter and Gross (1989) reported no interaction between

inherited sterility (IS) and diapause with regard to several traits, although crosses involving moths that emerged from diapaused pupae produced significantly fewer eggs. In contrast, diapausing twospotted spider mites *Tetranychus urticae* Koch appeared more tolerant to irradiation than non-diapausing mites (Lester and Petry 1995).

4.2.6. Nutritional State

Pre- or post-irradiation starvation, or the nutritional state, may influence radiosensitivity (Wharton and Wharton 1959, Stahler and Terzian 1963, Drummond et al. 1966). For example, to achieve 100% sterility, male and female lone star ticks *Amblyomma americanum* (L.) required about 10 Gy before engorgement and 24 Gy after engorgement (Drummond et al. 1966). The data suggested an attenuation of radiation-induced lethality in a blood-fed organism, but the mechanism remains unknown. Beuthner (1975) did not find such differences in *Amblyomma variegatum* (F.), *Hyalomma anatolicum excavatum* Koch or *Rhipicephalus appendiculatus* Neumann.

4.2.7. Additional Factors

An insect's state of hydration, or moisture content, could potentially influence the effects of radiation, but probably this is applicable mostly to commodity disinfestation. Diurnal rhythms apparently can influence the induction of sterility by radiation. Rananavare et al. (1991) found that potato tuberworms *Phthorimaea operculella* (Zeller) irradiated in scotophase were less resistant than those treated in photophase. Finally, genetic differences related to geographical diversity within a species can potentially affect insect radiosensitivity (Fisher 1997, Azizyan 2003, Hallman 2003).

5. ARTHROPOD SPECIES SUBJECTED TO RADIOSTERILIZATION

The International Database on Insect Disinfestation and Sterilization (IDIDAS) (IDIDAS 2004) was developed to collect and share information about radiation doses for disinfestation and reproductive sterilization of arthropods, and to perform a comparative analysis and quality assurance check on existing data. IDIDAS was based on a literature review and analysis of more than 2750 references that were published during the past five decades. Due to space limitations, references are not included in this chapter, but can easily be obtained from the IDIDAS website.

In the past five decades, at least 217 species of arthropods of economic importance, found in 136 genera, 61 families, 7 insect orders and 2 arachnid orders, have been subjected to irradiation studies for the purposes of research, biological control, or pest suppression programmes integrating the SIT (Table 3). Of these, 31% are Diptera, 25% Lepidoptera, 24.5% Coleoptera, 9% Hemiptera, 5.5% Acari, 3% Dictyoptera, 1% Araneae, 0.5% Thysanoptera, and 0.5% Orthoptera. Out of 66 entries on Diptera from 15 families and 26 genera, 21 species belong to the Tephritidae, indicating the importance of this group in pest management and

international trade. The Culicidae and Pyralidae follow Tephritidae in terms of the number of species radiosterilized.

Potential sources of error in any compilation of records, such as this database, are numerous. One of the main difficulties derives from taxonomy, an evolving science; during the past 50 years the names of many pest species have been revised. Organisms for irradiation drawn from a cultured population should, therefore, be defined for posterity by lodging voucher specimens in an appropriately secure and curated collection. This is particularly important for groups subject to frequent taxonomic changes, such as the Tephritidae.

Table 3. Calculated mean and 95% confidence limits (upper L_2, lower L_1) (Sokal and Rohlf 1995) for radiosterilization doses for insects and related arthropods (data are for in-air irradiation of males treated either as pupae or nymphs, but mosquitoes and apple maggots treated as adults; other factors, e.g. radiation source, temperature, dose rate, and level of sterility achieved, were not necessarily consistent; references for data from IDIDAS (2004))

Order	Family	Number of genera	Number of species	Sterilization dose (Gy)		
				L_2	Mean	L_1
Acari	Acaridae	4	4	305	270	234
	Argasidae	2	2	302	198	93
	Ixodidae	4	7	33	32	31
	Tetranychidae	2	4	273	153	43
Araneae	Eresidae	1	1	150	150	150
	Pholcidae	1	1	20	20	20
Coleoptera	Anobiidae	3	4	71	43	15
	Bostrichidae	2	1	176	132	87
	Bruchidae	3	5	90	80	70
	Cerambycidae	1	1	90	80	70
	Chrysomelidae	2	2	100	54	28
	Coccinellidae	1	1	69	69	69
	Curculionidae	10	12	119	76	33
	Dermestidae	3	5	211	152	93
	Laemophloeidae	1	3	200	200	200
	Lyctidae	1	1	69	69	69
	Scarabaeidae	3	5	75	44	13
	Scolytidae	1	1	65	65	65
	Silvanidae	1	1	117	117	117
	Tenebrionidae	5	11	102	77	52
Dictyoptera	Blaberidae	1	1	5	5	5
	Blattellidae	1	2	32	32	32
	Oxyhaloidae	1	1	140	140	140

Table 3. Continued

Order	Family	Number of genera	Number of species	Sterilization dose (Gy)		
				L_2	Mean	L_1
Diptera	Agromyzidae	1	1	155	155	155
	Anthomyiidae	1	2	45	37	30
	Calliphoridae	3	5	40	30	20
	Chloropidae	1	1	45	45	45
	Culicidae	3	15	116	54	10
	Cuterebridae	1	1	200	150	100
	Drosophilidae	1	1	160	160	160
	Glossinidae	1	7	120	99	60
	Muscidae	4	6	30	26	20
	Oestridae	1	2	50	45	40
	Piophilidae	1	1	100	100	100
	Sarcophagidae	1	1	52	36	19
	Sciaridae	1	1	40	40	40
	Tachinidae	1	1	20	20	20
	Tephritidae	5	21	83	63	44
Hemiptera	Aleyrodidae	2	3	80	70	60
	Aphididae	2	2	10	10	10
	Cicadellidae	1	1	200	180	160
	Coreidae	2	2	80	80	80
	Delphacidae	2	2	50	50	50
	Lygaeidae	1	1	100	100	100
	Pentatomidae	2	3	60	60	50
	Pseudococcidae	2	1	160	160	160
	Pyrrhocoridae	1	1	70	70	70
	Reduviidae	3	3	150	80	10
Lepidoptera	Arctiidae	2	2	400	400	400
	Bombycidae	1	2	250	250	250
	Gelechiidae	3	3	200	200	150
	Lymantriidae	2	2	180	133	80
	Noctuidae	4	13	300	300	300
	Pieridae	1	1	350	350	350
	Plutellidae	1	1	200	200	200
	Pyralidae	11	16	389	260	131
	Sphingidae	1	1	100	100	100
	Thaumetopoeidae	1	1	40	40	40
	Tortricidae	8	12	330	278	226
Orthoptera	Acrididae	1	1	4	4	4
Thysanoptera	Thripidae	2	1	100	100	100

6. RADIATION DOSES FOR ARTHROPOD STERILIZATION

Arthropods are more radioresistant than human and other higher vertebrates (Table 4), but less resistant than viruses, protozoa and bacteria (Ravera 1967, Rice and Baptist 1974, Whicker and Schultz 1982, Blaylock et al. 1996, Harrison and Anderson 1996). One of the main reasons for the higher radioresistance is that arthropods have a discontinuous growth during immature stages, and cells become active only during the moulting process. This is encoded in Dyar's Rule, i.e. insects double their weight at each moult and thus their cells need to divide only once per moulting cycle (Hutchinson et al. 1997, Behera et al. 1999). The high resistance of most adult insects to radiation is attributed to the fact that they are composed of differentiated cells, which do not undergo replacement (Sullivan and Grosch 1953). Such cells are much more resistant to death or damage induced by irradiation than are dividing or undifferentiated cells.

Table 4. Ranges of LD_{50} for acute irradiation of organisms from different taxonomic groups (length of time for survival is usually set at 30 days for mammals, but longer times may be needed for other organisms) (Table from Bakri et al. 2005, reproduced with permission)

Group	Dose (Gy)	Reference
Bacteria, protozoa, viruses	100–10 000	Harrison and Anderson 1996
Insects	30– 1500	Whicker and Schultz 1982
Molluscs	50– 500	Ravera 1967
Higher plants	1.5–>130	Harrison and Anderson 1996
Fish	4– 100	Harrison and Anderson 1996
Amphibians	7– 22	Harrison and Anderson 1996
Reptiles	3– 40	Harrison and Anderson 1996
Birds	5– 20	Harrison and Anderson 1996
Humans	3	Rice and Baptist 1974

Radiation doses for sterilization, as reported in the literature (IDIDAS 2004), were selected using similar criteria, when available, for sterility level (full or as available), gender (male), and atmospheric condition (air). The developmental stage at irradiation was the pupa or nymph, except for mosquitoes and apple maggots *Rhagoletis pomonella* (Walsh), which were treated as adults. Other experimental parameters such as temperature, radiation source, dose rate, etc., may differ. Even compiling the data was difficult because of the absence of uniform experimental procedures and dosimetry, and the influence of various parameters. Dose values reported below may also differ from doses that are routinely used to sterilize

members of the reported taxa for the SIT, especially in cases where programmes irradiate insects in oxygen-reduced atmospheres. Therefore the doses presented should be considered only as guidelines for further investigation and to provide general introductory information.

6.1. Arachnidae

6.1.1. Acari

The mean dose to sterilize Acari species ranged from 32 to 270 Gy (Table 3). The fowl tick *Argas persicus* (Oken) (Argasidae), Chilean false red mite *Brevipalpus chilensis* Baker (Tenuipalpidae), grain mite *Acarus siro* L. (Acaridae), and brownlegged grain mite *Aleuroglyphus ovatus* (Troupeau) (Acaridae), are among the most resistant species. Hard ticks (Ixodidae), such as *Amblyomma* spp. and *Boophilus* spp., tend to be more sensitive than soft ticks (Argasidae). The radiation sensitivity of some tick species appears to change depending on whether the tick is engorged with blood or not (section 4.2.). Parthenogenesis occurs in many species of ticks and other arthropods, making the practical application of the SIT rather unlikely (Lance and McInnis, this volume).

6.1.2. Araneae

The only known cases of irradiation of spiders for sterilization were conducted to determine the pattern of sperm precedence (Lance and McInnis, this volume) in multiple-mated females. Kaster and Jakob (1997) used a 20-Gy dose to sterilize males of *Holocnemus pluchei* (Scopoli) (Pholcidae), whereas Schneider and Lubin (1996) applied a 150-Gy dose to *Stegodyphus lineatus* (Latreille) (Eresidae). These species showed last-male precedence and complete sperm mixing, respectively.

6.2. Insecta

6.2.1. Coleoptera

The mean sterilization dose for Coleoptera ranged from 43 to 200 Gy (Table 3). Curculionidae and Tenebrionidae, which represent the major groups of species that have been tested for radiation sterilization, both required a dose of about 76 Gy. The most resistant species belong to Laemophloeidae (200 Gy), and the most sensitive to Anobiidae (43 Gy). Some data in this order suggested a differential response of males and females towards sterilizing doses of radiation. Males may be less resistant than females, as in the case of the Japanese beetle *Popillia japonica* Newman (Ladd et al. 1973) and the beetle *Tribolium madens* (Charpentier) (Brower and Tilton 1973), or more resistant as in the case of the khapra beetle *Trogoderma granarium* Everts (Carney 1959, Nair and Rahalkar 1963).

The effects of gamma radiation on the boll weevil *Anthonomus grandis grandis* Boheman were thoroughly studied with a view to applying the SIT (Earle et al. 1979, Villavaso et al. 1989, Haynes 1993). Males were sterilized by about 80 Gy, but longevity was poor; egg-laying capacity was reduced at doses of 50 Gy or more,

but females continued to produce some fertile eggs until doses approached 200 Gy, a dose which rendered the weevils non-competitive (McKibben et al. 2001). Eventually methods were found to block egg hatch using a chitin synthesis inhibitor (diflubenzuron), and studies were conducted on reducing the negative effects of radiation using improved mass-reared strains, oxygen-reduced atmospheres, and fractionated radiation doses (Earle et al. 1979, Haynes 1993, McKibben et al. 2001). Competitive sterile boll weevils can now be delivered to the field, but the SIT remains more complicated and expensive than current pest management strategies, which employ pheromone traps and chemical control (McKibben et al. 2001).

The detrimental effects of radiation have also been one of the main obstacles in applying the SIT to the West Indian sweetpotato weevil *Euscepes postfasciatus* (Fairmaire). Digestive obstruction following the collapse of the epithelial tissue of the midgut was suggested as the cause of the short lifespan of gamma-irradiated adults (Sakurai et al. 2000). Nevertheless an AW-IPM programme in Kume Island, Japan, to eradicate this weevil using the SIT is making good progress (Shimoji and Miyatake 2002, Shimoji and Yamagishi 2004).

6.2.2. Dictyoptera

Several species of dictyopterans have been used in radiobiological studies exploring biochemical, physiological, and genetic properties (Shivaji and Rastogi 1974). In spite of the pest status of many cockroach species, there have been relatively few investigations related to pest suppression using sterile insects, due largely to potential problems with releasing large numbers of males into natural populations (Ross and Cochran 1963, Berndt 1978, Menon 1978, Ross et al. 1981, Gecheva and Apostolova 1986). In terms of sterility and mortality, cockroaches are among the most radiation-sensitive insects, with less than 5 Gy required in some cases to induce sterility (Wharton and Wharton 1959, Ross and Cochran 1963). Sexual differentiation in radiosensitivity was observed in *Blaberus craniifer* Burmeister, where males were less resistant than females (Gecheva and Apostolova 1986). In Dictyoptera, it is the adult stage that is most frequently used for sterilization with ionizing radiation.

6.2.3. Diptera

Radiation sterilization of dipterans generally requires doses from 20 to 160 Gy (Table 3). Drosophilidae and Agromyzidae are among the most radiation-resistant families of Diptera tested, whereas tachinids are the most sensitive. The late pupal (often pharate adult) stage is preferred for the radiation of most fly species because it is practical to handle and ship pupae, and an acceptable balance between competitiveness and sterility is achieved. In the Culicidae, due to the fragility of other stages, the adult is irradiated.

Tephritidae, the major family in this group that uses the SIT, require, on average, about 63 Gy for sterilization. Tephritids are relatively homogeneous with respect to radiation sensitivity — less than 100 Gy are needed to achieve complete sterility in the five major pest genera (*Anastrepha, Bactrocera, Ceratitis, Dacus, Rhagoletis*), and this confirms the generic recommendation of a dose in the range of 100–150 Gy

to disinfest agricultural commodities for international trade (Hallman 2000). Many AW-IPM programmes applying the SIT against major pest tephritids have used 100–150 Gy for sterilization, well over the 64-Gy family "average". In some early programmes (LaChance et al. 1967), this was a "precaution" to increase the security margin for sterilization, but the overdose often has lowered competitiveness to the point where it reduced the overall ability of irradiated flies to induce sterility into the wild population (Toledo et al. 2004). In recent programmes, these higher doses are usually associated with the use of hypoxia to enhance sterile male competitiveness (section 4.2.) (Fisher 1997).

6.2.4. Hemiptera

The mean sterilizing dose in the Hemiptera ranged from 10 to 180 Gy (Table 3), with *Circulifer tenellus* (Baker) females (Cicadellidae) being the most resistant species tested thus far (Ameresekere and Georghiou 1971), and *Brachycorynella asparagi* (Mordvilko) (Aphididae) adults being the most sensitive. In general, adult females required a gamma radiation dose of 50–60 Gy to achieve a high level of sterility. However, higher doses of up to 200 Gy (electrons in this case) were needed to achieve complete sterility in female *Myzus persicae* (Sulzer) (Aphididae) and *Pseudococcus comstocki* (Kuwana) (Pseudococcidae) (Dohino et al. 1997). Adult males typically required a dose between 60 and 150 Gy. For 4^{th}- and 5^{th}-instar nymphs, a lower dose was needed; 75 to 100% sterility was achieved with doses between 5 and 60 Gy. Patterns of relative radiosensitivity between females and males differ among species of Hemiptera (IDIDAS 2004).

Only 19 species belonging to 10 out of 53 families of Hemiptera have been subjected to radiation for sterilization. For several species, the feasibility of releasing sterile males for pest suppression was investigated (Shipp et al. 1966, Baldwin and Chant 1971, Tadic 1972, Dyby and Sailer 1999, Calvitti et al. 2000). Some hemipterans are facultatively parthenogenetic, but Steffan and Kloft (1973) argued that, with proper timing and climate, effective genetic control might still be possible.

6.2.5. Hymenoptera

The Hymenoptera include a number of serious pests, such as Africanized honey bees, and various Formicidae (ants) and sawflies. Since bees and ants are social insects with complex life histories, the application of the SIT has been limited to a few laboratory experiments (Sakamoto and Takahashi 1981). For male honey bees, the sterilizing dose is 80–100 Gy (Lee 1958). Most experimental irradiations of hymenopterans, e.g. the parasitic wasp *Bracon hebetor* (Say), have been conducted in conjunction with relatively basic radiobiological investigations. (For these reasons, the doses for sterilization or disinfestation of this group are not included in Table 3.)

6.2.6. Lepidoptera

Lepidopterans as a group are relatively resistant to radiation; mean doses for sterilization range from 40 to 400 Gy, with *Thaumetopoea pityocampa* (Denis and Schiffermüller) (Thaumetopoeidae) requiring the lowest documented average dose

of 40 Gy (Baccetti and Zocchi 1962), while the arctiid *Diacrisia obliqua* Walker has the highest recorded doses — 300 Gy and 400 Gy for complete sterility of pupae and adults, respectively (Khattak 1998). Successful lepidopteran AW-IPM programmes that integrate the SIT (Bloem et al., this volume) include the codling moth in Canada (Proverbs 1982), and the pink bollworm *Pectinophora gossypiella* (Saunders) in the USA (Henneberry and Clayton 1988).

In contrast to other insect orders, the F_1 progeny of irradiated male lepidopterans are typically more sterile than their parents. The sex ratio in the F_1 generation is biased toward males. Thus substerilized males can sire completely sterile offspring, and this phenomenon has been exploited in a number of programmes (Carpenter et al., this volume).

6.2.7. Orthoptera

Acrididae (Orthoptera), along with Blaberidae (Dictyoptera), are among the most radiosensitive insects known (less than 5 Gy needed for sterilization). This is in agreement with Willard and Cherry (1975) who suggested that large long-lived adults are more radiosensitive than small short-lived adults.

6.2.8. Thysanoptera

No species of Thysanoptera has been investigated for pest suppression directly using the SIT. However, in Japan, radiation sterilization has been investigated as a quarantine treatment to disinfest cut flowers of thysanopteran pests. Doses up to 400 Gy (electron beam) and 100 Gy (gamma rays) were given to suppress the pests *Frankliniella occidentalis* (Pergande) (EPPO 1994) and *Thrips* spp. (Dohino et al. 1996, Hayasi et al. 1999, Bansiddhi 2000), respectively.

7. CONCLUSIONS

Although radiation is such a key component of the SIT, it is generally not given the attention it deserves — in terms of dosimetry, and the choice of an appropriate dose to maximize the introduction of sterility into wild females. The development of accurate dose-response curves, using precise dosimetry for the target insect, is a prerequisite of any programme releasing sterile insects. The survey of the available literature presented here shows the wide variation in the response of the different insect species to radiation, and also highlights the need for accurate dosimetry.

8. REFERENCES

Adem, E., F. L. Watters, R. Uribe-Rendon, and A. de la Piedad. 1978. Comparison of Co-60 gamma radiation and accelerated electrons for suppressing emergence of *Sitophilus* spp. in stored maize. Journal of Stored Products Research 14: 135–142.

Ahmed, M. Y. Y., M. A. R. Daoud, E. S. El-Hawary, and S. A. Boshra. 1990. Susceptibility of the pupal stage of *Plodia interpunctella* to gamma irradiation as affected by radiation dose, age, sex and temperature, pp. 1119. *In* F. Fleurat-Lessard and P. Ducom (eds.), Proceedings, 5th Working Conference on Stored-Product Protection. Volume II.

Anwar, M., D. L. Chambers, K. Ohinata, and R. M. Kobayashi. 1971. Radiation-sterilization of the Mediterranean fruit fly (Diptera: Tephritidae): comparison of spermatogenesis in flies treated as pupae or adults. Annals of the Entomological Society of America 64: 627–633.

Ameresekere, R. V. W. E., and G. P. Georghiou. 1971. Sterilization of the beet leafhopper: induction of sterility and evaluation of biotic effects with a model sterilant (OM-53139) and Co60 irradiation. Journal of Economic Entomology 64: 1074–1080.

Ashraf, M., N. Chatha, K. Ohinata, and E. J. Harris. 1975. Melon flies: dosage-response and sexual competitiveness after treatment with gamma irradiation in a nitrogen atmosphere. Journal of Economic Entomology 68: 838–840.

Baccetti, B., and R. Zocchi. 1962. Prove di lotta contro la processionaria del pino mediante l'uso di radiazioni jonizzanti [Possible use of ionizing radiation against the pine processionary moth]. Redia 47: 161–168.

Bakri, A., N. Heather, J. Hendrichs, and I. Ferris. 2005. Fifty years of radiation biology in entomology: lessons learned from IDIDAS. Annals of the Entomological Society of America 98: 1–12.

Baldwin, W. F., and G. D. Chant. 1971. Use of nitrogen during irradiation to improve competitiveness in sterile males of *Rhodnius prolixus*, pp. 469–475. *In* Proceedings, Symposium: Sterility Principle for Insect Control or Eradication. International Atomic Energy Agency, and the Food and Agriculture Organization of the United Nations, 14–18 September 1970, Athens, Greece. STI/PUB/265. IAEA, Vienna, Austria.

Bansiddhi, K. 2000. Combination treatments with irradiation for controlling orchid thrips, *Thrips palmi* Karny. Report, 2nd research coordination meeting on irradiation as a phytosanitary treatment for food and agricultural commodities. FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria.

Bartlett, A. C., and R. T. Staten. 1996. The sterile insect release method and other genetic control strategies. http://ipmworld.umn.edu/chapters/bartlett.htm

Baumhover, A. H., A. J. Graham, B. A. Bitter, D. E. Hopkins, W. D. New, F. H. Dudley, and R. C. Bushland. 1955. Screwworm control through release of sterilized flies. Journal of Economic Entomology 48: 462–466.

Behera, M. K., R. Behera, and B. Patro. 1999. Application of Dyar's rule to the development of *Macrosiphoniella sanborni* (Gill.) (Homoptera: Aphididae). Agricultural Science Digest (Karnal) 19: 179–182.

Berndt, K. P. 1978. Genetic control of *Blattella germanica*. Angewandte-Parasitologie 19: 41–50.

Beuthner, U. 1975. On the sterile male release technique: sterilization by gamma-radiation (60Co) in *Amblyomma variegatum* (Fabricius 1794), *Hyalomma anatolicum excavatum* (Koch 1844), and *Rhipicephalus appendiculatus* (Neumann 1901). Thesis Dissertation, Freie University of Berlin, Berlin, Germany.

Blaylock, B. G., C. W. Theodorakis, and L. R. Shugart. 1996. Biological effects of ionizing radiation, pp. 39–50. *In* B. Amiro, R. Avadhanula, G. Johansson, C. M. Larsson and M. Luning (eds.), Proceedings, Symposium: Ionizing Radiation. Swedish Radiation Protection Institute (SSI) and Atomic Energy Control Board (AECB) of Canada, 20–24 May 1996, Stockholm, Sweden. SSI, Stockholm, Sweden.

Bloem, S., K. A. Bloem, J. E. Carpenter, and C. O. Calkins. 1999. Inherited sterility in codling moth (Lepidoptera: Tortricidae): effect of substerilizing doses of radiation on insect fecundity, fertility and control. Annals of the Entomological Society of America 92: 222–229.

Bracken, G. K., and C. D. Dondale. 1972. Fertility and survival of *Achaearanea tepidariorum* (Araneida: Theridiidae) on a diet of chemosterilized mosquitoes. Canadian Entomologist 104: 1709–1712.

Brower, J. H., and E. W. Tilton. 1973. Comparative gamma radiation sensitivity of *Tribolium madens* (Charpentier) and *T. castaneum* (Herbst). Journal of Stored Products Research 9: 93–100.

Bushland, R. C., and D. E. Hopkins. 1951. Experience with screw-worm flies sterilized by X-rays. Journal of Economic Entomology 44: 725–731.

Bushland, R. C., and D. E. Hopkins. 1953. Sterilization of screw-worm flies with X-rays and gamma rays. Journal of Economic Entomology 46: 648–656.

Calvitti, M., P. C. Remotti, and U. Cirio. 2000. The sterile insect technique in the integrated pest management of whitefly species in greenhouses, pp. 185–192. *In* K. H. Tan (ed.), Proceedings: Area-Wide Control of Fruit Flies and Other Insect Pests. International Conference on Area-Wide Control

of Insect Pests, and the 5th International Symposium on Fruit Flies of Economic Importance, 28 May–5 June 1998, Penang, Malaysia. Penerbit Universiti Sains Malaysia, Pulau Pinang, Malaysia.

Carney, G. C. 1959. Differential response of male and female adults of *Trogoderma granarium* Everts towards sterilizing doses of gamma-radiation. Nature 183: 338–339.

Carpenter, J. E., and H. R. Gross. 1989. Interaction of inherited sterility and diapause in the corn earworm (Lepidoptera: Noctuidae). Journal of Economic Entomology 82: 1354–1357.

Carpenter, J. E., J. R. Young, A. N. Sparks, H. L. Cromroy, and M. A. Chowdhury. 1987. Corn earworm (Lepidoptera: Noctuidae): effects of substerilizing doses of radiation and inherited sterility on reproduction. Journal of Economic Entomology 80: 483–489.

Casarett, A. P. 1968. Radiation biology. American Institute of Biological Sciences; U.S. Atomic Energy Commission. Prentice-Hall, Englewood Cliffs, NJ, USA.

Cavalloro, R., and G. Delrio. 1974. Sterilisation of *Dacus oleae* Gmel. and *Ceratitis capitata* Wied. with gamma radiation and fast neutrons. Redia 55: 373–392.

Chand, A. T., and S. S. Sehgal. 1978. Sensitivity to gamma radiation of *Corcyra cephalonica* eggs related to their age. Entomon 3: 1, 7–9.

Cleland, M. R., and G. M. Pageau. 1985. Electrons versus gamma rays — alternative sources for irradiation processes, pp. 397–406. *In* Proceedings, Symposium: Food Irradiation Processing. International Atomic Energy Agency and the Food and Agriculture Organization of the United Nations, 4–8 March 1985, Washington, DC, USA. STI/PUB/695. IAEA, Vienna, Austria.

Codex Alimentarius. 1983. Recommended international code of practice for the operation of irradiation facilities used for the treatment of foods. CAC/RCP 19–1979, Rev. 1–1983. http://www.codexalimentarius.net/

Cogburn, R. R., E.W. Tilton, and J. H. Brower. 1973. Almond moth: gamma radiation effects on the life stages. Journal of Economic Entomology 66: 745–751.

Dey, S. K., and G. K. Manna. 1983. Differential stage sensitivity to X-rays in a bug *Physopelta schlanbuschi*. National Academic of Science Letter 16: 101–103.

Dohino, T., K. Tanabe, and T. Hayashi. 1994. Comparison of lethal effects of electron beams and gamma rays on eggs of two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). Research Bulletin of the Plant Protection Service, Japan. Number 30: 69–73.

Dohino, T., K. Tanabe, S. Masaki, and T. Hayashi. 1996. Effects of electron beam irradiation on *Thrips palmi* Karny and *Thrips tabaci* Lindeman (Thysanoptera: Thripidae). Research Bulletin Plant Protection Japan 32: 23–29.

Dohino, T., S. Masaki, T. Takano, and T. Hayashi. 1997. Effects of electron beam irradiation on sterility of Comstock mealybug, *Pseudococcus comstocki* (Kuwana) (Homoptera: Pseudococcidae). Research Bulletin Plant Protection Service Japan 33: 31–34.

Dongre, T. K., M. R. Harwalkar, S. P. Nene, and S. R. Padwal-Desai. 1997. Radio-sensitivity of different developmental stages of pulse beetle (*Callosobruchus maculatus*). Journal Food Science and Technology 34: 413–415.

Drummond, R. O., J. G. Medley, and O. H. Graham. 1966. Engorgement and reproduction of lone star ticks (*Amblyomma americanum* L.) treated with gamma radiation. International Journal of Radiation Biology 10: 183–188.

Dyby, S. D., and R. I. Sailer. 1999. Impact of low-level irradiation on fertility and fecundity of *Nezara viridula* (Hemiptera: Pentatomidae). Journal of Economic Entomology 92: 945–953.

Earle, N. W., L. A. Simmons, and S. L. Nilakhe. 1979. Laboratory studies of sterility and competitiveness of boll weevils irradiated in an atmosphere of nitrogen, carbon dioxide, or air. Journal of Economic Entomology 72: 687–691.

(EBFRF) Electron Beam Food Research Facility. 2004. Institute of Food Science and Engineering, Texas A&M University. http://ifse.tamu.edu/centers/ebeam.html

Economopoulos, A. P. 1977. Gamma-ray sterilization of *Dacus oleae* (Gmelin). Effect of nitrogen on the competitiveness of irradiated males. Zeitschrift für Angewandte Entomologie 83: 1, 86–95.

Elias, P. S., and A. J. Cohen. 1977. Radiation chemistry of major food components: its relevance to the assessment of the wholesomeness of irradiated foods. Elsevier, Amsterdam, The Netherlands.

(EPPO) European and Mediterranean Plant Protection Organization. 1994. Irradiation of cut flowers to control insects and mites. Phytosanitary Procedure Number 49. EPPO Bulletin 24: 318.

(FAO/IAEA/USDA) Food and Agriculture Organization of the United Nations/International Atomic Energy Agency/United States Department of Agriculture. 2003. Manual for product quality control and shipping procedures for sterile mass-reared tephritid fruit flies. Version 5.0. IAEA, Vienna, Austria. http://www.iaea.org/programmes/nafa/d4/index.html

(FAO/IAEA/WHO) Food and Agriculture Organization of the United Nations/International Atomic Energy Agency/World Health Organization. 1999. High-dose irradiation: wholesomeness of food irradiated with doses above 10 kGy. Joint FAO/IAEA/WHO Study Group, Technical Report Series 890. World Health Organization, Geneva, Switzerland.

Farrell, J. P., S. M. Seltzer, and J. Sillverman. 1983. Bremsstrahlung generators for radiation processing. Radiation Physics and Chemistry 22: 469–478.

(FDACS) Florida Department of Agriculture and Consumer Services. 2004. Florida Accelerator Services and Technology, Bureau of Methods Development and Biological Control, Division of Plant Industry. http://www.doacs.state.fl.us/pi/methods/fast.htm

Fisher, K. 1997. Irradiation effects in air and in nitrogen on Mediterranean fruit fly (Diptera: Tephritidae) pupae in Western Australia. Journal of Economic Entomology 90: 1609–1614.

Fletcher, B. S., and A. Giannakakis. 1973. The selection of age is also affecting adult males of *Dacus (Strumeta) tryoni*. Journal of Economic Entomology 66: 62–64.

Flint, H. M., B. Wright, H. Sallam, and B. Horn. 1975. A comparison of irradiated or chemosterilized pink bollworm moths for suppressing native populations in field cages. Canadian Entomologist 107: 1069–1072.

Gecheva, G. G., and V. M. Apostolova. 1986. Attempts at sterilization of the cockroach *Blaberus craniifer* with gamma-radiation. Ekologiia, Bulgaria 18: 40–44.

Gonzalez, A. J. 1999. Strengthening the safety of radiation sources and the security of radioactive materials: timely action. IAEA Bulletin 41: 2–17.

Guerra, A. A., D. A. Wolfenbarger, D. E. Hendricks, R. D. Garcia, and J. R. Raulston. 1972. Competitiveness and behaviour of tobacco budworms sterilized with reserpine and gamma irradiation. Journal of Economic Entomology 65: 966–969.

Hallman, G. J. 1998. Ionizing radiation quarantine treatments. Anais da Sociedade Entomologica do Brasil 27: 313–323.

Hallman, G. J. 2000. Expanding radiation quarantine treatments beyond fruit flies. Agricultural and Forestry Entomology 2: 85–95.

Hallman, G. J. 2003. Ionizing irradiation quarantine treatment against plum curculio (Coleoptera: Curculionidae). Journal of Economic Entomology 96: 1399–1404.

Hamed, M., and S. U. Khattak. 1991. Effect of larval irradiation on development of cabbage butterfly *Pieris brassicae* (L.). Pakistan Journal of Zoology 23: 115–117.

Harrison, F. L., and S. L. Anderson. 1996. Taxonomic and development aspects of radiosensitivity, pp. 65–88. *In* B. Amiro, R. Avadhanula, G. Johansson, C. M. Larsson and M. Luning (eds.), Proceedings, Symposium: Ionizing Radiation. Swedish Radiation Protection Institute (SSI) and Atomic Energy Control Board (AECB) of Canada, 20–24 May 1996, Stockholm, Sweden. SSI, Stockholm, Sweden.

Hayasi, T., S. Todoriki, H. Nakakita, T. Dohino, and K. Tanabe. 1999. Effectiveness of electron irradiation as a quarantine treatment of cut flowers, pp. 49–56. *In* Irradiation as a quarantine treatment of arthropod pests. IAEA-TECDOC-1082. International Atomic Energy Agency, Vienna, Austria.

Hayes, W. J., Jr. 1968. Toxicological aspects of chemosterilants, pp. 315–347. *In* G. C. Labecque and C. N. Smith (eds.), Principles of insect chemosterilization. Appleton-Century-Crofts, New York, USA.

Haynes, J. W. 1993. Fractionated irradiation regimens for sterilizing boll weevils. Journal of Agricultural Entomology 10: 89–95.

Henneberry, T. J., and T. E. Clayton. 1988. Effects of gamma radiation on pink bollworm (Lepidoptera: Gelechiidae) pupae: adult emergence, reproduction, mating, and longevity of emerged adults and their F_1 progeny. Journal of Economic Entomology 81: 322–326.

Hooper, G. H. S. 1971. Sterilization and competitiveness of the Mediterranean fruit fly after irradiation of pupae with fast neutrons. Journal of Economic Entomology 64: 1369–1372.

Hooper, G. H. S. 1975. Sterilization of *Dacus cucumis* French (Diptera: Tephritidae) by gamma radiation. II. Effect of dose rate on sterility and competitiveness of adult males. Journal Australian Entomological Society 14: 175–177.

Hooper, G. H. S. 1989. The effect of ionizing radiation on reproduction, pp. 153–164. *In* A. S. Robinson and G. Hooper (eds.), World crop pests, Volume 3A, Fruit flies, their biology, natural enemies and control. Elsevier, Amsterdam, The Netherlands.

Hutchinson, J. M. C., J. M. McNamara, A. I. Houston, and F. Vollrath. 1997. Dyar's Rule and the investment principle: optimal moulting strategies if feeding rate is size-dependent and growth is

discontinuous. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 352: 113–138.

(IAEA) International Atomic Energy Agency. 1992. Radiation safety of gamma and electron irradiation facilities. Safety Series, Number 107. IAEA, Vienna, Austria.

(IAEA) International Atomic Energy Agency. 1996a. International basic safety standards for protection against ionizing radiation and for the safety of radiation sources. Joint publication of the FAO, IAEA, ILO, OECD/NEA, PAHO and WHO. Safety Series Number 115. IAEA, Vienna, Austria.

(IAEA) International Atomic Energy Agency. 1996b. Lessons learned from accidents in industrial irradiation facilities. IAEA, Vienna, Austria.

(IAEA) International Atomic Energy Agency. 2002a. Natural and induced radioactivity in food. IAEA-TECDOC-1287. IAEA, Vienna, Austria.

(IAEA) International Atomic Energy Agency. 2002b. Dosimetry for food irradiation. Technical Reports Series Number 409. IAEA, Vienna, Austria.

(IAEA) International Atomic Energy Agency. 2003. Practice specific model regulations: radiation safety of non-medical irradiation facilities. IAEA-TECDOC-1367. IAEA, Vienna, Austria.

(IAEA) International Atomic Energy Agency. 2004. Gafchromic® dosimetry system for SIT. Standard operating procedure. Version 1.0b. FAO/IAEA Report. IAEA, Vienna, Austria. http://www.iaea.org/programmes/nafa/d4/index.html

(IDIDAS) International Database on Insect Disinfestation and Sterilization. 2004. http://www-ididas.iaea.org/IDIDAS/default.htm

Ignatowicz, S. 1997. Susceptibility of diapausing larvae of the khapra beetle, *Trogoderma granarium* Everts, to gamma radiation. Annals of the Warsaw Agricultural University 31: 47–52.

Ilao, L. L. 1977. Effect of different radiation doses and dose rates on 2-day-old pupae of the oriental fruit fly, *Dacus dorsalis* Hendel. Araneta Research Journal 24: 34–38.

Ismail, I. I., A. K. M. El-Nahal, A. H. Kame, and T. M. Mostafa. 1987. Effect of gamma radiation on the different stages of the Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae). Insect Science and its Application 8: 327–329.

(ISO/ASTM) International Organization for Standardization/American Society for Testing and Materials. 2004a. Standard guide for dosimetry for sterile insect release programs, ISO/ASTM 51940. Annual book of ASTM standards 12.02. ASTM International, Philadelphia, PA, USA.

(ISO/ASTM) International Organization for Standardization/American Society for Testing and Materials. 2004b. Standard guide for selection and calibration of dosimetry systems for radiation processing. ISO/ASTM 51261. Annual book of ASTM standards 12.02. ASTM International, Philadelphia, PA, USA.

(ISO/ASTM) International Organization for Standardization/American Society for Testing and Materials. 2004c. Standard guide for dosimetry in radiation research on food and agricultural products. ISO/ASTM 51900. Annual book of ASTM standards 12.02. ASTM International, Philadelphia, PA, USA.

(ISO/ASTM) International Organization for Standardization/American Society for Testing and Materials. 2004d. Standard practice for dosimetry for a self-contained dry-storage gamma-ray irradiator. ISO/ASTM 52116. Annual book of ASTM standards 12.02. ASTM International, Philadelphia, PA, USA.

(ISO/ASTM) International Organization for Standardization/American Society for Testing and Materials. 2004e. Standard guide for use of radiation-sensitive indicators. ISO/ASTM 51539. Annual book of ASTM standards 12.02. ASTM International, Philadelphia, PA, USA.

Jacquet, P., and A. Leonard. 1983. Studies on the differential radiosensitivity of the male and female pronuclei in the mouse zygote, C4–08. *In* J. J Broerse, G. W. Barendsen and A. J. Van der Kogel (eds.), Proceedings, Congress: Radiation Research, Somatic and Genetic Effects. The 7th International Congress of Radiation Research, 3–8 July 1983, Amsterdam, The Netherlands. Martinus Nijhoff Publishers, Amsterdam, The Netherlands.

Kaster, J. L., and E. M. Jakob. 1997. Last-male sperm priority in a haplogyne spider (Araneae: Pholcidae): correlations between female morphology and patterns of sperm usage. Annals of the Entomological Society of America 90: 254–259.

Khattak, S. U. 1998. Induction of sterility and mortality in soybean hairy caterpillar, *Diacrisia obliqua* Walker by gamma radiation. International Journal of Tropical Agriculture 16: 123–129.

Kiritani, K. 1963. Oviposition habit and effect of parental age upon the post-embryonic development in the southern green stink bug, *Nezara viridula*. Japanese Journal of Ecology 13: 88–96.

Klassen, W., and F. Matsumura. 1966. Resistance to a chemosterillant, Metepa, in *Aedes aegypti,* mosquitoes. Nature 209: 1155–1156.

Knipling, E. F. 1979. The basic principles of insect population suppression and management. Agriculture Handbook Number 512. SEA, USDA, Washington, DC, USA.

Knipling, E. F. 1982. Present status and future trends of the SIT approach to the control of arthropod pests, pp.3–23. *In* Proceedings, Symposium: Sterile Insect Technique and Radiation in Insect Control. Food and Agriculture Organization of the United Nations, and the International Atomic Energy Agency, 29 June–3 July 1981, Neuherberg, Germany. STI/PUB/595. IAEA, Vienna, Austria.

Kuznetsova, V. G., and M. B. Petropavlovskaia. 1976. Behavior of holokinetic chromosomes in the spermatogenesis of bugs. Tsitologiia 18: 702–711.

LaChance, L. E., and C. K. Graham. 1984. Insect radiosensitivity: dose curves and dose fractionation studies of dominant lethal mutations in the mature sperm of 4 insect species. Mutation Research 127: 49–59.

LaChance, L. E., and A. P. Leverich. 1962. Radiosensitivity of developing reproductive cells in female *Cochliomyia hominivorax*. Genetics 47: 721–735.

LaChance, L. E., and J. G. Riemann. 1973. Dominant lethal mutations in insects with holokinetic chromosomes. 1. Irradiation of *Oncopeltus* (Hemiptera: Lygaeidae) sperm and oocytes. Annals of the Entomological Society of America 66: 813–819.

LaChance, L. E., C. H. Schmidt, and R. C. Bushland. 1967. Radiation-induced sterilization, pp. 147–196. *In* W. W. Kilgore and R. L. Doutt (eds.), Pest control. Academic Press, New York, USA.

Ladd, T. L., R. T. Canfield, and A. J. Forgash. 1973. Sterilization of Japanese beetles by gamma radiation. Journal of Economic Entomology 66: 1047–1048.

(LAF) Linear Accelerator Facility. 2004. Department of Animal Science, Iowa State University. http://www.ag.iastate.edu/centers/linac/

Lee, W. R. 1958. The dosage response curve for radiation induced dominant lethal mutations in the honeybee. Genetics 43: 480–492.

Lindquist, A. W. 1955. The use of gamma radiation for the control or eradication of the screwworm. Journal of Economic Entomology 48: 467–469.

Mansour, M. 2003. Gamma irradiation as a quarantine treatment for apples infested by codling moth (Lepidoptera: Tortricidae). Journal of Applied Entomology 127: 137–141.

Mau, R. F. L., W. C. Mitchell, and M. Anwar. 1967. Preliminary studies on the effects of gamma irradiation of eggs and adults of the southern green stink bug, *Nezara viridula* (L.). Proceedings of the Hawaiian Entomological Society 19: 415–417.

Mayas, I. 1975. The effect of fractionation of the sterilising dose of gamma-rays on the emergence, fertility and competitiveness of the Mediterranean fruit fly, *Ceratitis capitata* Wied., pp. 145–152. *In* Proceedings, Symposium: The Use of Isotopes in Pesticides and Pest Control. March 1974, Beirut, Lebanon. National Council for Scientific Research, Beirut, Lebanon.

McKibben, G. H., E. J. Villavaso, W. L. McGovern, and B. Grefenstette. 2001. United States Department of Agriculture — research support, methods development and program implementation, pp. 101–136. *In* W. A. Dickerson, A. L. Brashear, J. T. Brumley, F. L. Carter, W. J. Grefenstette and F. A. Harris [eds.], Boll weevil eradication in the United States through 1999. Cotton Foundation Publisher, Memphis, TN, USA.

Menon, M. 1978. Changes in pheromone production, release, mating behaviour and reproductive ability of the gamma-irradiated cockroach *Nauphoeta cinerea* (Olivier). International Journal of Radiation Biology and Related Studies in Physics, Chemistry and Medicine 33: 541–549.

Mitchell, W. C., and R. F. L. Mau. 1969. Sexual activity and longevity of the southern green stink bug, *Nezara viridula*. Annals of the Entomological Society of America 62: 1246–1247.

Moursy, L. E., N. M. Eesa, L. K. Cutkomp, and B. H. E. Subramanyam. 1988. Effects of gamma radiation and a chemosterilant applied to late nymphal instars of the large milkweed bug (Hemiptera: Lygaeidae). Journal of Economic Entomology 81: 459–462.

Nair, K. K., and G. W. Rahalkar. 1963. Studies on the effects of gamma radiation on the different developmental stages of the khapra beetle, *Trogoderma granarium* Everts, pp. 465–477. *In* Proceedings, Symposium: Radiation and Radioisotopes Applied to Insects of Agricultural Importance. Food and Agriculture Organization of the United Nations, and International Atomic Energy Agency, 22–26 April 1963, Athens, Greece. STI/PUB/74. IAEA, Vienna, Austria.

Nair, K. K., and G. Subramanyam. 1963. Effect of variable dose-rates on radiation damage in the rust-red flour beetle, *Tribolium castaneum* Herbst, pp. 426–429. *In* Proceedings, Symposium: Radiation and Radioisotopes Applied to Insects of Agricultural Importance. Food and Agriculture Organization

of the United Nations, and International Atomic Energy Agency, 22–26 April 1963, Athens, Greece. STI/PUB/74. IAEA, Vienna, Austria.

North, D. T. 1975. Inherited sterility in Lepidoptera. Annual Review of Entomology 20: 167–182.

Offori, E. D., and K. H. Czock. 1975. The use of 'fast' neutrons and gamma radiation to sterilize the tsetse fly *Glossina tachinoides* Westw. The International Journal of Applied Radiation and Isotopes 26: 257–260.

Ohinata, K., D. L. Chambers, M. Fujimoto, S. Kashiwai, and R. Miyabara. 1971. Sterilization of the Mediterranean fruit fly by irradiation: comparative mating effectiveness of treated pupae and adults. Journal of Economic Entomology 64: 781–784.

Ohinata, K., M. Ashraf, and E. J. Harris. 1977. Mediterranean fruit flies: sterility and sexual competitiveness in the laboratory after treatment with gamma irradiation in air, carbon dioxide, helium, nitrogen or partial vacuum. Journal of Economic Entomology 70: 165–168.

Ohinata, K., M. Fujimoto, H. Higa, N. Tanaka, and E. J. Harris. 1978. Mediterranean fruit fly: gamma irradiation in nitrogen and packaging for sterile insect release program in Los Angeles. Journal of Economic Entomology 71: 610–612.

Piedade-Guerreiro, J., and J. Gomes da Silva. 1983. Sterilization of the fruit fly, *Ceratitis capitata* (Wied.) by ionizing radiations, pp. 386–396. *In* R. Cavalloro (ed.), Proceedings, Symposium: Fruit Flies of Economic Importance. CEC/IOBC International Symposium, 16–19 November 1982, Athens, Greece. A. A. Balkema, Rotterdam, The Netherlands.

Pizzarello, D. J., and R. L. Witcofski. 1967. Basic radiation biology. Lea and Febiger Publisher, Philadelphia, PA, USA.

Proverbs, M. D. 1969. Induced sterilization and control of insects. Annual Review of Entomology 14: 81–102.

Proverbs, M. D. 1982. Sterile insect technique in codling moth control, pp. 85–99. *In* Proceedings, Symposium: Sterile Insect Technique and Radiation in Insect Control. Food and Agriculture Organization of the United Nations, and International Atomic Energy Agency, 29 June–3 July 1981, Neuherberg, Germany. STI/PUB/595. IAEA, Vienna, Austria.

Purnell, R. E., J. D. Dargie, B. Gilliver, A. D. Irvin, and M. A. Ledger. 1972. Some effects of irradiation on the tick *Rhipicephalus appendiculatus*. Parasitology 64: 429–440.

Rananavare, R. D., M. R. Harwalkar, and G. W. Rahalkar. 1991. Influence of modifying factors on induction of sterility and mating ability of potato tuberworm, *Phthorimaea operculella* (Zeller). Journal of Nuclear Agriculture and Biology 20: 199–205.

Ravera, O. 1967. The effect of X-ray on the demographic characteristics of *Physa acuta* (Gasteropoda: Basommatophora). Malacologia 5: 95–109.

Rice, T. R., and J. P. Baptist. 1974. Ecologic effects of radioactive emissions from nuclear power plants, pp. 373–439. *In* L. A. Sagan (ed.), Human and ecological effects of nuclear power plants. Charles C. Thomas Publisher, Springfield, IL, USA.

Riemann, J. G., and H. M. Flint. 1967. Irradiation effects on midguts and testes of the adult boll weevils *Anthonomus grandis*, determined by histological and shielding studies. Annals of the Entomological Society of America 60: 289–308.

Ross, M. H., and D. G. Cochran. 1963. Some early effects of ionizing radiation on the German cockroach, *Blattella germanica.* Annals of the Entomological Society of America 56: 256–261.

Ross, M. H., C. B. Keil, and D. G. Cochran. 1981. The release of sterile males into natural populations of the German cockroach *Blattella germanica*. Entomologia Experimentalis et Applicata 30: 241–253.

Ruhm, M. E., and C. O. Calkins. 1981. Eye-color changes in *Ceratitis capitata* pupae, a technique to determine pupal development. Entomologia Experimentalis et Applicata 29: 237–240.

Sakamoto, C. H., and C. S. Takahashi. 1981. Biological effects of gamma radiation on Africanized bees, pp. 284–287. *In* Proceedings: XXVIIIth International Congress of Apiculture, Acapulco, Mexico. Apimondia Publishing House, Bucharest, Romania.

Sakurai, H., Y. Murakami, T. Kohama, and T. Teruya. 2000. Sterilizing mechanism of gamma-radiation in the female of West Indian sweet potato weevil, *Euscepes postfasciatus*. Research Bulletin of the Faculty of Agriculture, Gifu University, Japan, Number 65: 13–20.

Schneider, J. M., and Y. Lubin. 1996. Infanticidal male eresid spiders. Nature 381: 655.

Seo, S. T., D. L. Williamson, and M. Fujimoto. 1987. *Ceratitis capitata* (Diptera: Tephritidae): colorimetric method to estimate age and rate of development of pupae for the sterile-insect technique. Journal of Economic Entomology 80: 1087–1090.

Shimoji, Y., and T. Miyatake. 2002. Adaptation to artificial rearing during generations in the West Indian sweetpotato weevil, *Euscepes postfasciatus* (Coleoptera: Curculionidae). Annals of the Entomological Society of America 95: 735–739.

Shimoji, Y., and M. Yamagishi. 2004. Reducing rearing cost and increasing survival rate of West Indian sweetpotato weevil, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae) on artificial larval diet. Applied Entomology and Zoology 39: 41–47.

Shipp, E., A. W. Osborn, and P. B. Hutchinson. 1966. Radiation sterilization of sugar-cane leafhoppers of the family Delphacidae. Nature 211: 98–99.

Shivaji, S., and S. C. Rastogi. 1974. X-ray induced changes in the activities of acid and alkaline phosphatase in *Periplaneta americana*. Current Science 43: 757–758.

Smittle, B. J. 1993. Irradiation of *Anastrepha suspensa* (Diptera: Tephritidae): new irradiation facility. Florida Entomologist 76: 224–227. http://fulltext10.fcla.edu/DLData/SN/SN00154040/0076_002/98p0217h.pdf

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. The principles and practice of statistics in biological research, 3rd ed. W. H. Freeman and Company, New York, NY, USA.

Sparrow, A. H., L. A. Schairer, and R. C. Sparrow. 1963. Relationships between nuclear volumes, chromosome numbers and relative radiosensitivities. Science 141: 163–166.

Sparrow, A. H., A. G. Underbrink, and R. C. Sparrow. 1967. Chromosomal and cellular radiosensitivity. I. The relationship of D_0 to chromosome volume and complexity in seventy-nine different organisms. Radiation Research 32: 915–945.

Stahler, N., and L. A. Terzian. 1963. The response of blood-fed *Aedes aegypti* to gamma radiation. Journal of Economic Entomology 56: 416–417.

Steffan, A. W., and W. J. Kloft. 1973. The possibilities of using the sterile male technique for aphid control — a theoretical discussion, pp. 129–136. *In* Proceedings, Panel: Computer Models and Application of the Sterile-Male Technique. Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture, 13–17 December 1971, Vienna, Austria. STI/PUB/340. IAEA, Vienna, Austria.

Sullivan, R. L., and D. S. Grosch. 1953. The radiation tolerance of an adult wasp. Nucleonics 11: 21–23.

Tadic, M. 1972. Sterilisation of overwintering adults of the sun pest (*Eurygaster* sp.). Zastita Bilja 23: 65–71.

Tamhankar, A. J., and K. Shantharam. 2001. Sterile insect technique (SIT) for control of spotted bollworm of cotton: field experiments confirm dose fractionation improves mating competitiveness of sterile males, pp. 250–252. *In* N. Ramamoorthy, M. Ananthakrishnan and A. N. Nandakumar (eds.), Proceedings, Conference: Applications of Radioisotopes and Radiation Technology in the 21st Century. National Association for Applications of Radioisotopes and Radiation in Industry, 12–14 December 2001, Mumbai, India. NAARRI, Mumbai, India.

Thornburn, C. C. 1972. Isotopes and radiation in biology. Butterworth and Co. (Publishers) Ltd., London, UK.

Toledo, J., J. Rull, A. Oropeza, E. Hernandez, and P. Liedo. 2004. Irradiation of *Anastrepha obliqua* (Diptera: Tephritidae) revisited: optimizing sterility induction. Journal of Economic Entomology 97: 383–389.

Villavaso, E. J., J. L. Roberson, and R. W. Seward. 1989. Effectiveness of sterile boll weevils (Coleoptera: Curculionidae). Journal of Economic Entomology 82: 472–476.

Vreysen, M. J. B. 1995. Radiation induced sterility to control tsetse flies. The effect of ionizing radiation and hybridisation on tsetse biology and the use of the sterile insect technique in integrated tsetse control. Ph.D. Dissertation, University of Wageningen, Wageningen, The Netherlands.

Walker, M. L., W. L. McLaughlin, J. M. Puhl, and P. Gomes. 1997. Radiation-field mapping of insect irradiation canisters. Applied Radiation and Isotopes 48: 117–125.

Watters, F. L. 1979. Potential of accelerated electrons for insect control in stored grain, pp. 278–286. *In* Proceedings: Second International Working Conference on Stored-Product Entomology, 10–16 September 1978, Ibadan, Nigeria. IWCSPP, Manhattan, KS, USA.

Wharton, D. R. A., and M. L. Wharton. 1959. The effect of radiation on the longevity of cockroach, *Periplaneta americana* L., as affected by dose, age, sex and food intake. Radiation Research 11: 600–615.

Wharton, M. L., and D. R. A. Wharton. 1957. The production of sex attractant and oothecae by the normal and irradiated American cockroach, *Periplaneta americana* L. Journal of Insect Physiology 1: 229–239.

Whicker, F. W., and V. Schultz. 1982. Radioecology: nuclear energy and the environment. CRC Press, Boca Raton, FL, USA.

Willard, W. K., and D. S. Cherry. 1975. Comparative radiosensitivity in the class Insecta. Journal of Theoretical Biology 52: 149–158.

Williamson, D. L., S. Mitchell, and S. T. Seo. 1985. Gamma irradiation of the Mediterranean fruit fly (Diptera: Tephritidae): effects of puparial age under induced hypoxia on female sterility. Annals of the Entomological Society of America 78: 101–106.

Yanders, A. F. 1959. Dose rate effect in *Drosophila melanogaster.* Radiation Research 10: 30–36.