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Ionizing Radiations in Entomology

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Abstract

Radiation in the form of particles (α or β particles and neutrons) or electromagnetic waves (gamma or X-rays) can induce biological effects in insect cells like in other living cells. Ionization and chemical damages to organic molecules can be caused directly (mostly by particulate types of radiation) or indirectly by free radicals. Radioinduced ions and radicals, most of them coming from water radiolysis, may react with neighboring molecules to produce secondary DNA radicals or even chain reactions, particularly in lipids, and most of the significant biological effects results from damage to DNA. Currently, more than 300 species of arthropods, mostly of economic importance, have already been subjected to irradiation studies for basic research, pest control applications, and disinfestation of commodities (quarantine and phytosanitary purposes). This chapter focused on insect sterilization and disinfestation by ionizing radiations in view of the socioeconomic impacts. The release of insects that are sterile after exposure to radiation aiming to control or eradicate pest populations revealed to be a revolutionary tactic in the area-wide management of pests, and many successful cases with the application of the sterile insect technique can be found around the globe. The use of ionizing radiations to inhibit the spread of quarantine insects represents an important alternative postharvest control, and the development of generic radiation treatments has resulted in a significant increase in the international use of phytosanitary irradiation for trade in horticultural products and other commodities

Keywords: Radiation, sterile insects, phytosanitary irradiation

1. Introduction

The radioentomology can be defined as a branch of science that deals with the effects of ionizing radiations over insects and the study of insects using nuclear techniques. Radioentomological studies have been extremely useful in elucidating many entomological problems that were previously considered hard to solve or even insoluble due to limitations posed by conventional methods available.

The first radiobiological experiments performed with insects were initiated at the end of the 19th century. One of the first bioassays was performed by Professor Axenfelt in 1897 with house flies, but due to the methodology used, the results were not conclusive [1]. In 1911, Hunter made a series of experiments exposing several arthropods to X-rays, like *Sitophilus oryzae* L., *Culex pipiens* L., and some species of ticks, but no effects upon fertility or the tested life stages were observed. In the fall of 1912, Morgan and Runner performed experiments at Florida with the cigarette beetle *Lasioderma serricornis* F. with an X-ray machine aiming to sterilize cigar boxes in commercial scale. Their results, however, were also negative, as the beetle presented normal development.

According to Runner [2], the negative results from previous tests were caused by the fact that the equipments used were too rudimental. Most X-ray tubes that were tested were unable to operate continuously without neither fluctuation of intensity nor alteration of penetration power, being impossible to establish precisely the radiation dosage. Runner then executed new experiments with *L. serricornis*, using a device improved by W.D. Coolidge, whose X-ray tubes received a pure electron discharge, intensity and penetration power did not vary, and start and running voltages were the same. All these characteristics resulted in a homogeneous irradiation, and sterilization could be reached with high doses.

More detailed investigations on the genetic effects caused by ionizing radiations began with Muller's demonstration that genetic damage and a larger number of dominant lethal mutations could be induced in *Drosophila melanogaster* Meigen by X-rays [3]. He demonstrated, for instance, that an X-ray dose around 49 Gy applied on spermatic cells of *D. melanogaster* increased 100-fold the mutation frequency per generation.

However, entomologists became really aware of the extension of Muller's discovery only after 1950, when Muller made a great effort to publicize the biological effects of radiation. That moment of the 20th century could be considered as the rising of radioentomology.

Currently, there are almost 3000 references in literature, published continuously for the past seven decades. One of the most complete sources of information about radiation effects on the major groups of insects is the International Database on Insect Disinfestation and Sterilization (IDIDAS; <http://www-ididas.iaea.org/ididas/>). This website was developed with the aim to collect data of radiation doses for sterilization and disinfestations of arthropods, also performing a comparative analysis and quality assurance check on existing data [4]. IDIDAS have provided scientists a basis for literature searches to better plan experiments and became a comprehensive entry to the scientific literature for regulatory authorities to evaluate sterilization or disinfestation methods.

Over 300 species of arthropods, mostly of economic importance, have already been subjected to irradiation studies for basic research, pest control applications, and disinfestation of commodities (quarantine and phytosanitary purposes) [4]. In addition, insects may be labeled with stable or radioactive isotopes for radioecology or feeding studies. Nevertheless, this chapter will focus on insect sterilization and disinfestation by ionizing radiations in view of the socioeconomic impacts.

2. Effects of ionizing radiations in insects and radiation sources

Ionizing radiations can be emitted in the decay process of unstable nuclei or by de-excitation of atoms in nuclear reactors, X-ray devices, cyclotrons, and other equipments. Radiation in the form of particles (α or β particles and neutrons) or electromagnetic waves (gamma or X-rays) can induce random biological effects in cells of insects likewise to other living cells [5, 6].

The chemical damage to organic molecules from the absorbing medium through which the radiation pass can be caused directly (mostly by particulate types of radiation) or indirectly by free radicals (i.e., atoms or molecules carrying at least one unpaired orbital electron in the outer shell), secondary electrons, or other charged particles [7]. The radioinduced ions and radicals, most of them coming from the water radiolysis, may react with neighboring molecules to produce secondary DNA radicals or even chain reactions, particularly in lipids. Most significant biological effects result from damage to DNA, which is the critical target in living organisms. Some radioinduced lesions in DNA are single-strand breaks in the phosphodiester linkage, double-strand breaks, base damage, protein–DNA cross-links, and protein–protein cross-links. The double-strand breaks in DNA double helix are believed to be the most important type of lesion produced in chromosome by ionizing radiation, cracking the chromatin into different pieces that may result in cell killing or mutation. Examples of lethal aberrations to the cell are the dicentric and ring (which are chromosome aberrations) and the anaphase bridge (a chromatid aberration). Two relevant aberrations that are usually not lethal to the cell are symmetrical translocation and small deletions. These changes and mutations left in the genetic code will influence base pairing, coding, transcription, and gene expression [5, 7].

According to the law of Bergonie and Tribondeau, cells that are dividing are more radiosensitive. Thus, cells that have a high mitotic rate and a long mitotic future, such as the reproductive cells, stand among the most radiosensitive cells [8]. Radioinduced changes in DNA of germ cells of insects can result in physiologically compromised gametes, aspermia, infertility, and even inability to mate. Sterilization can also be a result of fragmentation in germ cell chromosomes that generated random dominant lethal mutations, translocations, deletions, and other aberrations, which will lead to the production of imbalanced gametes and early zygotic death. The later type of sterilization is explored by the sterile insect technique (SIT), a genetic control method that relies essentially on the transfer of competitive sperm from released irradiated males to wild females [9, 10].

Somatic cells are more radioresistant than germ cells since they are usually differentiated cells, which explains why lethal radiation doses must be higher than sterilizing doses [11]. In general,

insects are less resistant to radiation than bacteria, protozoa, and viruses, although more radioresistant than higher vertebrates [12, 13, 14]. Dyar's rule serve to explain this difference in sensitivity to radiation, as insects have a discontinuous growth and most of the cells divide only during the molting process [15].

The radiosensitivity varies widely among and within insect orders (Figure 1) [11]. Bakri et al. [4] highlights that the comparison of radiosensitivity between insect species must clearly take into account the end result measured, like sterilization, death, or inability to reach the next life stage. Lepidopterans exhibit more resistance to be sterilized by ionizing radiation (mean sterilization doses ranging between 40 and 400 Gy) [11] because some species may present a more complex sperm transfer, spermatophore formation, lower ability for mating after irradiation, production of eupyrene and apyrene sperm, and resistance to the induction of dominant lethal mutations due to the presence of holokinetic chromosomes (diffuse centromere) [16].

Besides the inherent differences in radioresistance between species and insect orders, many other factors can influence the sensitivity to radiation. These factors can be physical or biological conditions.

The other biological conditions that can influence insect radiosensitivity are as follows:

- a. Age/developmental stage: in general, adults are more radioresistant than pupae, which in turn are more resistant than larvae and eggs [11].
- b. Sex: female insects are usually more radiosensitive than males [17].
- c. Size and weight: large long-lived adults of some species, with higher moisture content, may be more radiosensitive than small short-lived adults [18].
- d. Nutritional stage: starvation may increase the radiosensitivity [19].
- e. Diapause: diapausing larvae of some species could be more radiosensitive [20].
- f. Genetic differences: strains of some species adapted to diverse environments could develop different radioresistances [21].

The main physical factors that can modify insect radiosensitivity are as follows:

- a. Atmosphere: radioinduced damages are fewer with hypoxia [22].
- b. Temperature: radioresistance may increase at lower temperatures [23].
- c. Irradiation dose rate: as the dose rate is lowered and the exposure time extended, more sublethal damage can be repaired [7].
- d. Dose fractionation: when splitting a radiation dose in time, cells are allowed to repair sublethal damage during the intervals between doses [24].
- e. Radiation type: radiations with a higher linear energy transfer (LET), like α particles and neutrons, are more effective in inducing biological effects [7].

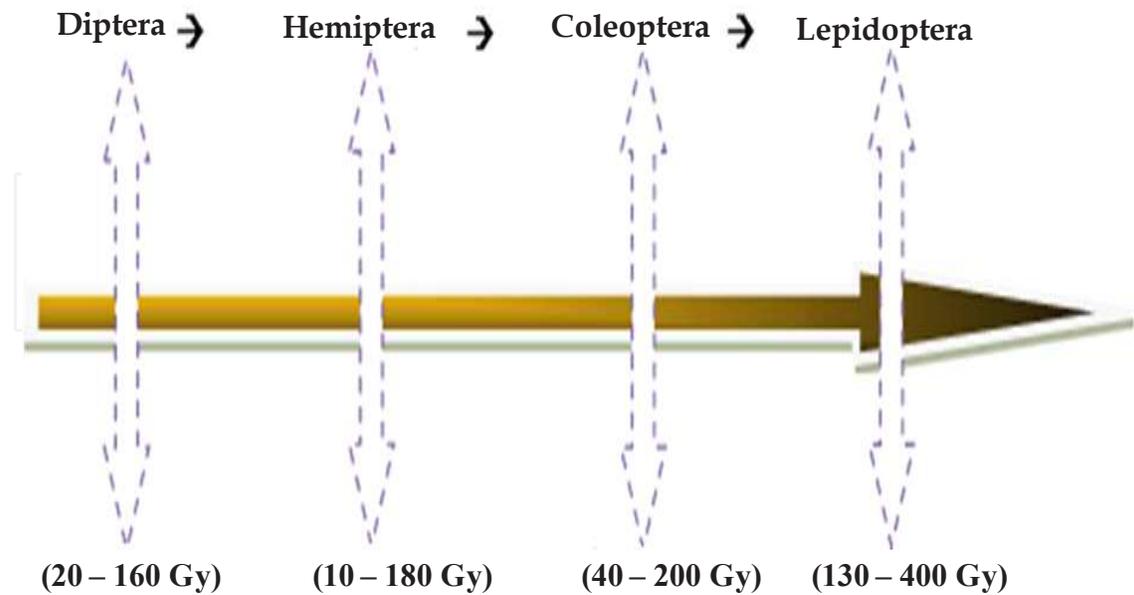


Figure 1. Decrease in radiosensitivity based on estimated sterilization doses for different insect orders (IDIDAS, 2015).

As aforementioned, radiations with a high LET are more effective in inducing biological effects, but their penetration can be limited. A typical alpha particle, for example, has high LET, but its penetration range is of only about 3 cm in air or 0.04 mm in tissue [7]. Neutrons also produce dense ionized tracks, but they can travel great distances in air as they carry no charge, requiring thick hydrogen-containing materials, such as concrete or water, to block them. Nevertheless, the application of neutron in radioentomological projects and pest control is constrained due to the easy induction of radioactivity in irradiated materials and the availability of neutron sources, which are usually restricted to nuclear reactors.

Researchers have preferably applied gamma or X-rays and high-energy electrons in studies involving pest control and disinfestation of commodities. As these radiations have similar relative biological effectiveness (RBE), most studies have indicated not significant differences in the biological damage induced by them for most doses and insect life stages [25, 26]. The insects are not rendered radioactive when irradiated with these sources by ensuring that the incident radiation is below 10 million electron volts (MeV) for high-energy electrons and less than 5 MeV for photons (gamma or X-rays) [27].

High-energy electrons are generated by electron accelerators, not involving any type of radioisotope. Likewise, most X-ray machines do not use radioisotopes, and X-rays are generated basically by the rapid deceleration of a beam of electrons before a material of high atomic number (e.g., tungsten or gold). The major advantages of these radiation sources are that no radioactive waste is produced, no radiation is produced when switched off, and the dose rate from electron accelerators can be hundred times greater than from gamma irradiators [11].

Despite these advantages, the types of irradiator used most frequently by radioentomologists for the past four decades have been those equipped with the radioisotopes ^{60}Co or ^{137}Cs as source of gamma rays. ^{60}Co has a half-life of 5.3 years and emits two gamma photons of 1.17 and 1.33 MeV, while ^{137}Cs has a half-life of 30.1 years and emits a monoenergetic photon of 0.66 MeV. The gamma irradiators used in pest control programs or for disinfestation of commodities are commonly of two types: large-scale panoramic irradiators or self-contained dry storage irradiators (Figure 2). The choice of radiation source is based considering basically costs, penetration, and irradiated material throughput [11]. Panoramic irradiators allow the irradiation of entire rooms and large number of samples or products can be irradiated at the same time. In self-contained irradiators, such as the most common irradiator used for insect sterilization, the Gammacell-220 (MDS Nordion International Inc., Ottawa, Canada), the canister containing the samples is lowered from the loading position to the shielded chamber with the radiation sources. The production of the Gammacell-220 was discontinued since 2008. On its place, appeared new models whose irradiation chamber contains a single source, lowering the overall costs, and the sample rotates through its own axis in front of the radiation source.



Figure 2. Types of gamma irradiators used in pest control trials or for disinfestation of commodities at the Center for Nuclear Energy in Agriculture (CENA, São Paulo, Brazil): (left) large-scale panoramic Gammabeam-650 irradiator; (right) self-contained Gammacell-220 irradiator.

3. Sterile insect technique

One of the main applications of ionizing radiations in Entomology is the production of sterile insects by the sterile insect technique (SIT). The SIT can be defined as a control tactic that uses

area-wide inundative releases of sterile insects to reduce the fertility of a field population of the same species [28]. This technique is usually used as one of the components of area-wide integrated pest management programs, where the density of the target insect pest population is initially reduced by other control methods, like cultural or chemical control [29, 30].

The idea of releasing insects of the same species to introduce sterility into wild populations was independently conceived on the 1930s by three researchers: A.S. Serebrovskii at the USSR, F.L. Vanderplank at Tanzania, and E.F. Knipling from the United States [31]. Serebrovskii used chromosomal translocations to induce inherited partial sterility in *Musca domestica* L. and *Calandra granaria* L., but his research was not continued in the USSR during World War II [32]. Vanderplank tried to use hybrid sterility to combat tsetse flies, after obtaining low fertility from cross-matings between *Glossina morsitans* Westwood and *Glossina swynnertoni* Austen, but the detailed results were not published until his death [33]. At the United States Department of Agriculture (USDA), Knipling and colleagues [31, 34, 35, 36] exploited Muller's discovery that ionizing radiation could induce dominant lethal mutations, and their studies continued despite the tribulations during the World War II, resulting in an approach that was applied to eradicate the New World Screwworm, *Cochliomyia hominivorax* Coquerel, from the United States and Central America.

The SIT does not apply to all insects species. Innumerable factors must be considered before the adoption of the technique: (a) the species must reproduce sexually (even low levels of parthenogenesis can derail the technique); (b) the technique can be impractical for species that are vector of serious diseases, nuisance pests, or those which are highly destructive in the adult stage; (c) mass rearing procedures must be available; (d) the released sterile insects must present adequate dispersion; (e) the sterilization must not compromise the competitiveness of the males; (f) females must preferably mate only once or irradiated sperm must be very competitive; and (g) the population density of the target pest must be low, making economically feasible the release of a dominant population of sterile males over an extended period of time [34, 37].

Knipling et al. [38] realized that the degree of sterility introduced into the wild population by the sterile males must be sufficiently high to overcome the rate of increase of the wild females in order to provoke an overall reduction in the target population. As the ratio of sterile to fertile insects increases asymptotically as the density of the wild population declines to low levels, Knipling advocated that the sterile insects should be released when the wild population was at a seasonal low or after its decimation by weather events or other control methods. Most of the successful programs that released sterile insects were applied when field populations were at low densities [29].

Basically, the SIT involves the mass rearing of the target species, exposing the insects to ionizing radiation to induce sexual sterility, and then releasing the irradiated insects into the target population. The released sterile males mate with wild females, preventing the generation of a fertile offspring [10, 39].

The production of high quality insects in sufficient numbers using mass-rearing techniques is one of the main steps of the technique [40]. Methods to rear insects on artificial diets have been

developed for more than 1000 species so far [41–45]. The production must be timely and cost effective, taking advantage of economies of scale whenever possible [46–49], and maximum attention must be paid to the factors that can affect quality of the insects produced [50].

Since the 1950s, most of the insect pest control programs that integrate the SIT have applied radioisotope irradiators loaded either with ^{60}Co or ^{137}Cs , sterilizing the insects, therefore, with gamma rays [11, 51, 52]. Sterilization doses for hundreds of insect species can be found at IDIDAS database [53]. As absorbed dose is a key parameter for the success of the technique, the facilities that sterilize insects must have an accurate dosimetry system [11]. Due the growing complexities of the transboundary shipment of radioisotopes and the fear of “dirty bombs” after the September 11 attacks, some studies have supported the adoption of other practical alternatives for the sterilization of insects, such as X-ray irradiators [26, 54–58].

Studies aiming to develop procedures for handling and chilling adult insects or to provide food and water prior to release are continually performed. After sterilization, the insects can be released via static-release receptacles, ground-release methods, or most commonly from the air [59]. One of the most efficient methods of release is the aerial release of chilled irradiated insects or bags containing the adults, especially when aircraft flight paths are guided by a global positioning system (GPS) linked to a computer-controlled release mechanism [59, 60].

The SIT has been used mostly against species that are highly harmful to agriculture or public health or which elimination would have significant economic benefits. Currently, about 38 facilities are making research on SIT or sterilizing millions of insects per week for national area-wide integrated pest control programs [53]. Effective programs integrating the SIT have been performed against screwworm flies, tropical fruit flies, some species of tsetse flies, the pink bollworm *Pectinophora gossypiella* Saunders, and the codling moth *Cydia pomonella* L.

One of the best examples of application of the SIT was the phenomenal successful eradication campaigns conducted against the New World Screwworm, *C. hominivorax*, in the American continent. This fly can be sterilized as pupae 24 h before adult emergence with 40 Gy [61, 62]. The economic losses to livestock caused by *C. hominivorax* in the United States during the 1930s were significant [63]. After the field pilot tests at the Sanibel Island (1951–1953) and the Curaçao Island (1954) [64], eradication campaigns using suppression techniques and sterile insects were implemented in the Southeastern (1957–1959) and Southwestern (1962–1966) United States. As fertile flies continued infesting the United States coming from Central America, the eradication campaigns advanced through Mexico. Using sterile flies reared in the mass-rearing facility from COMEXA (*Comisión México-Americana para la Erradicación del Gusano Barrenador del Ganado*) at Tuxtla Gutiérrez, Mexico, the eradication of *C. hominivorax* was achieved until the Isthmus of Tehuantepec in 1984. With the interest of Central American countries and as fewer sterile flies would be required to maintain a buffer zone at Panama (150 million sterile flies/week were needed in the Isthmus of Tehuantepec, while only 40 million/week would be required in Panama), national eradication campaigns continued with the aerial release of more than 20 million sterile flies/week [65] during more than two decades (Figure 3). Panama was finally declared free from *C. hominivorax* in 2006 and a biological barrier of 30,000 km², maintained by the weekly release of 50 million sterile flies, was set at the Darien Gap [65, 66]. With this eradication effort, all warm-blooded animals became free of this deadly parasite in

the United States, Mexico, Belize, Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica, Panama, some Caribbean Islands, and additionally Libya, North Africa, after an outbreak [36, 67]. The economic benefits of these campaigns trespassed US\$1 billion per year [68].

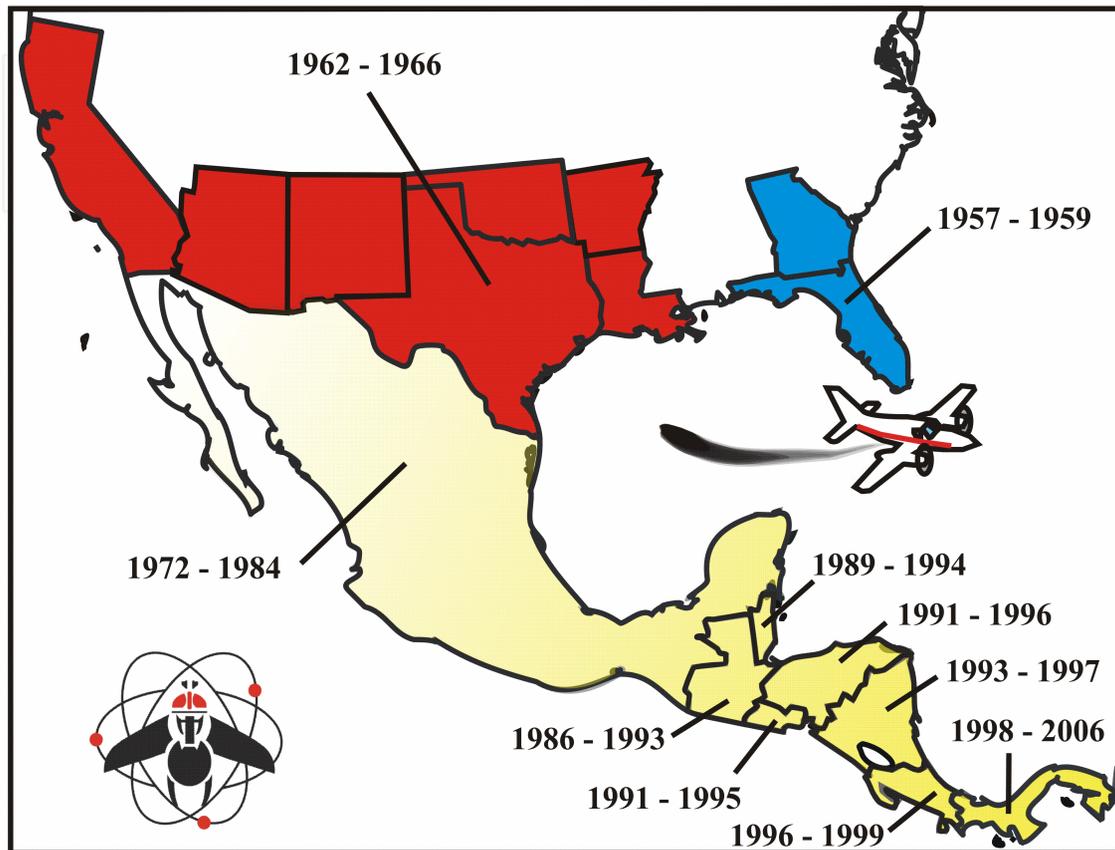


Figure 3. Expansion of the eradication campaigns that used aerial releases of irradiated flies against the New World Screwworm in North and Central America.

Many species of fruit flies are major economic pests due to the direct and indirect damages caused to fruit growers and difficulties imposed to international trade of fruits and vegetables [69]. Because of that, some species, especially tephritid fruit flies, have been target of programs that integrate the SIT. Fruit flies from the Tephritidae family can be generally sterilized at 90–150 Gy, and *Bactrocera* spp. are usually sterilized at 30–90 Gy [11, 53]. The first large-scale program stopped the invasion of the Mediterranean fruit fly (medfly) *Ceratitis capitata* Wiedemann from Central America into southern Mexico in the 1970s [70, 71]. After the invasion of Costa Rica by the medfly in 1955 and its expansion up to southern Mexico in 1976, the Government of Mexico started working with Guatemala and the United States to establish a large area-wide program using the SIT against this pest [71]. Using 500 million sterile flies/week from the rearing facility at Metapa, Mexico, and, currently, almost 2 billion sterile males/week [69, 72] from the biofactory located at El Piño, Guatemala, the MOSCAMED program has kept the United States, Mexico, and half of Guatemala free of the medfly for over 35 years. To prevent the establishment of the medfly in the continental United States through infested

imported fruits, sterile males are regularly released in the Los Angeles Basin and Florida [31, 73]. During the 1980s and 1990s, the SIT was employed to eradicate the melon fly *Bactrocera cucurbitae* Coquillett in all of Japan's southwestern islands [74]. Significant SIT programs against the medfly and *Anastrepha* species have also been developed in several provinces of Argentina, some of which have become pest-free areas [75, 76].

Sterilization doses for flies from the Glossinidae family range from 50 to 120 Gy [53], and some SIT trials have been conducted on tsetse flies, which are vectors of trypanosomosis ("sleeping sickness"), in African countries during the 1970s and 1980s. However, as most programs had not been conducted area-wide, the pest-free status of most of the areas could not be maintained [31]. For example, three tsetse species (*G. morsitans submorsitans* Newstead, *Glossina palpalis gambiensis* Vanderplank, *G. palpalis palpalis* Robineau-Desvoidy) were eradicated at the same time in 3,000 km² from Burkina Faso through insecticide application and trapping suppression, followed by ground release of irradiated adults [77]. *G. palpalis palpalis* was eradicated in 1,500 km² of Nigeria with traps and insecticide-impregnated targets followed by ground releases of sterile adults [78]. In 1994–1997, *Glossina austeni* Newstead was eradicated from Unguja Island of Zanzibar (1,650 km²) by using attractive devices, treating livestock with insecticide and aerial releases of irradiated adults, ceasing the transmission of trypanosomosis [79, 80]. The government of Ethiopia started the Southern Tsetse Eradication Project (STEP) in 2009, aiming to eradicate two species of tsetse flies over a 25,000 km² area in the Southern Rift Valley [81, 82], and after area-wide suppression activities, the mass-rearing facility in the Kality suburb of Addis Ababa had supplied in 2012 up to 60,000 sterile males/week to be released over the Deme Basin region. Since 2012, very good progress is also being made in the eradication of *G. palpalis gambiensis* on the Niayes area in Senegal with aerial releases of sterile males [60], and the annual increases of cattle sales after eradication were estimated in more than € 2,800/km² for the farming communities.

Despite some difficulties when applying the SIT against moths [83], like high mean sterilization doses (usually higher than 100 Gy) and appropriate air-handling and filtering in the mass-rearing facilities, radiobiological studies have been conducted for more than 30 lepidopteran species [84] and two SIT programs are still operational.

Since 1968, the pink bollworm, *Pectinophora gossypiella* Saunders, has been excluded from the San Joaquin Valley, USA, by a containment program [85] (<http://www.cotton.org/tech/pest/bollworm/index.cfm>), which releases adults that emerge from pupae irradiated with 100–150 Gy at the rearing facility in Phoenix, Arizona. The cost of this program has been around US \$12.5/ha/season for each cotton grower (but control costs would increase by US\$200/ha per grower if the program was not in place, besides an additional 2.2 million kg of pesticide that would have to be used every year) [83].

Populations of the codling moth, *Cydia pomonella* L., from British Columbia are being kept at insignificant levels since 1997 and individuals of this pest have not been detected in 37% of the orchards since 2009 due to the Okanagan-Kootenay suppression program that integrates the SIT (newly emerged males are partially sterilized with 100–250 Gy and chilled moths are released). Growers used to pay a tax of US\$169/ha/year, and the application of insecticides in the province was reduced 82% since then [83, 86, 87].

4. Radiation as quarantine treatment against insect pests

One major concern in exporting agricultural commodities is to prevent the introduction or spread of exotic quarantine pests. Phytosanitary measures are used to disinfest commodities of pests, providing quarantine security [88]. The fumigant gas methyl bromide used to be the most common treatment for agricultural commodities [89] due the low cost, effectiveness against a wide range of insects, rapid dispersion, and minimal impact on commodity quality [90]. However, with the imminent phasing out of methyl bromide as mandated by the Montreal Protocol [91], the interest in alternative phytosanitary treatments has raised [92, 93]. The use of ionizing radiations as a way to inhibit the spread of quarantine insects represents an important alternative postharvest control, reducing the need for chemical fumigants and other toxic products [94].

Hallman [95] stated that the objective of using ionizing radiations as a phytosanitary treatment is not to obtain acute mortality of the insects but to prevent development or reproduction, as most commodities do not tolerate the usual dose ranges required to achieve immediate mortality (usually ≥ 1 kGy). Actually, the U.S. Food and Drug Administration (FDA) has approved radiation up to 1 kGy to control insects in foods and to extend the shelf life of fresh fruits and vegetables [96]. Thus, a phytosanitary irradiation treatment must be effective against the most tolerant insect stage that could be present on the commodity [97], and the inhibition of further development should be considered as a measure of efficacy of phytosanitary irradiation [98].

Some regulators may consider this a disadvantage since other commercially applied quarantine treatments, which are generally based on heat, cold or methyl bromide fumigation, do reach acute mortality. When inspectors find live quarantine pests from these treatments, the entire consignment can be rejected or retreated regardless of certification of treatment because the inspectors may assume that the treatment was not properly done, the shipment was contaminated with infested commodity or the cargo was reinfested after treatment. Furthermore, live adults found in survey traps could trigger restrictive and costly regulatory responses in importing countries [99].

Nevertheless, phytosanitary irradiation can be a viable commercial insect control technique. The advantages of radiation include the fact that pest insects cannot develop resistance, the absence of residual radioactivity, and few significant changes in the physicochemical properties of the treated products for most doses applied [100].

Another advantage of phytosanitary irradiation compared with other treatments is the possibility of using generic doses (i.e., one dose serves for a group of insects and commodities, although not all have been tested for efficacy), which facilitate the development and application of the treatment [94].

Radioentomologists are constantly looking for a generic radiation dose to serve as quarantine treatment, i.e. a dose that could control a broad group of pests without adversely affecting the quality of a wide range of commodities [101]. This dose would necessarily be set at the minimum absorbed dose required for the most tolerant organism within the insect group

considered [102]. Due the high radiotolerance of the Angoumois grain moth (*Sitotroga cerealella* Olivier), Hallman and Phillips [102] suggested that a generic dose of 600 Gy for all insects in ambient atmospheres would be efficacious to attend quarantine purposes. Currently, some of the generic phytosanitary irradiation treatments are 150 Gy for all hosts of Tephritidae, 150 Gy also for mangoes and citrus fruits exported from Mexico to the United States, 250 Gy for all arthropods on mango and papaya shipped from Australia to New Zealand [103], 300 Gy for all arthropods on mango shipped from Australia to Malaysia, 350 Gy for all arthropods on lychee shipped from Australia to New Zealand, and 400 Gy is applied for Mexican guavas, Indian mangoes, and dragon fruit (*Hylocereus undatus* Britton and Rose) from Vietnam exported to the United States [94, 99]. Hallman [88] also presented a number of cases indicating the usefulness of generic doses for important pest groups such as mealybugs, scales, and weevils.

In 2006, the USDA approved irradiation at a generic dose of 150 Gy for any tephritid fruit fly and 400 Gy for all insects except pupae and adult of Lepidoptera [88, 104, 105]. Subsequent studies lead the USDA-APHIS to approve minimum doses for 23 insect pests [106], including 10 tephritid fruit fly species, 6 lepidopteran species, 4 curculionid species, and 1 mite species. These approved specific doses for fruit flies range between 60 and 150 Gy, between 100 and 250 Gy for lepidopterans, between 92 and 300 Gy for Coleoptera, and 300 Gy for the spider mite [106].

The International Plant Protection Convention (IPPC) also accepted the 150 Gy minimum absorbed dose for Tephritids as an international standard for phytosanitary treatment of these quarantine pests, including it in the International Standards of Phytosanitary Measures (ISPM #28) together with 13 species-specific treatment procedures [107]. The IPPC, however, did not accept the generic dose of 400 Gy for all insects (except pupae and adult of lepidopterans). The IPPC does not approve at first some irradiation treatments due to perceived problems with the study or the presence of live adults after irradiation (an issue that must be carefully addressed).

The development of methods to determine whether quarantine pests have been irradiated could help to resolve the issue of presence of live adults after exposure to radiation. Biomarkers based on the molecular processes of irradiation-induced DNA damage and repair would have internationally broad application to confirm the irradiation status of pests found on commodities and for the detection of sterile insects. Siddiqui et al. [108] discovered a protein in the Queensland fruit fly, *Bactrocera tryoni* Froggatt, that was modified due to radiation, with a higher amount of modified protein at higher radiation doses. The authors also tested the doses approved for disinfestation and SIT. Leifert et al. [109] reported highly specific antibodies that allowed the sensitive detection of proteins from irradiated *B. tryoni* using even standard commercial technologies, such as western blot or ELISA assays.

According to Follett [110], current research on phytosanitary irradiation is focused on development of specific doses for quarantine lepidopterans not covered by the generic treatments, shortening treatment time through the reduction of dose levels for specific pests and commodities, the development of generic doses below 400 Gy for economically important groups of quarantine insects other than fruit flies, and deep investigations on commodity tolerance

and novel methods to reduce damages and extend shelf life. The author also discussed that future research should be dedicated to reduce the present barriers to the wider use of phytosanitary irradiation, like the 1 kGy limit, restrictions on the use of modified atmosphere and the small number of countries that approve the use of phytosanitary irradiation. For example, the development of small-scale X-ray machines could provide farmers and packinghouses with in-house treatment capability, accelerating the adoption of phytosanitary irradiation. A recent change in U.S. import regulations has permitted the irradiation upon entry, allowing exporting countries to explore new markets without investing in expensive irradiation facilities [111].

5. Conclusion

The use of ionizing radiations allowed the rise of a new branch of the study of insects in the middle of the 20th century, the radioentomology. The release of insects that are sterile after exposure to radiation aiming to control or eradicate pest populations revealed to be a revolutionary tactic in the area-wide management of pests, and many successful cases of the application of the sterile insect technique can be found around the globe. Furthermore, the development of generic radiation treatments has resulted in a significant increase in the international use of phytosanitary irradiation for trade in horticultural products and other commodities.

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