

# Use of Radiation and Isotopes in Insects

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## 1. Introduction

One of the first successful experiments to verify the effects of an ionizing radiation over an insect (*Lasioderma serricorne* F., the cigarette beetle) was performed by Runner in 1916. Soon afterwards, Muller demonstrated in 1927 that rays from radioactive substances could induce genetic damage and a larger number of dominant lethal mutations in *Drosophila melanogaster* Meigen, which were expressed through a reduction in the hatchability of the eggs laid by treated females or fathered by treated males. However, the economic entomologists became really aware that sterility in insects could be quite easily achieved through ionizing radiations only after 1950, when Muller made a great effort to publicize the biological effects of radiation. This was the beginning of a new branch of Science, the Radioentomology.

More than 2800 references in literature were published along the past six decades. Over 300 species of arthropods, mostly of economic importance, have already been subjected to irradiation studies for basic research, pest control applications (e.g., the autocidal control known as the Sterile Insect Technique-SIT, and in support of biological control programs), and for disinfection of commodities (for quarantine and phytosanitary purposes) (Bakri et al., 2005). Besides that, insects may be labelled with stable or radioactive isotopes for ecology or nutrition studies.

## 2. Mode of action of radiation in insects

In general, the mode of action of ionizing radiations in living cells consists basically in a chain of oxidative reactions along the radiation path and the formation of free damaging peroxy-radicals, which alter irreversibly the organic molecules. At the cytological level, sterilization is the result of the germ cell chromosome fragmentation (dominant lethal mutations, translocations, and other chromosomal aberrations), leading to the production of imbalanced gametes and, subsequently, inhibition of mitosis and the death of fertilized eggs. Beside the reproductive sterility induced by direct lesion of the genetic material by radiation, LaChance et al. (1967) reported that there are other causes of reproductive sterility that might have a cytological or physiological basis.

According to the law of Bergonie and Tribondeau, cells are most sensitive to radiation when they are dividing. The most radiosensitive cells are, therefore, those with a high mitotic rate, with a long mitotic future and that are of the stem or germ cell type (Casarett, 1968).

Apparently, neither DNA content, chromosome number, nor chromosome arm number can be responsible for the differences in radiosensitivity of cells (Jacquet & Leonard, 1983). Even though, there is a relationship between the interphase nuclear volume and cell sensitivity to radiation, that is used in vertebrate animals and plants to predict their sensitivity to chronic

irradiation, which express that the larger the nuclear volume, the greater the sensitivity (Casarett, 1968; Sparrow et al., 1963).

The radiosensitivity of the mitotically active reproductive cells has different sterilization and killing susceptibility regarding the developmental stage and the division phase. According to Proverbs (1969), spermatocytes and spermatogonia are more radiosensitive than the spermatids and spermatozoa. Dey & Manna (1983) found that the chromosomes of the bug *Physopelta schlanbuschi* were more sensitive to X rays at the spermatogonial metaphase and anaphase I. The sensitivity of the mitotically active reproductive cells in female insects can be increased by the presence of nurse cells, which possesses polytene chromosomes with huge nucleus of unraveled chromatin when undergoing endomitosis (LaChance & Leverich, 1962; LaChance & Bruns, 1963). Thus, female insects are, in general, more radiosensitive than males (Bakri et al., 2005; Hooper, 1989).

Somatic cells are less sensitive to radiation than stem or gonial cells, as they are generally differentiated cells that have lost their ability to divide at the adult stage, what explains why lethal doses are usually higher than the sterilizing doses for insects (Bakri et al., 2005). The effect of radiation on somatic cells can be expressed by the development of abnormalities, reduction of adult lifespan, flight ability, mating propensity, nutrition, and, ultimately, death of the insect (FAO/IAEA/USDA, 2003).

### 3. Radioresistance in insect orders

Insects are more radioresistant than higher vertebrates, but less resistant than bacteria, protozoa and viruses (Harrison & Anderson, 1996; Rice & Baptist, 1974; Ravera, 1967; Whicker & Schultz, 1982). One of the reasons for this is the Dyar's Rule, *i.e.* arthropods have a discontinuous growth and most cell divisions happen only during the moulting process (Behera et al., 1999). The sensitivity to radiation varies widely among the insect orders, *e.g.* some species from Orthoptera are sterilized at doses below 5 Gy, whilst some Lepidoptera requires more than 300 Gy (IDIDAS, 2010).

According to Bakri et al. (2005), more than 200 species of insects of economic importance from at least eight taxonomic orders have been irradiated for radiobiological studies, integrated pest management (IPM) programs or phytosanitary purposes. Almost 80% of these species belongs to only three orders, *i.e.* Diptera, Lepidoptera and Coleoptera. The radiation dose values for sterilization summarized in the Table 1 were chosen to serve as standard criteria in analysing the differences in radiosensitivity among the insect orders.

ORDER	Sterilization Doses (Gy)
Coleoptera	13 - 200
Dictyoptera	5 - 140
Diptera	10 - 200
Hemiptera	10 - 200
Hymenoptera	80 - 100
Lepidoptera	40 - 400
Orthoptera	4 - 30
Thysanoptera	100 -200

Table 1. Estimated sterilization doses reported for nymphs or pupae of insects from different taxonomic orders (IDIDAS, 2010).

In Diptera, doses ranging from 20 to 160 Gy are usually required for sterilization. Tachinids are the most sensitive, whereas some species from the Drosophilidae family are quite radioresistant. Most fly species are often irradiated at the late pupal stage due to the easiness in handling and even shipping. In the Culicidae family, most of the studies were conducted treating the adults instead of pupae because of their higher resistance in handling and to dehydration. Most of the Area-Wide IPM programs integrating the SIT targeted fruit flies from the Tephritidae family, which are generally sterilized at 90-150 Gy (Bakri et al., 2005; IDIDAS, 2010).

Lepidopterans are relatively more resistant to radiation, with sterilization doses ranging from 40 to 400 Gy. Major differences between lepidopteran and the other insect orders are that their chromosomes usually show diffuse centromere (holokinetic) and also do not present the classical breakage-fusion-bridge cycle which is characteristic of the dominant lethal radioinduced in Diptera, for instance. These two characteristics increase the tolerance of lepidopteran chromosomes to telomere loss without the drastic effects that this has on chromosomes of other orders (Robinson, 2005). As fully sterilizing doses reduce significantly the competitiveness of sterile moths, Proverbs (1962) proposed the use of substerilizing doses which revealed to lead to a F<sub>1</sub> progeny that is more sterile than the parents and whose sex ratio is biased toward males. Such F<sub>1</sub> sterility phenomenon has been exploited in some Area-Wide IPM programs using the SIT against the pink bollworm (*Pectinophora gossypiella* Saunders, pupae sterilized at 100-150 Gy), the codling moth (*Cydia pomonella* L., newly emerged males are partially sterilized at 100-250 Gy), the cactus moth (*Cactoblastis cactorum* Berg, adults partially sterilized at 200 Gy) and the diamondback moth (*Plutella xylostella* L., pupae partially sterilized at 100 Gy) (IDIDAS, 2010).

The sterilization doses for Coleoptera range from 13 to 200 Gy. In Coccinellidae, the same family of the important natural enemy *Cycloneda sanguine* L., a sterilization dose of 35 Gy was reported for pupae of the Mexican bean beetle, *Epilachna varivestis* Mulsant (Heneberry et al., 1964). Since the boll weevil *Anthonomus grandis grandis* Boheman was first recorded in the USA in 1892, it was estimated to have caused billion of dollars in economic damages and it was responsible for almost one-third of the insecticides applied in US agriculture. Therefore, it was the species of this group most studied, aiming the application of the SIT. The males could be sterilized at 80 Gy, but the competitiveness and longevity of the weevils were severely reduced. Fecundity of females was reduced at 50 Gy, but they kept laying fertile eggs up to 200 Gy. Nevertheless, these problems were solved if the insects were sterilized after being treated with the anti-leukemia drug busulfan or under low doses of fractionated radiation, and egg hatch was avoided by using chitin synthesis inhibitor (Haynes & Smith, 1992; Klassen & Earle, 1970; McKibben et al., 2001). Other detrimental impacts of radiation, such as the collapse of the epithelial tissue of the midgut, were also reported for the West Indian sweetpotato weevil, *Euscepes postfasciatus* Fairmare, target of an Area-Wide-IPM program in Japan (Kumano et al., 2010).

The sterilizing dose values reported in Table 1 were difficult to combine due basically to the inexistence of neither standard dosimetry nor even experimental procedures. Furthermore, several factors can also affect insect radiosensitivity, especially the biological factors (developmental stage, age, sex, size, weight, nutritional stage, diapauses and genetic differences) and physical conditions (atmosphere, temperature and irradiation dose rate) (Bakri et al., 2005).

## 4. Radioisotopes in the sterile insect technique and biological control

### 4.1 The sterile insect technique

The use of sterile insects (Sterile Insect Technique – SIT) to control or eradicate pest populations was a revolutionary initiative in Entomology conceived at the beginning of the twenty century.

During the 1930's, the New World Screwworm fly (NWS), *Cochliomyia hominivorax* Coquerel, was a serious pest of warm-blooded animals, including humans, in North America. The larval stages of the NWS are obligate parasites, feeding on the living flesh of the host (myiasis), what caused serious livestock production losses. In 1935, an infestation with 230,000 myiasis cases happened in USA (James, 1947) and Parish (1942) reported 6,148 myiasis cases during the screwworm seasons from 1936 to 1940 on Texas. The economic losses at the southeast and southwest regions of USA were estimated in US\$ 20 million/year and US\$ 50-100 million/year respectively (Baumhover, 1966). In view of this, scientists of the US Department of Agriculture (USDA) started summing efforts to control this plague.

Melvin & Bushland (1936) developed techniques to colonize and an artificial diet to rear NWS, what made available large number of insects for studies. E.F. Knipling then observed that females of NWS usually mated only once and realized that if sexual sterility could be induced in males and if large numbers of them could be continuously released in the field for several successive generations, the wild NWS population could decrease up to the suppression level (Knipling, 1955). Furthermore, if the wild population was isolated, the ratio number of sterile flies: number of fertile flies would become so high that probably not even a single fertile mating would occur and that wild population would finally be eradicated.

In 1927, H.J. Muller demonstrated that ionizing radiation could induce in *Drosophila* dominant lethal mutations that could be verified by the reduction in viability of the eggs laid by irradiated females or normal females mated with irradiated males. But only in 1946, A.W. Lindquist called Knipling's attention to the fact that Muller had reported a means of sterilizing insects. Bushland & Hopkins (1951) then conducted the first NWS irradiations at the X-ray Therapy Section of Brooke Army Hospital and found that, when 6 day old pupae were exposed to 50 Gy, the adult flies that emerged were sterile and could compete almost equally with non-irradiated insects.

The first field evaluation pilot test was performed during 1951-1953 at the Sanibel Island (47 km<sup>2</sup>), 4 km from the coast of Florida, using <sup>32</sup>P-labelled flies for a release-recapture experiment. The percentage of radioactive egg masses was also assessed. The results corroborated the laboratory studies and just after 8 weeks of releases (ca. 39 sterile males/km<sup>2</sup>/week), 100% of the egg masses collected from wounded sentinel goats were sterile. Nevertheless, eradication was not achieved because wild fertile flies continued reinfesting the island from the mainland (Baumhover et al., 1955).

To prove the SIT reliability once for all, an eradication trial was initiated on the Curaçao Island (435 km<sup>2</sup>), 6.5 km from Venezuela coast, on 1954. The NWS flies were reared in Florida and irradiated pupae were packaged in paper bags, air shipped to Curaçao and released by air twice per week. The sterile males started being released (ca. 155 sterile males/km<sup>2</sup>/week) on August 1954 and eradication had been accomplished within just 14 weeks (Baumhover et al., 1955).

The success of the NWS eradication experiment on the Curaçao Island led to the implementation of eradication programs in the Southeastern (1957-1959) and Southwestern (1962-1966) United States. In 1966, the entire USA was declared free of the NWS, but the

country remained vulnerable to the influx of fertile flies from Mexico. So, in 1972, the *Comisión México-Americana para la Erradicación del Gusano Barrenador del Ganado* (COMEXA) was created. In 1977, a mass-rearing facility with capacity to produce 500 million sterile flies/week, built at Tuxtla Gutiérrez, Mexico, reached full production. By 1984, the NWS had been eradicated from USA to the Isthmus of Tehuantepec, Mexico. The eradication operations continued through Central America in the following years with new countries been declared free: Mexico (1991), Belize and Guatemala (1994), El Salvador (1995), Honduras (1996), Nicaragua (1999) and Costa Rica (2000). Panama was declared free from the NWS in 2001 and a buffer zone of 30,000 km<sup>2</sup> was set at the Darien Gap by the release of 40-50 million sterile males (Klassen & Curtis, 2005; Wyss, 2000). The estimated annual producer benefits in the USA, Mexico and Central America were US\$ 796 million, US\$ 292 million and US\$ 77.9 million respectively (Wyss, 2000).

According to the International Plant Protection Convention (IPPC), the SIT can be defined currently as “a method of pest control using area-wide inundative releases of sterile insects to reduce fertility of a field population of the same species” (FAO, 2005).

Sterility in insects can be induced by chemosterilants when added to rearing diets, sprayed over the insect or even in attractant baits in the field. However, most of the chemosterilants are mutagenic or teratogenic, what leads to human health and environmental issues, especially the integrity of ecological food chains (Hayes, 1968), besides the fact that the insects can develop resistance (Fitt, 2008). Thus, for the past five decades, exposure to ionizing radiation has been the main method of inducing sterility in mass-reared insects for Area-Wide IPM programs that integrate the SIT.

Once the absorbed dose is achieved, the irradiation process reaches high accuracy. Other advantages of the irradiation are: insects can be irradiated inside packaging materials, the sterile insects may be released immediately after the irradiation, radiation does not leave residues that could be harmful to humans or the environment, temperature rise in the irradiation process is usually insignificant (Bakri et al., 2005). The induction of radioactivity in irradiated material for SIT operations is avoided by ensuring that the energy applied is bellow 5 million electron volts (MeV) for photons (gamma or X rays) or 10 MeV for electrons (FAO/IAEA/WHO, 1999; IAEA, 2002).

In the 1950's, the scientists used more X rays to investigate the effects of ionizing radiation in insects. Bushland & Hopkins (1953) reviewed initial literature about the effects of X rays on arthropods. However, the penetration and dose rate achieved by most X ray machines at that time were much lower than that of isotopic sources, limiting drastically the number of insects that could be sterilized per batch (Lindquist, 1955).

Most of the facilities dedicated to produce sterile insects to Area-Wide IPM programs have used irradiators with gamma radiation from the radioisotopes <sup>60</sup>Co or <sup>137</sup>Cs. The <sup>60</sup>Co is produced when natural cobalt (100% <sup>59</sup>Co) absorbs neutrons in nuclear reactors, while the <sup>137</sup>Cs can be obtained by chemical separation from spent nuclear fuel such as plutonium or uranium. These radioisotopes can be then encapsulated in stainless steel to become source pencils, for example. The half-lives of the <sup>60</sup>Co and <sup>137</sup>Cs are 5.27 and 30.07 years respectively. The <sup>60</sup>Co emits photons with two energies (1.17 and 1.33 MeV), while <sup>137</sup>Cs emits a monoenergetic photon of 0.66 MeV. Therefore, cesium sources require almost four times more activity than cobalt sources to provide the same throughput (Bakri et al., 2005).

The commercially available irradiators for SIT programs are usually of two types, self-contained or large scale panoramic type, both having as irradiation source several source pencils arranged in many different ways, but typically in a circular array or in a plane or a

single rod. So far, the self-contained irradiators were the most commonly used in SIT facilities. Generally, the radiation source is kept inside a protective shielded chamber, which receives the material to be irradiated by a mechanism that rotates or lowers the canister of insects from the load position to the irradiation position. The canister must be moved (as in a turntable) or hold in a way that the dose delivered gets relatively uniform. The operator controls the dose delivered to the insects by positioning them correctly in the canister and calculating the time of exposure, since the dose rate from  $^{60}\text{Co}$  or  $^{137}\text{Cs}$  pencils changes with time and is determined by the current activity of the source. For example, cobalt sources have their activity reduced by 12% annually, so the operator has to compensate this loss by increasing the exposure time (Bakri et al., 2005). When the time of exposure becomes too long, the irradiation source must be replaced and reload with new pencils of high activity.

After the September 11<sup>th</sup> attacks, the fear of terrorism have provoked an increase in delays and denials of transboundary shipments of radioisotopes, what is constraining the reloading of existing sources and the acquisition of new ones. Between September 2007 and March 2008, for instance, almost 70 reports of delays and denials of shipments of radioactive materials were forwarded to the IAEA, being 13 related to  $^{60}\text{Co}$  (Mastrangelo et al., 2010). In addition, the production of the isotopic irradiator most commonly used at SIT facilities, the self-contained Gamma Cell 220  $^{60}\text{Co}$  irradiator (MDS Nordion International Inc., Ottawa, ON, Canada) has been discontinued. Therefore, due to the growing complexities of the transboundary shipment of radioisotopes and the fear of “dirty bombs”, there are serious doubts about the future availability of small scale irradiators (Mastrangelo et al., 2010).

Two alternatives to gamma radiation are high-energy electrons (with energy < 10 MeV) and X rays (generated from electron beams with energies below 7.5 MeV) (US FDA, 2004). The high energy electrons are generated by electron accelerators and X rays can come from breaking radiation (*i.e.*, rapid deceleration of a beam of electrons before striking a material with a high atomic number, or “bremsstrahlung”), in which way that any radioactive materials are involved. Since they have relative biological effectiveness (RBE) normalized to gamma rays close to one for most insect life stages and doses, many studies have demonstrated that they produce similar effect on insects (Adem et al., 1978; Bushland & Hopkins 1953; Dohino et al., 1994; Lindquist, 1955; Mastrangelo et al., 2010). Actually, almost a hundred low-energy self-contained X ray irradiators are already operating successfully at medical institutes in USA and at two SIT facilities. Such machines have also other advantages as no radiation is produced when switched off, no generation of radioactive waste, better public acceptance and simpler regulatory requirements. It seems that these new technologies may address the demands of national Area-Wide IPM programs that are in expansion around the world.

Currently, about 36 facilities are producing millions of sterile insects per week for national Area-Wide Integrated Pest Control programs or making research on SIT against screwworms, fruit flies, moths and tsetse flies (IDIDAS, 2010). One of the largest biofactories of the world (part of the MOSCAMED program) is located at El Piño, Guatemala, where is produced almost 2 billion sterile males per week of the Mediterranean fruit fly, *Ceratitidis capitata* Wiedemann. At Metapa de Domínguez, Mexico, a mass-rearing facility produces the fruit flies *Anastrepha ludens* Loew (300 million/week), *Anastrepha obliqua* Macquart (30 million/week) and the parasitoid *Diachasmimorpha longicaudata* Ashmead (50 million/week) (Rull et al., 1996). These programs generate substantial direct and indirect benefits to the horticulture industry, health sector and the overall society.

As a result of the containment barrier at the Mexico-Guatemala border by the MOSCAMED program, Mexico's gross revenue from horticultural products have tripled since 1994 to more than US\$ 3.5 billion/year, with a economic return over these years of 167 dollars for each dollar invested in the program (Enkerlin, 2005). The pink bollworm, *Pectinophora gossypiella* Saunders, has been excluded of the San Joaquin Valley, USA, by an ongoing SIT containment program since 1968, which cost approximately US\$ 12.5/ha/season for each cotton grower, but it was estimated that growers pest control costs would increase by US\$ 200/ha if the program was not in place, besides an additional 2.2 million kg of pesticide that would have to be used every year (Bloem et al., 2005). In 1994-1997, the population of a tsetse fly, *Glossina austeni* Newstead, vector of trypanosomosis ("sleeping sickness"), was eradicated by the SIT from Unguja Island of Zanzibar, what allowed a local increase in the amount of small farmers raising indigenous cattle (from 31% in 1985 to 94% in 2002) and in the proportion of domestic versus imported cattle slaughtered for meat (29% to 66% on 1986-1995) (Feldman et al., 2005).

#### 4.2 Biological control

The biological control can be defined as the action of predators, parasites or pathogens in maintaining a pest population density at a lower average than would occur in their absence, eventually reaching a level below the crop's economic damage threshold (De Bach, 1964). This control method differs from the other forms of pest control by acting in a density-dependent manner with the pest population at the point that may become, in many agroecosystems, a self-sustained strategy, restoring the functional biodiversity (Altieri, 1994). As such, the biological control is one of the most environmentally and sustainable control tactics for insect pests and can be applied as part of IPM programs (Altieri, 1994). Basically, the applied biological control can be done through conservation management and classical or inundative techniques.

The conservation approach consists on practices to attract, protect or enhance the natural enemies' populations, as mixing plant cultivars or providing flowering borders which increase the diversity of habitats and serve as alternative food resource (Rabb et al., 1976). In classical biological control, exotic biological agents (e.g., predators, parasitoids or entomopathogens) are introduced into the target area usually in inoculative releases, keeping as main concern the host specificity of the exotic agent (Louda et al., 2003). The third type of biological control, the inundative tactic, involves the mass-rearing and release of the biological agent, exotic or not, in very large numbers, often several times each season as they generally do not establish permanently in the environment. Constraints of the later tactic include the high cost of rearing, adequate quality control and political regulations which complicate shipping and trade (van Lenteren, 2003). Nuclear techniques present several potential applications that can increase the trade, safety, efficiency and cost effectiveness of biological control tactics (Hendrichs et al., 2009).

Reproductively inactivated host insects can be placed in the field at strategic locations as sentinels to monitor wild populations of indigenous biological agents or to explore new exotic agents (Jordao-Paranhos et al., 2003). Sterile F<sub>1</sub> larvae from irradiated *Lymantria dispar* L. had been used to monitor the density and type of parasitoids and entomopathogens in forests (Novotny & Zubrik, 2003). Sterilized *Musca domestica* L. pupae can be used in traps to monitor wild populations of pteromalid parasitoids under conditions of livestock production (Zapater et al., 2009).

Radiation can also be useful in screening classical biological agents under field conditions, when host specificity doubts still remain (Hendrichs et al., 2009) after pre-release studies under quarantine conditions. In other words, the exotic biological agent could be radiosterilized and then released in the field without the risk of establishing any permanent breeding population, allowing assessment of host associations under actual field conditions (Carpenter et al., 2001). As example under evaluation, the exotic herbivore *Episimus unguiculus* Clarke was in quarantine in Florida for the eventual biological control of the Brazilian pepper tree *Schinus terebinthifolius* Raddi (Moeri et al., 2009).

Radiosterilized preys or hosts could also be released in the field prior to the pest outbreak to serve as supplemental food to increase the native population of natural enemies or inoculatively released biological agents. Sterile eggs from irradiated cotton bollworm *Helicoverpa armigera* Hubner and diamondback moth *Plutella xylostella* L. served as hosts for wild egg parasitoids (Wang et al., 2009). In sugarcane fields, the provision of irradiated host eggs to *Trichogramma chilonis* Ishii early in the season allowed building-up its populations and such approach is providing now the control of many sugarcane borers species in a 40,000 ha sugarcane area in Pakistan (Fatima et al., 2009).

Radiation can increase rearing efficiency and parasitoid quality or make non-habitual hosts (i.e., cheaper to mass rear) by suppressing host immune system. Some physiological processes in the host (e.g., defence mechanisms and hormone metabolism) can be selectively modified by radiation (Vey & Causse, 1979). The immune response (haemolymph melanisation and haemocytic encapsulation) of haemolymph from *Galleria mellonella* L. was severely reduced when irradiated, and *G. mellonella* larvae irradiated at 65 Gy were found to serve as potential hosts for the rearing of *Venturia canescens* Gravenhorst (Genchev et al., 2007). Irradiated *Sitotroga cerealella* Olivier eggs, when used as prey substitute, increased larvae viability, fecundity and the sex ratio of the predator *Chrysoperla carnea* Stephens (Hamed et al., 2009). Irradiation of the wasp *Glyptapanteles liparidis* Bouché was used to study the action of its polydnavirus and venom, which were injected along with the sterilized eggs, in *L. dispar* larvae (Hoch et al., 2009). Radiation hormesis was reported in the parasitoids *Habrocon hebetor* Say, *T. chilonis* and *V. canescens* after exposure to very low doses of radiation (Genchev et al., 2008; Wang et al., 2009).

The limited shelf life of prey and hosts may limit their use during mass production of biological agents, but, in some cases, radiation can delay normal insect development, extending the time window for feeding or host parasitisation. The parasitisation period of third instar *Anastrepha* spp. larvae irradiated at 45 Gy was extended, as the efficiency of the parasitism by *Diachasmimorpha longicaudata* Ashmead increased (Cancino et al., 2009b). Irradiation of the carambola fruit fly *Bactrocera carambolae* Drew & Hancock eggs extended the larval period suitable for parasitisation by *Psytalia incise* Sylvestri and *Fopius vandenboschi* Fullaway (Kuswadi et al., 2003). The irradiated parasitoid *Cotesia flavipes* Cameron could be stored as pupae up to 2 months at 10 °C without apparent loss of quality (Fatima et al., 2009).

Insect mass-rearing facilities usually produce significant amounts of by-products (e.g., large batches of sub-standard insects and specimens from the quality control tests). Instead of being discarded, these by-products can be processed or irradiated to rear biological agents (Hendrichs et al., 2009; Nakashima et al., 1996). In fruit fly mass-rearing facilities, discarded larvae or pupae could be used to rear some kinds of parasitoids (Cancino et al., 2009a).

Irradiation can overcome problem with trade barriers related to shipment of biological agents, since accidental inclusion of hitchhikers or fertile pest specimens in the shipments



are possible, by eliminating the need to sort parasitoids from non-parasitized hosts and avoiding the emergence of adult pests from non-parasitized immature stages (Hendrichs et al., 2009). Irradiation can be used to eliminate the risk of introducing fertile spider mites *Tetranychus urticae* Koch, which are provided along with several shipped species of predatory mites (Baptiste et al., 2003). Fruit fly larvae of *A. obliqua*, *A. serpentina* Wiedemann and *A. ludens* are routinely irradiated in the mass-rearing of tens of millions of parasitoids (Cancino et al., 2009a), ensuring shipments clean of the adult stage of these pests.

The release of sterile or half sterile insects together with biological agents has been known to have synergistic effects for population suppression when applied simultaneously, because the sterile insects impact on the adult stage, while the biological agents target mostly the immature stages (Knipling, 1992). Saour (2009) demonstrated the synergistic effects of combining F<sub>1</sub> sterility with egg parasitoids, by releasing *Trichogramma principium* Sugonyaev & Sorokina together with moths irradiated at 250 Gy, what reduced significantly *Phthorimaea operculella* Zeller progeny. Field-cage evaluations in citrus orchards in South Africa revealed that releases of irradiated moths combined with releases of *Trichogrammatoidea cryptophlebiae* Nagaraja provided synergistic suppression of false codling moth populations (Carpenter et al., 2004).

## 5. Radiation as quarantine treatment against insect pests

In international agricultural markets, the use of radiation as a method for the prevention of quarantine insects represents an important alternative post-harvest pest control, reducing the need for chemical fumigants and other similar toxic products. The US 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 (US FDA, 2004).

The advantages of radiation include the no resistance development by pest insects, the absence of residual radioactivity and few significant changes in the physicochemical properties or the nutritive value of the treated products (Lapidot et al., 1991). A major disadvantage is that it is the only commercially applied quarantine treatment that does not result in significant acute mortality. This issue is very important because when inspectors find live quarantine pests from the major phytosanitary treatments, which are based on heat, cold or methyl bromide fumigation, the entire consignment is rejected or retreated regardless of certification of treatment. In this case, the inspectors assume that the treatment was not properly done, the shipment was contaminated with infested commodity or that the cargo was reinfested after treatment. In addition, live adults found in survey traps could trigger restrictive and costly regulatory responses in importing countries (Hallman et al., 2010).

Nevertheless, Hallman (2004a) stated that the objective of irradiation is not acute mortality but prevention of development or reproduction, as most commodities do not tolerate the usual dose ranges required to reach it (usually  $\geq 1$  kGy). Therefore, the inhibition of further development must be considered as a measure of efficacy of phytosanitary irradiation (FAO, 2003). The US Animal and Plant Health Inspection Service (APHIS) has not objected to live adults because of a comprehensive process of validation and certification of irradiation treatment facilities with monitoring of dosimetry and dose application during preclearance programs (Hallman et al., 2010).

The APHIS and the International Plant Protection Convention (IPPC) have approved phytosanitary irradiation treatments for more than 20 insect pest species (FAO, 2009). Tephritid fruit flies are one of the most invasive quarantine pests, attacking 21 of the 24

fresh commodities exported to the United States and to sterilize or disrupt normal development of early stages, doses ranging from 70 to 100 Gy are sufficient for *Anastrepha* species, whilst *Bactrocera* spp. and other species may require doses in the range of 100 to 150 Gy (Follett, 2009). After fruit flies, tortricid moths are the most important pests of quarantine concern for fruit and vegetables. Several studies have shown that a dose of 200 Gy could be sufficient to control codling moth (*Cydia pomonella* L.), *Ecdytoplopha aurantiana* Lima and oriental fruit moth (*Grapholita molesta* Busck) (Arthur, 2004; Hallman, 2004b; IDIDAS, 2010; Mansour, 2003). Curculionid weevils are another important group of pests and available studies suggest that a dose  $\leq$  150 Gy may be sufficient to control cowpea weevil (*Callosobruchus chinensis* L.), *Euscepes postfasciatus* Fairmaire and boll weevil (*Anthonomus grandis* Boheman) (Davich & Lindquist, 1962; Follett, 2006; Gao et al., 2004).

Hallman et al. (2010) analyzed several factors that could affect phytosanitary irradiation efficacy, such as hypoxia, insect life stage, host, dose rate, temperature, diapause and genotypes. After dose itself, hypoxia can be considered the most important factor that abates the effects of radiation on living organisms because lesser radioinduced radicals are produced (Hallman & Hellmich, 2010; von Sonntag, 1987). Cryptically-feeding Tephritidae and Curculionidae that occur as immature inside host plants (practically hypoxic conditions) may present increased radiotolerance (Hallman & Loaharanu, 2002). As radiotolerance also increases as insects develop, a phytosanitary irradiation treatment must be effective against the most tolerant stage that could be present on the commodity (Hallman, 2000). About the differences in hosts, dose rates and temperatures, their characteristics are not so relevant as long as the required minimum dose is absorbed (APHIS, 2005). Jessup et al. (1992) reported the desinfestation of six different fruits from *Bactrocera tryoni* Froggatt third instars at 75 Gy. Temperature did not affect radiation efficacy for a tephritid and a crambid within the cold storage range for fresh commodities (Hallman 2004b; Hallman & Hellmich, 2009). Insects in diapause may be more susceptible to radiation (Hallman, 2000). According to Hallman et al. (2010), most studies that made direct comparisons among populations of the same species did not show significant differences in response to radiation. Cornwell (1966) analyzed 35 irradiated strains of *Sitophilus granaries* L. and found no differences in sterility. The adult emergence of laboratory and wild strains of three tephritid species irradiated as third instars did not differ significantly (Follett & Armstrong, 2004).

Quarantine entomologists are constantly looking for a generic radiation quarantine treatment, which could be able to control a broad group of pests without adversely affecting the quality of a wide range of commodities (Follett & Neven, 2006). Such doses would necessarily be set at the minimum absorbed dose required for the most tolerant organism within that group (Hallman & Phillips, 2008). In 2006, the APHIS accepted as phytosanitary treatments the generic doses of 150 Gy for all Tephritidae and 400 Gy for all insects other than Lepidoptera for commodities entering the United States (USDA-APHIS, 2006). The generic dose of 150 Gy is currently being used for mangoes and citrus fruit exported from Mexico to the United States, and the dose of 400 Gy is applied for Mexican guavas, Indian mangoes and dragon fruit (*Hylocereus undatus* Britton & Rose) from Vietnam, all exported to the United States (Hallman et al., 2010). Australia is also using a generic treatment (250 Gy for insects) to send mangoes and litchi to New Zealand (MAF, 2009).

Hallman & Phillips (2008) suggested that a generic dose of 600 Gy for all insects in ambient atmospheres would be efficacious to attend quarantine purposes, owing to the high radiotolerance of the Angoumois grain moth (*Sitotroga cerealella* Olivier).

## 6. Isotopes as markers for insects

By the late 1940s, isotopic releases from nuclear operations had demonstrated the utility of radiotracers for studying the dynamics of biological systems, and by the early 1950s ecologists were using radioisotopes to new areas of experimental research. Labeling insects with radiotracers in order to study dispersal, population densities, behavior and food intake became a very popular insect-marking method from the 1950s to the 1970s (Table 2).

	Type of Study	Radioisotope	References
Pest Management	Synthetic diets for mass-rearing		Radeleff et al. (1952); Strong & Landes (1965)
	Efficiency of Sterile Males	$^{32}P$	Baumhover et al. (1955); Haisch (1970)
	Efficiency of Predators		Smith (1965); Van Dinther & Mensink (1971); Edney et al. (1974); Moore et al. (1974)
Ecology	Energy Flow in Communities	$^{134}Cs$ , $^{45}Ca$	Crossley (1963); Odum (1963); Reichle (1967); Williams & Reichle (1968)
	Heterotrophic Productivity	$^{22}Na$ , $^{86}Rb$	Van Hook et al. (1970); Kowal & Crossley (1971)
Nutrition	Growth	$^{32}P$	Radeleff et al. (1952); Gordon (1972); Klein & Kogan (1974); Rapport & Turner (1975); Baily (1976)
	Food Utilization	$^{14}C$ , $^{32}P$	Evans (1939); Day & Irzykiewiez (1953); Oertel et al. (1953); Kasting & McGinnis (1965); Waldbauer (1968); Dietz & Lambremont (1970); Devine (1978)

Table 2. Radiotracers most commonly used in entomological studies.

Labeling procedures involved rearing the insects in labeled larval or adult diet, spraying, submersion, attaching radioactive objects to the body, and even indirect methods, in which a plant or animal was rendered radioactive (Barnes, 1959; Radeleff et al., 1952). The detection methods included trapping, killing, and examining specimens at bait stations with a Geiger counter or radioautography (Dissanaike et al., 1957; Jensen & Fay, 1951).

Screwworm *Cochliomyia hominivorax* larvae had been successfully reared on a ground meat medium labeled with  $0.5 \mu\text{C}$  of  $^{32}P/g$  (Radeleff et al., 1952). Long & Lilly (1958) attached pieces of radioactive wire made of  $^{60}Co$  and gold plated to the outside of the wireworm

*Melanotus communis* Gyllenhal with a plastic cement and, after their release into soil, their location was determined by an end-window Geiger tube and rate meter. Strips of  $^{182}\text{Tl}$ , glued to the prothorax of the insect, had been used to label coccinellid larvae (Banks, 1955). Barnes (1959) sprayed foliage with a solution of corn protein hydrolysate with  $50\ \mu\text{C}$  of  $^{32}\text{P}/\text{mL}$  to label the walnut husk fly *Rhagoletis completa*, and 15% of the flies captured in the orchard were labeled, with flies averaging 8022 counts/minute.

When screwworm larvae were reared in wounds of goats which had received intravenously injection of  $^{32}\text{P}$  at  $0.1\ \mu\text{C}/\text{g}$  body weight, the adult flies showed 2500 counts/minute (Radeleff et al., 1952). Plants that had grown in water containing  $100\ \mu\text{C}$  of  $^{35}\text{S}/\text{L}$  were used to mark Lepidoptera larvae (Kettlewell, 1952).

Schoof & Siverly (1954) labeled the American cockroach, *Periplaneta americana* L., in order to assess its movements within a sewage system. The authors captured about 6500 insects and sprayed them with a solution containing  $^{32}\text{P}$  and casein as sticker. Almost 14% of the insects were recovered and, curiously, they noticed that only a single specimen was found elsewhere in the sewage system, leading to the conclusion that the involvement of *P. americana* in disease transmission out of the sewage system was practically negligible.

The advantages of using radioisotopes rather than conventional markers were the relative permanence (dyes often rubbed off and molting usually eliminates external labels), the rapidity of checking, and the possibility of tracing the labeled insects that were out of sight (e.g., underground). Several tracers were studied, such as  $^{60}\text{Co}$ ,  $^{89}\text{Sr}$ ,  $^{65}\text{Zn}$ ,  $^{144}\text{Ce}$ ,  $^{131}\text{I}$  and  $^{45}\text{Ca}$ , but  $^{32}\text{P}$  was far the most applied radioisotope for tagging due to its short half-life, safety, activity and easy of detection (O'Brien & Wolfe, 1964).

One of the earliest examples of using inorganic  $^{32}\text{P}$  as a label was reported by Hasset & Jenkins (1949), who reared *Aedes aegypti* mosquitoes in water containing up to  $1\ \mu\text{C}/\text{mL}$  and obtained adults with up to 10,000 counts/minute each. Fredeen et al. (1953) applied  $0.2\ \mu\text{C}$  of  $^{32}\text{P}/\text{mL}$  in vats used to rear blackflies, and 800,000 larvae were labeled and released, with counts up to 50,000 counts/minute each. Odum (1963) used  $^{32}\text{P}$  to isolate individual food chains of several natural communities.

The short half-life of 14 days of  $^{32}\text{P}$ , however, was a disadvantage in studies where prolonged observations were necessary. Cerium-144 was used as persistent label for fleas, mosquitoes, cockroaches, ticks and other insects, as it has a half-life of 282 days and its daughter,  $^{144}\text{Pr}$ , has a half-life of 18 minutes, but emits an energetic beta particle of 2.97 MeV, which could be easily detected (Quan et al., 1957). Babers et al. (1954) needed to make observation on *A. grandis* for over a 5 month period and found that dipped weevils from a solution of  $\text{Co}^{60}\text{Cl}$  ( $5\ \mu\text{C}/\text{mL}$ ) with detergent averaged 710 counts/minute each. Although the radioisotope  $^{35}\text{S}$  has a convenient half-life of 87 days, it is a weak beta emitter (0.17 MeV) and, therefore, a poor choice for labeling (Kettlewell, 1952).

A requirement for the use of such tracers was that the behavior of the labeled insect should not be affected, and some studies investigated the conditions that could affect the tagged insects, such as age, stage, radiotracer concentrations and so on. Hasset & Jenkins (1951) performed a detailed study of the conditions affecting mosquitoes labelled with  $^{32}\text{P}$  and compared stages,  $^{32}\text{P}$  concentrations and age. Younger larvae were too radiosensitive and pupae absorbed less phosphorus. The maximum concentration in water was  $0.1\ \mu\text{C}/\text{mL}$  and females took up three times as much as males, concentrating the  $^{32}\text{P}$  about 75 times over the concentration in the medium. Toxicity to the insect was also a serious problem to be considered in many studies (Quarterman et al., 1955). The radioisotopes  $^{45}\text{Ca}$  and  $^{131}\text{I}$  were very toxic when fed to adult house-flies at  $1\ \mu\text{C}/\text{mL}$  of milk, whereas  $^{32}\text{P}$  was satisfactory (Quarterman et al., 1954).

Jensen & Fay (1951) compared the effectiveness of feeding  $^{32}\text{P}$  to adults and to larvae of houseflies and secondary screwworms (*Cochliomyia macellaria* F.) and observed that larvae fed on a medium with  $0.1\ \mu\text{C}$  of  $^{32}\text{P}/\text{g}$  gave adults averaging 100 counts/minute, whilst adults fed on milk with  $1\ \mu\text{C}/\text{mL}$  gave counts of 1100 for males and 2000 for females.

Much work was done on the measurement of the feeding rate of insects, as in the transfer of food within colonies, water intake and transfer of plant juices between plants.

One of the methods used for feeding rate determination, which was proved very fruitful for studying especially primary consumers or predators, was based on the radiotracer conservation through the system "food-insect body-removed products". If insects were fed with uniformly labelled food, the food uptake could be calculated from the amount of tracer measured in the body and that measured in the products removed by secretion (like honey dew), excretion, respiration of  $\text{CO}_2$ , (whether  $^{18}\text{O}$  or  $^{14}\text{C}$  was used), water transpiration (whether  $^{18}\text{O}$  or  $^3\text{H}$  was used), egg production and others (Buscarlet, 1983; Kasting & McGinnis, 1965). In grain beetles fed on tapioca labelled with inulin- $^{14}\text{C}$ , which was not assimilated by the insect, the ingestion rate could be estimated from the  $^{14}\text{C}$  turnover rate constant and from the expendable solids (Devine, 1978).

Oertel et al. (1953) studied food transmission in bee colonies. Drone bees were exposed in a cage with unlabeled sucrose syrup available and separated by a screen from worker bees which had fed on sucrose- $^{14}\text{C}$  syrup. The drone bees became radioactive as a result of being fed sucrose by the workers. Alibert (1959) studied the termite *Calotermes flavicollis* F. and verified a quick transmission of food between the workers: in 35 hours, all insects were labeled.

McEnroe (1961) used  $^{32}\text{P}$  to evaluate the water intake of individual mites. *Tetranychus telarius* L. was fed on water containing inorganic  $^{32}\text{P}$  and the author verified that it took in from 1.3 to  $4.6\ \mu\text{mL}$  in one hour (about 25% of the mite's body weight).

Many researchers had been interested in following the feeding behavior of phytophagous insects, in particular disease vectors as aphids. Miss Hamilton (1935) described the use of the  $\alpha$ -emitter, polonium, as a tracer in *Myzus persicae* Sulzer transmission, measuring its activity with a gold-leaf electroscope. Day & Irzykiewicz (1953) fed aphids either on leaves from cabbages cultivated in a  $^{32}\text{P}$  solution or through a plastic membrane on a sucrose solution labeled with  $^{32}\text{P}$  and verified that *Myzus* took up to  $69\ \mu\text{g}$  of plant material in only one hour (35% of its weight; data computed from the average radioactivity in leaf tissues and the radioactivity taken up by the insects) and up to 7% of the total uptake was excreted in an hour. When imbibing sucrose, *Myzus* took up only about 3% of its plant value, and, finally, the authors shown that the aphids did reinject imbibed material (but only up to 0.5% of the imbibed dose was reinjected in a day).

Lawson et al. (1954) assessed the transmission of tobacco plant juices by *Myzus* sp. by feeding the aphids on tobacco plants grown in soil treated with  $^{32}\text{P}$  phosphoric acid and then placing them on unlabeled plants. Several spots of radioactivity were shown after 6 days, not caused by external honeydew or by absorption of honeydew, and translocation of radioactivity to other leaves of the plant was also found.

Labeled insects were also used as devices to study parasites. Larvae of the mosquito *Armigera obturbans* Walker were reared in water containing  $1\ \mu\text{C}$  of  $^{32}\text{P}/\text{mL}$ , and the subsequent adults, averaging  $7 \times 10^5$  counts/min., were let to feed on cows or men infected with microfilaria. The filarial larvae, *Setaria digitata* Linstow, gave 174 counts/minute, which was sufficient to allow tracking its passage through host tissues either by counting or radioautographically (Dissanaike et al., 1957).

Recent stricter environmental protection laws coupled with the development of simpler, less expensive and reliable methods have reduced the usefulness of the radioactive isotopes as insect markers.

A substitute for many radionuclide methods is the stable isotope methods, as they pose no health or environmental risks. Isotopic signatures are natural differences in stable isotope composition of organisms caused by discrimination against the heavier isotopes during some biological processes. Over the past twenty years, much progress was made in isotope ratio mass spectrometry in terms of detection, accuracy and automation. Stable isotopes do not decay and occur naturally in the environment. Other advantages are the analysis costs (depending on the isotope and the matrix, the cost per sample may range from US\$ 5-100.00), shipping stable isotope samples is simple, safe and inexpensive (IAEA, 2009).

Natural isotopic signatures are already used in wide range of research areas and, therefore, were standardized to an internationally accepted scale. Because of the large differences in the abundances of the carbon and nitrogen isotopes ( $^{12}\text{C} \approx 1.1\%$ ,  $^{13}\text{C} \approx 98.9\%$ ,  $^{15}\text{N} \approx 0.3663\%$  and  $^{14}\text{N} \approx 99.63\%$ ),  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$  ratios are generally expressed in the delta notation in parts per thousand (per mil ‰) relative to the international standard Vienna PeeDee Belemnite (VPDB) and atmospheric nitrogen, respectively (IAEA, 2009). These stable isotope markers meet the usual criteria for use in insect studies: retention, no effect on behavior, durability, easily applied, clearly identifiable, and not expensive (Hagler & Jackson, 2001).

The isotopic signature of an organism is mainly dependent on what it eats and as natural processes also lead to distinctive isotopic signals, the formation of the so called isotopic landscapes (*i.e.*, isoscapes) is even useful in tracing insect movement, mating patterns, and in studies about the use of natural resources (Hershey et al., 1993; Helinski et al., 2007; Peterson, 1987). For example, some insects that are reared on C4 sugar based diets will be isotopically different in  $^{13}\text{C}$  signatures from the wild populations that naturally feed on C3 plants (Hood-Nowotny et al., 2006).

While most of the fruit fly species feed on C3 plants in the wild, which have a carbon isotope signature of around -28‰ versus VPDB, almost all mass-rearing facilities use cane sugar in the larval and adult diet, which is a C4 sugar source (with a signal of around -11‰ versus VPDB). Hood-Nowotny et al. (2009) demonstrated that this difference in isotopic signatures between wild and released factory-reared flies could be a reliable and intrinsic secondary marker to complement existing marking methods from Area-Wide IPM programs.

Insects can also be marked by adding an enriched compound to the diet, such as  $^{15}\text{N}$  labelled glycine (Caquet, 2006; Fisher et al., 2003; Markow et al., 2000; McNeill et al., 1998; Nienstedt et al., 2000; Nienstedt et al., 2004). The natural enemies, *Cotesia plutellae* Kurdjumov and *Hippodamia convergens* Guérin-Meneville, that foraged at the flowers of  $^{15}\text{N}$ -marked plants showed detectable quantities of the marker (Steffan et al., 2000). Plant material enriched with  $^{15}\text{N}$  was added to the diet of navel orangeworms *Amyelois transitella* Walker and both the orangeworms and wasps *Goniozus legneri* Gordh that parasitized them gave detectable levels of  $^{15}\text{N}$  in their systems (Steffan et al., 2000).

Among the disadvantages of the stable isotopes methodology are the cost of isotope ratio mass spectrometers (some cost more than US\$ 100,000), the controlled environment required by the equipment and skilled personnel to use the mass spectrometer (IAEA, 2009).

## 7. Conclusion

Radioisotopes allowed the rise of an entire new branch of the study of insects, the Radioentomology. Even today, the Sterile Insect Technique and phytosanitary irradiation

treatments protect horticultural markets and livestock of many countries. Furthermore, radiotracers had a very important role in revealing the characteristics and dynamics of several biological systems.

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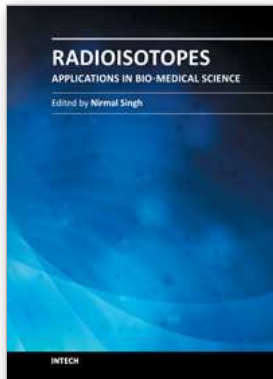
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## **Radioisotopes - Applications in Bio-Medical Science**

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