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Mortality and sterility consequences of X-ray radiation on the granary weevil, *Sitophilus granarius*

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Abstract

The efficacy of X-ray radiation as a method of protection of maize grain against the granary weevil *Sitophilus granarius* (L.) (Coleoptera Dryophthoridae) was evaluated in laboratory tests. Irradiation was applied at five doses: 10, 50, 70, 100 and 200 Gy. After each irradiation treatment samples were divided into two parts. One group (n = 50) was left unchanged, while in the other group 25 treated adults were replaced by 25 untreated adults, in an attempt to determine the sterility effect. Mortality of *S. granarius* adults in the unchanged group was estimated after 48 h and 7, 14, 21 d of exposure. After the 21 d counts, all the exposed adults were removed and progeny production was assessed 45 days later. The mortality consequences of X-ray radiation on the granary weevil was highly influenced by the time after exposure and the dose applied. At doses of 50 and 70 Gy, the efficacy accepted in plant protection (insect mortality: 95%) was recorded only on 21 d. During the application of 100 and 200 Gy doses, high mortality values (83.5 and 97.5%) were observed on 14 d. Additionally, the use of multiple doses at a minimum treatment of 50 Gy was effective against *S. granarius* in maize under operational conditions. Our results confirmed the complete sterilizing effect of the doses of 70 Gy and above. The autocide effect of 50 and 70 Gy doses were demonstrated when irradiated specimens were mixed into groups of fertile specimens. Consequently, these doses might be successfully applied to put sterile insect technique (SIT) into practice.

Key words: mortality, sterility, reproduction, *Sitophilus granarius*, X-ray radiation.

Introduction

The granary weevil *Sitophilus granarius* (L.) (Coleoptera Dryophthoridae) is a typical cosmopolitan pest. It can cause significant damage to stored grains, and may drastically decrease yields (Fava and Burlando, 1995). The females lay 125 eggs on average (Jávor, 1990) and the larvae consume the inside of the grain kernels (Stejskal and Kucerova, 1996). Damaged grain has reduced nutritional value, low percentage germination, reduced weight and lowered market value. Environmentally friendly, cheap and effective methods are needed to reduce *S. granarius* damage in stored grains.

Residual insecticides are widely used to control insects in stored grain but residues and development of resistance in certain species have been of some concern (Arthur, 1996). As options become more limited, countries may increasingly be expected to turn to irradiation as an alternative treatment (Pszczola, 1997). Moreover, as it has been demonstrated repeatedly (Tuncbilek, 1995), the residue-free character is an advantage of irradiation disinfestations over chemical fumigation (Trematerra, 2013). Therefore, pest sterility and mortality induced by various types of irradiation might be a good solution (Mastrangelo and Walder, 2011; Karunakaran and Jayas, 2014). Mostly ionizing radiation (e.g. γ-rays and X-rays) has been used to manage storage pests (Tilton *et al*., 1966; Watters and MacQueen, 1967; Brower *et al*., 1973; Hallman, 2013), but there were also attempts with a variety of other irradiation types, such as radio waves, radar, infrared, microwaves and ultraviolet (Adem *et al.*, 1978; Hasan and Khan, 1998 Abdelaal and El-Dafrawy, 2014; Echereobia *et al*., 2014). Insect irradiation is safe and reliable when established safety and quality-assurance guidelines are followed. The key processing parameter is the absorbed dose, which must be controlled to ensure that treated insects are sufficiently sterile in their reproductive cells and yet able to compete for mates with wild insects (Bakri *et al*., 2005a). However, the dose of efficacy was, among other things, influenced by oxygen concentration, temperature and substance (Hasan and Khan, 1998; Bakri *et al*., 2005a). Beside, the treated grain and food can be used for foraging and human nutrition without all health risk (Renu and Chidanand, 2013; Kotwaliwale *et al*., 2014).

Weevils are known to be sensitive to irradiation (Tilton and Brower, 1987). Brown *et al*. (1972) found a dose of 10 Gy to be sufficient to cause sterility in adults of *S. granarius*. According to Aldryhim and Adam (1999), the same dose induces decreased reproductivity and a 70 Gy dose causes sterility at the pupal and four-week-old adult stages. The sterilizing effect of a 70 Gy dose was also confirmed in the case of various arthropod pests (Tsan *et al*., 2002; Tandon *et al*., 2009). Jefferies (1962) found the dose sufficient to cause complete sterility for adults of *S. granarius* to also cause sterility when applied to other life stages. Young adults would not produce progeny when treated with doses of 100 Gy or above. The same results were found by Brown *et al*. (1972). Tuncbilek (1995) found that a dose of 80 Gy caused complete sterility to adults of *S. granarius.* Zewar and Abdel-Salam (1988) found that irradiation of *S. granarius* at 160 Gy decreased food consumption rates on wheat, sorghum and rice by 92.4, 99.3 and 100%, measured after five weeks. On the other hand, at a rather high dose (300 Gy) an immediate lethal effect can be observed between treated *S. granarius* specimens (Aldryhim and Adam, 1999; Tsan *et al*., 2002; 2003).

The physiological cause of this phenomenon is the release of free-radicals and a consequent molecular membrane damages (Machlin and Bendich, 1987).

Our objective was to study the effects of different X-ray radiation doses on the mortality and the progeny vitality of *S. granarius*. The summarizing report of the previous researches can be found in IDIDAS (2012). However, the concrete irradiation dose has not already been determined in connection with *S. granarius*. The sterility and mortality effects of wider irradiation doses has not been studied. This research endeavours to better understand and contribute to the more accurate clarification of the environmental physical effects and the counteraction of the important stored pests. This study aims at determining the effects of X-ray radiation on *S. granarius* adults and their reproductive ability.

Materials and methods

Doses of 0 (control), 10, 50, 70, 100 and 200 Gy were used on sixteen replicates at each dose. Untreated, clean and infestation-free maize grain was used for experimentation. Each sample (100 g maize) was placed in a small glass vial (4 dl) and 50 healthy *S. granarius* adults of mixed sex (mated and unmated) and age were added. The vials were covered with well ventilated textiles and placed in incubators at 26 ± 2 °C and 60 ± 7% RH.

Irradiation was carried out by the high energy ionizing radiation of a Siemens Primus linear accelerator. Large (40 × 40 cm) field size 15 MV energy ionizing radiation were used. Eight glass vials were irradiated at the same time. The vials were placed on a glass plate above a 3 cm water layer (figure 1). For achieving a homogenous dose distribution the ionizing radiation crossed the water layer from beneath. A CT based radiation treatment plan (made by a commercial XiO treatment planning system which calculates absorbed dose on Computer Tomography image sets) was used to determine the dose for the different samples: 10, 50, 70, 100 and 200 Gy were prescribed as the mean doses of the target volumes. Each treatment was made separately. Deviation from the prescribed dose was less than ± 7% in 90% of the target volumes.

Each treatment was divided into two parts immediately after the X-ray radiation. Two times eight sample groups were formed by treatments. One of the groups was left unchanged [intact group (IG)], while in the other group [mixed group (MG)] 25 treated adults were replaced by 25 untreated adults with the purpose to determine a sterility effect. Direct mortality was only examined in the intact groups. Dead adults were counted after 48 h and 7, 14, 21 d. After the 21 day count, from both parts of each sample, the adult insects (dead and alive) were removed, after which the vials returned to the incubator for another 45 d. After this period, the emerged *S. granarius* adults were counted, classified as dead or alive and removed from the vials. These adults were observed for survival and progeny as an indication of reproductive ability.

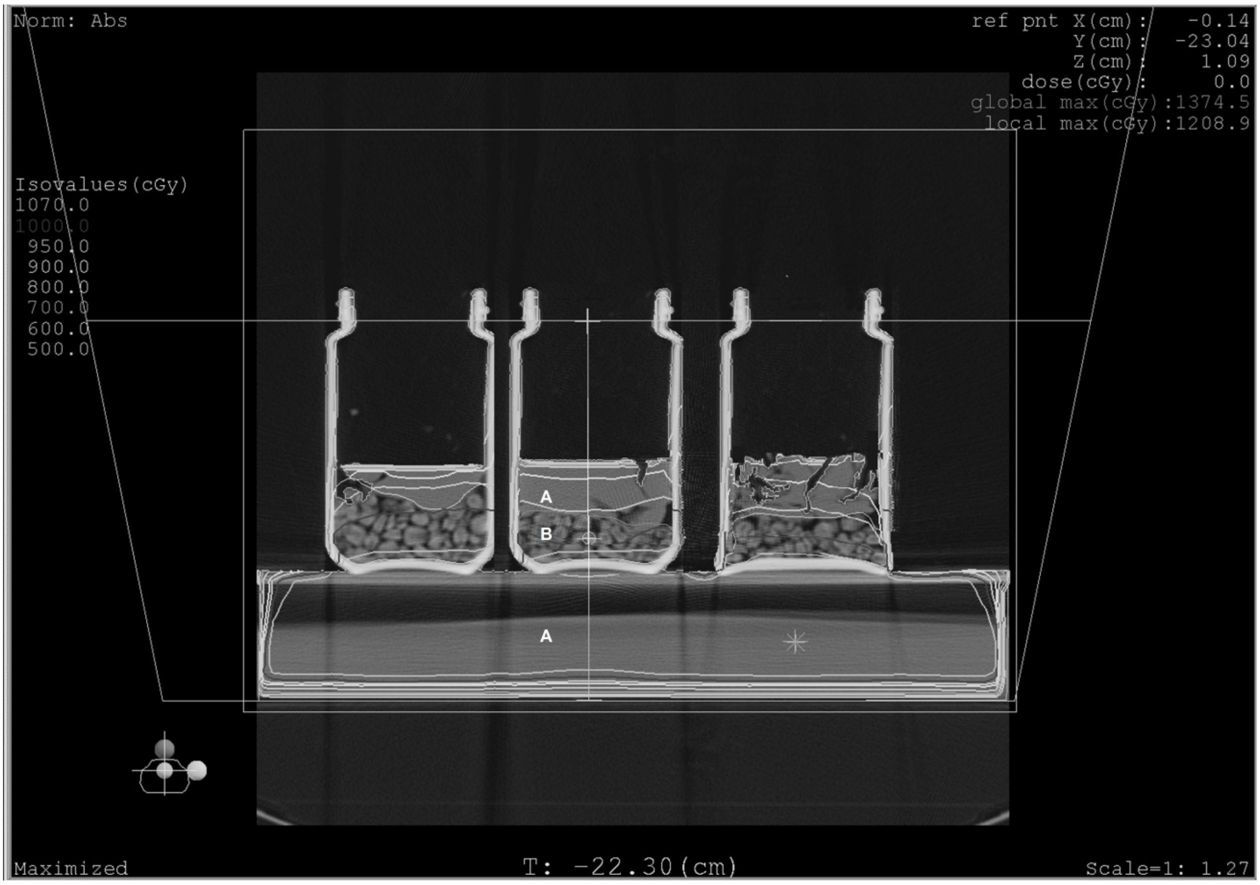

Figure 1. Ct-based radiation treatment plan of the samples. A: water layer; B: infested maize grain by granary weevils.

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Mortality was evaluated as a function of both time after exposure and dosage rate. The data were analyzed using one-way ANOVA in SPSS 11.5 software, with weevil mortality as the response variable and time after exposure and dose rate as the main effects. Means were separated by using the Tukey (HSD) test, at $P \leq 0.05$. Numbers of progeny were also examined statistically by one-way ANOVA (Tukey test, $P \leq 0.05$), and the effect of sterile insect technique (SIT) (Bakri *et al.*, 2005a) (distinction of progeny numbers between IG and MG) was analyzed by Student t-probe ($P \leq 0.05$) using Microsoft Excel 2007.

Results

Insect mortality is shown in figure 2 as a function of treatments. As expected, the mortality of insects increased in the control samples with the progress of the experiment. An increase of insect mortality caused by higher irradiation doses was evident from 7 d and onward, with the highest applied dose (200 Gy) inducing the highest mortality. There was a significant difference between the mortality of doses 10 and 50 Gy. We did not detect any significant increase of insect mortality at 10 Gy irradiation during the whole period of the experiment. In contrast, at a 200 Gy dose the increase of mortality was already observed on 7 d. However, in the case of doses 50 Gy and above, the most remarkable perishing percentages were recorded on 14 d. High mortality values occurred by 14 d at 100 (83.5%) and 200 Gy (97.5%). In the last week (21 d), only the 50 Gy treatment showed a significant increase of mortality (31.5%). Almost complete mortality of the experimental insects was recorded on 21 d at 50, 70 and 100 Gy, while the irradiated insects suffered complete mortality at 200 Gy.

The mortality of *S. granarius* was found to be clearly correlated with the doses of radiation and time after exposures. We observed a significant difference of mortality caused by increasing the irradiation doses in all time after exposures. The statistical relationship was extremely strong between the two examined factors (uniformly: $P = 0.000$). The increase of weevil mortality caused by longer time after exposures was also statistically confirmed in the case of all dose rates (uniformly: $P = 0.000$). The results of the dose-independent combined tests were similar.

With increasing the irradiation doses the adult progeny decreases in both groups (table 1). After forty five days, the total adult progeny production was 50% in the treated samples as compared to the initial control weevil populations. By our additional hypothesis, at all doses examined, progeny production was higher in mixed groups (MG) than in the intact groups (IG). In addition, a complete suppression of progeny production was achieved by 70 Gy and higher doses in IG. A further destructive effect in the IG was only confirmed by statistical examination. On the other hand, in all cases, the effect of irradiation treatment on the number of dead progeny adults was statistically not confirmed.

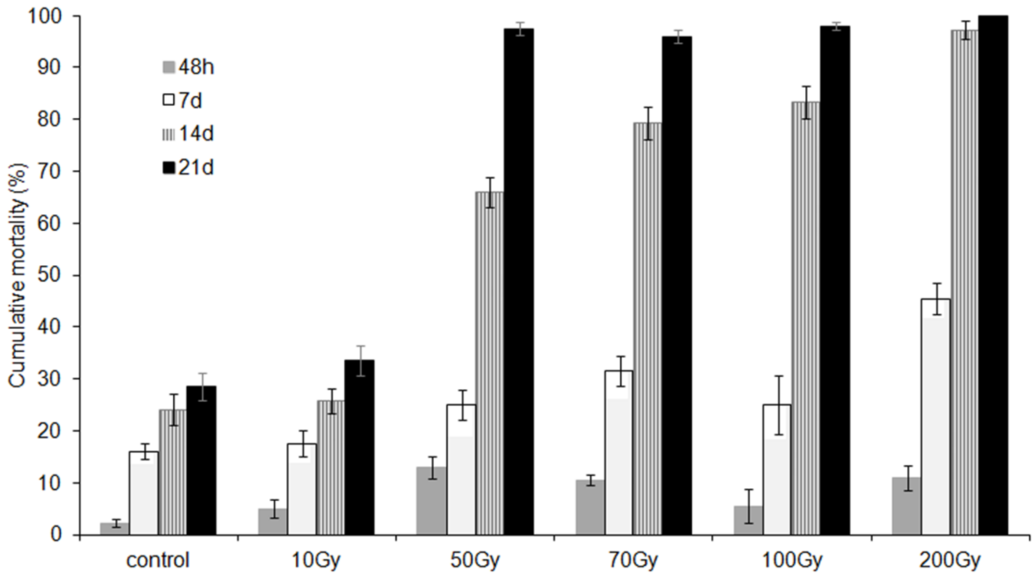

Figure 2. Mortality of *S. granarius* adults (mean ± SE) irradiated with five different dose rates of X-ray radiation, as a function of time after exposures.

Table 1. Progeny production of *S. granarius* (mean number of adults ± SE) and percentage (± SE) of dead progeny on X-ray irradiated maize, 45 days after the removal of exposed *S. granarius* adults, and the statistic relationships (P ≤ 0.05). Significant correlations in Italics.

X-ray dose (Gy)	I G (intact group)		M G (mixed group)		Student-t value
	No. progeny	% dead adults of progeny	No. progeny	% dead adults of progeny	
0 (control)	25.4 ± 5.65	5.72 ± 2.56			
10	10.33 ± 3.49	42.85 ± 13.82	20.91 ± 9.26	50.51 ± 8.11	0.311
50	1.47 ± 1.39	35.40 ± 6.45	21.11 ± 5.93	45.79 ± 12.62	*0.009*
70	0	0	17.92 ± 7.5	41.66 ± 5.70	*0.047*
100	0	0	4.48 ± 4.49	33.33 ± 9.83	0.350
200	0	0	6.52 ± 3.24	95.54 ± 3.22	0.457
P	*0.001*	0.455	0.305	0.375	

The effect of SIT was only determined by means of a statistical comparison of the progeny of the differently treated mixed groups. Statistically significant distinctions (P ≤ 0.05) were registered in the case of 50 and 70 Gy. At doses of 10, 100 and 200 Gy no significant differences were detected.

Discussion

By increased irradiation doses, the adult mortality of *S. granarius* increased and its progeny on maize grains decreased. However, its mortal effect requires time after exposure. The efficacy usually accepted in plant protection (insect mortality: 95%) did not occur in 50 and 70 Gy doses until 21 d.

The use of multiple doses has enabled us to identify a minimum treatment of 50 Gy which would be effective against *S. granarius* in maize under operational conditions. Treatments of 50 and 70 Gy are required to ensure zero replacement which equals sterility and later death of all adults within a shipping transit time of 28 days (Aldryhim and Adam, 1999). However, a quick efficacy (97.5% on 14 d) might also be realized with the help of the highest dose (200 Gy). In the latter case the sterilizing effect of irradiation may of course be waived. Obviously, the radiation tolerant properties of the insect can be perceived as well (Sullivan and Grosh, 1953; Bakri *et al*., 2005b).

By testing the mixed (irradiated and non-irradiated) groups, this study is the first to explore the sterilizing effects of X-ray radiation on the progeny of *S. granarius.* A lower dose of irradiation does not induce an immediate death of adults, but it can prevent an increase of population through its lethal effect on immature stages, the sterilization of adults and a reduction of adult longevity (Buscarlet, 1983).

Our results confirmed the complete sterilizing effect of 70 Gy and higher doses. The autocide effect of the doses of 50 and 70 Gy was demonstrated when irradiated specimens were mixed into groups of fertile specimens. For the purpose of the implementation of the sterile insect technique, doses other than what is discussed above might not be suitable, as lower doses are inefficient while higher doses destroy the experimental population (Trematerra, 2013; Enu and Enu, 2014). However, the damages and the number of individuals of the progeny of a fertile population may be successfully reduced with the method of adding a group of viable, properly sterilized specimens.

The SIT can be a very perspective solution especially in the protection of the stored product pests. However, there are several conditions of the successful utilization: the special clarification of the irradiation technique; the isolated target population; the mass propagation of the target insects, which ensures the prevalence of the sterile specimens (Bakri *et al*., 2005a; Mastrangelo and Walder, 2011; Karunakaran and Jayas, 2014).

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