Effect of gamma irradiation of gravid *Tetranychus desertorum, T. urticae* and *Oligonychus ilicis* (Trombidiformes: Tetranychidae) females on the viabilities and durations of F_1 life stages

Andre Ricardo Machi[1,2,], Valter Arthur[2,1], Gabriel Adrian Sarriés[3], and Sonia Maria De Stefano Piedade[3]*

Abstract

The objective of the study was to evaluate—as an alternative phytosanitary treatment—the effects of gamma irradiation of gravid spider mite females on the viabilities and durations of F_1 eggs and deutonymphs. The study focused on *Tetranychus urticae* Koch, *Tetranychus desertorum* Banks and *Oligonychus ilicis* (McGregor) (Trombidiformes: Tetranychidae). Each mite species was identified under a stereomicroscope, and individual female mites of each species were each taken with a fine-tipped brush and transferred to a Petri dish—i.e., 1 female mite individualized per dish with 32 dishes per species. Each dish with a *Tetranychus* spp. female was provided with a jack bean (*Canavalia ensiformis* [L.] DC) (Fabales: Fabaceae) leaf, and each dish with an *O. ilicis* female was provided with a coffee (*Coffea arabica* L.; Gentianales: Rubiaceae) leaf. Gravid female mites were irradiated in a Gammacell 220 cobalt-60 source—dose rate 0.486 kGy/h—with doses of 0 (control), 100, 200, 300, and 400 Gy with 32 replications per mite species. Evaluations were made every 24 h for 22 d after irradiation by counting the numbers of eggs, deutonymphs, and adults and by recording the mortality in each stage. Statistical analyses were done with generalized linear models with either the quasi-binomial distribution or the Gaussian distribution—as appropriate—for analyses of mortality rates and durations (d) of the F_1 eggs and deutonymphs of the 3 mite species. A 3 × 5 factorial experimental design was used in which factor A (3 levels) pertained to the species and factor B (5 levels) pertained to the 4 radiation doses plus the control. For Factor B polynomial regressions of dose rates on biological effects were used. The results showed progressive decreases in egg and deutonymph viabilities that were proportional with progressive increases in radiation doses. Against *O. ilicis* 200 Gy prevented all F_1 neonates from developing beyond the first instar. Against *T. urticae* and *T. desertorum* 300 Gy prevented all F_1 neonates from developing beyond the first instar. Irradiation of gravid females caused the durations of the F_1 egg stage and the F_1 deutonymph stage to be prolonged.

Key Words: phytosanitation; radiation; ionizing radiation; *Oligonychus ilicis; Tetranychus urticae*; *Tetranychus desertorum*

Resumen

El objetivo del estudio fue evaluar—como un tratamiento fitosanitario alternativo—el efecto de la irradiación gamma de hembras grávidas sobre la viabilidad y la duración de los huevos y deutoninfas de la generación F_1. El estudio se centró en *Tetranychus urticae* Koch, *T. desertorum* Banks y *Oligonychus ilicis* (McGregor) (Trombidiformes: Tetranychidae). Se identificó cada especie de ácaro bajo un microscopio estereoscópico y hembras individuales de cada especie fueron sacadas cada una con un pincel de punta fina y se transfirieron a una placa de Petri — un ácaro hembra individual por plato con 32 platos por especie. Se proporcionó cada plato con una hembra de *Tetranychus* spp. con una hoja de canavalia (*Canavalia ensiformis* [L.] DC) (Fabales: Fabaceae), y cada plato con una hembra de *O. ilicis* fue provisto con una hoja de café (*Coffea arabica* L.) (Gentianales: Rubiaceae). Se irradiaron las hembras grávidas en un fuente de Gammacell 220 de cobalto-60 — tasa de dosis 0,486 kGy /h — con dosis de 0 (control), 100, 200, 300, y 400 Gy con 32 repeticiones por especie de ácaro. Se realizaron las evaluaciones cada 24 h durante 22 d después de la irradiación contando el número de huevos, ninfas (deutoninfas), y adultos, y registrando la mortalidad en cada estadio. Se realizaron los análisis estadísticos mediante el uso de modelos lineales generalizados, ya sea con la distribución quasi-binomial o la distribución Gaussiana—como apropiado—para el análisis de la tasa de mortalidad y de la duración (d) de los huevos y deutoninfas de la F_1 de las 3 especies de ácaros. Se utilizó un diseño experimental factorial 3 × 5 en la que el factor A (3 niveles) pertenecía a la especie y el factor B (5 niveles) se refería a las 4 dosis de radiación más el control. Para el Factor B, regresiones polinómicas de las tasas de dosis en los efectos biológicos fueron utilizados. Los resultados mostraron una disminución progresiva en la viabilidad de los huevos y las deutoninfas que fueron proporcionales con el aumento progresivo en las dosis de radiación. Contra *O. ilicis,* 200 Gy impidió el desarrollo de todas las larvas recién nacidas de la F_1 más allá del primer estadio. Frente a *T. urticae* y *T. desertorum*, 300 Gy impidió el desarrollo de todas las larvas recién nacidas de F_1 más allá del primer estadio. La irradiación de las hembras grávidas causó la prolongación de la duración de los estadios de huevos y deutoninfas de la $F_{1.}$

Palabras Clave: fitosanitación; radiación; radiación ionizante; *Oligonychus ilicis; Tetranychus urticae; Tetranychus desertorum*

[1]University of São Paulo, Center for Nuclear Energy in Agriculture (CENA), Department of Environmental and Radiobiology. Piracicaba–SP, 13400-970, Brazil
[2]University of São Paulo, Department Radiation Technology Center, Institute of Nuclear Energy Research (IPEN). Lineu Prestes Avenue, 2242. University City – São Paulo – SP, 05508-000, Brazil
[3]University of São Paulo, Department of Exact Sciences of the ESALQ / USP. Luiz de Queiroz College of Agriculture. Padua Dias Avenue, 11. Piracicaba- São Paulo – SP, 13418-900, Brazil
*Corresponding author; E-mail: armachi@cena.usp.br

Brazil is one of the world's 3 largest producers of fruits, with a production that exceeds 40 million tonnes per yr, behind only India and China (SEAB 2016). However, the productivity, quality and profitability of those agricultural products depend largely on the damage caused by insects, mites, and pathogens and the costs of their control (Levine & D'Antonio 2003).

The existence of phytosanitary problems—which entail various threats to production, productivity, commercialization and access to export markets—explains and justifies the great national and international efforts underway to modernize agriculture with the imperatives of combating pests, diseases and weeds, as well as increasing levels and quality of production (Barros et al. 2009).

In Brazil, various species of insects and mites have direct (i.e., losses of quantity and quality) and indirect impacts (i.e., low prices for agricultural products in the world markets). Pimentel et al. (2005) estimated losses of more than 100 billion US dollars to crops and pastures in several countries caused by alien invasive species. The distribution of these losses is variable, because the socioeconomic differences of the country, deficiencies in available scientific information, and techniques to estimate the potential economic impacts of these pests are limited (Bento 1999), with reports existing only for a small number of species (Vilela et al. 2001).

Brazil is increasing its exports every yr, aiming to enlarge even more the external market. Producers and exporters are looking for measures to increase the safety and shelf life of fruits. Among the phytosanitary treatments used are chemical methods; however, a major chemical tool, fumigation with methyl bromide—a substance that destroys the stratospheric ozone layer—has been removed from the market in developed countries and now is being removed from the market in developing countries (UNEP 2006). Due to these problems, irradiation is rapidly becoming an alternative quarantine treatment for the control of plant pests—just as with food preservation where this alternative method is already being used with success.

Indeed, phytosanitary irradiation has been used successfully for the control of insects infesting perishable commodities (Arthur et al. 2012; Hallman 2011). But the phytosanitary irradiation of mites (Arachnida: Acari: Trombidiformes) of agricultural importance has been little studied. These pests attack almost every type of crop in Brazil, including mango, strawberry, citrus, lychee, cucumber, and eggplant, among others. Studies on the phytosanitary irradiation of mites are justified by the fact that the Order Trombidiformes includes a large number of species that cause losses of many millions of US dollars every yr, and whose combat requires the use of acaricides for phytosanitary pest control (Flechtmann 1979).

The life stages of spider mites (Tetranychidae) consist of the egg, larva, protonymph, deutonymph and adult. The mode of reproduction of spider mites is arrhenotoky in which fertilized eggs develop into females and non-fertilized eggs into males. Spider mite eggs tend to hatch 2–3 d after being laid, and may become sexually mature adults in another 5–6 d. One female may lay up to 20 eggs per d and live for 2–4 wk laying several hundred eggs.

The objective of this paper was to evaluate the effects of gamma radiation on *Tetranychus urticae* Koch, *T. desertorum* Banks, and *Oligonychus ilicis* (McGregor) (Trombidiformes: Tetranychidae) in order to determine if irradiation may be used as an effective phytosanitary treatment.

Materials and Methods

The research was performed in the Laboratório de Radiobiologia e Ambiente, Centro de Energia Nuclear na Agricultura (CENA/USP), na Universidade de São Paulo, Piracicaba - SP. The research was performed in 2 stages. In the first stage, tests were made to determine minimum and maximum lethal doses of eggs oviposited by irradiated females, as well as to assess the subsequent effects in the F_1 generation on the egg and deutonymph stages of each mite species. In the second stage the effects of irradiating gravid females on the F_1 eggs and deutonymphs were studied. The final step was to estimate the reproductively sterilizing dose of gamma radiation for each of the 3 species, which entailed the evaluation of the viability of F_1 eggs oviposited by irradiated female mites.

MITE SOURCE, REARING, AND PREPARATION

The mite cultures used in this research were obtained from colonies maintained in the laboratory for more than 3 yr in the Departamento de Acarologia, Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ / USP), Piracicaba-SP and Instituto Biológico de Campinas (IB), Campinas-SP.

The colonies of *T. urticae*, *T. desertorum,* and *O. ilicis* were placed in plastic trays measuring 40 × 27 × 11 cm surrounded by hydrophobic cotton and entomological glue (Stickem®) to prevent possible escape of mites. Thereafter, individuals of each species were placed in separate 1.5 × 1.5 × 1.5 m wooden cages covered with organdy fabric and maintained in a greenhouse. The 2 *Tetranychus* species were provided with jack bean (*C. ensiformis*) plants, and *O. ilicis* was provided with coffee (*C. arabica*) leaves.

Plants materials infested with mites were used to obtain gravid adult females. Thereafter, each species of mite was identified under a stereomicroscope and a single female mite was transferred with a fine tip brush to a Petri plate (a total of 32 plates) containing either a jack bean leaf for each of the 2 *Tetranychus* species and a coffee leaf for *O. ilicis*.

IRRADIATION PROCESS

After oviposition by each of the gravid *Tetranychus* spp. females, the eggs were transferred to Petri dishes with jack bean leaves. The sex of the mites was determined after they had developed into deutonymphs. After the female deutonymphs had developed into adults the latter were mated in single pair matings. Subsequently these gravid females were irradiated.

For irradiation a Gammacell-220 cobalt-60 irradiator with a dose rate 0.486 kGy/h was used. Dosimetry was done with radiochromic film (Gammachrome with a dose range of 0.1–3 kGy). The readings were made with a spectrophotometer (Genesys 20). The certificate of dosimetry was made by the Institute for Energy and Nuclear Research–IPEN. The traceability of measurement of dose was maintained by comparison with the international service assurance dose offered by the International Atomic Energy Agency in Vienna, Austria.

Each Petri dish to be irradiated contained 1 gravid female. The Petri dishes—arranged in stacks of 5—were centralized inside the irradiator to maximize dose uniformity. Six dosimeters were positioned as follows: 1 on top of the stack, 1 at the bottom of the stack and 4 equally-spaced at lateral positions. The uncertainty in each Petri dish was ± 1.6%. The variation of measured doses was of ± 1.5% in the Gammacell-220 source. Each species of mite was irradiated at doses of 0 (control), 100, 200, 300, and 400 Gy, with a total of 32 gravid females per replicate and 3 replicates of each treatment, i.e., 96 females per treatment.

SAMPLE EVALUATION AND STATISTICAL ANALYSES

After irradiation, the Petri dishes were placed in a climatic chamber with a temperature of 25 ± 5 °C and RH of 70 ± 5%. Every 24 h for a period of 22 d after irradiation the numbers of eggs, nymphs and adults, and adult mortality were recorded.

A 3 × 5 factorial experimental design was used in which factor A (3 levels) pertained to the species and factor B (5 levels) pertained to the 4 radiation doses plus the control. For Factor B polynomial regressions of dose rates on biological effects were used. The analyses were conducted using EXCEL 2010® and the Statistical Analysis System® (SAS), version 9.3 (SAS Institute 2016).

Results

PERCENT VIABILITY F_1 EGGS AND DURATIONS OF LIFE STAGES

The interactions between the mite species and irradiation doses (Table 1) were highly significant ($P < 0.001$). Irradiation of females of all 3 species of mites with progressively greater doses induced progressively greater durations (d) in the period of maturation of the F_1 eggs (Table 1) and progressively decreased the percent viability of eggs (Figs. 1, 2 and 3) of the 3 species of mites.

Tetranychus urticae

Gamma irradiation of gravid females prolonged the time to egg hatch of F_1 eggs by 1.2 d at 100 Gy and by 8.9 d at 200 Gy compared to the control, but the time to hatching did not differ statistically between the 100 Gy treatment and the control (Table 1). F_1 eggs oviposited by females irradiated with the intermediate dose of 200 Gy remained in the egg stage for a significantly longer duration than those either in the 100 Gy treatment or the control. When females were irradiated with either 300 or 400 Gy, the lifespans of the neonates were very brief and they did not develop into deutonymphs (Table 1). Thus irradiation of gravid females caused both a reduction in the viability of the F_1 eggs (Fig. 1) and a prolongation in the duration of the F_1 egg stage.

Also in the case of deutonymphs that developed from the F_1 eggs of irradiated females, the duration of the deutonymph stage was prolonged. The increased durations (d) of the deutonymphal stage in the 100 and 200 Gy treatments differed significantly from the control. Only 37.5% of the F_1 deutonymphs in the 100 Gy treatment survived and less than 1% survived in the 200 Gy treatment, while in the 300 and 400 Gy treatments none of the F_1 protonymphs developed into deutonymphs (Table 2).

Tetranychus desertorum

Irradiation of gravid females of the red spider mite, *T. desertorum,* caused the viability of their F_1 eggs to be reduced to a lesser extent with each the 4 doses than that of the F_1 eggs of *T. urticae* at corresponding doses (Figs. 2 and 3). In the 100 and 200 Gy treatments, the durations of the F_1 egg stage were almost 2-fold and 3-fold longer, respectively, than the control, and these differences were significant. When females were irradiated with either 300 or 400 Gy, the lifespans of the F_1 neonates were very brief—i.e., 1.7 and 0.9 d, respectively—and they did not develop into deutonymphs (Table 1). Thus irradiation of gravid females caused both a reduction in the viability of the F_1 eggs (Fig. 2) and a prolongation of the F_1 egg stage.

Also in the case of *T. desertorum* deutonymphs that developed from the F_1 eggs of irradiated females, the duration of the deutonymph stage was prolonged. Only 22.4% of the F_1 deutonymphs in the 100 Gy treatment survived, while in the 200, 300 and 400 Gy treatments all F_1 deutonymphs died (Table 3).

Oligonychus ilicis

Gamma irradiation of gravid females prolonged the time until hatching of F_1 eggs, so that in the 100 Gy treatment the duration of the F_1 egg stage was greatly extended from 3.98 d in the control to 7.50 d, i.e., a difference of 3.52 d (Table 1). However, in treatments with larger doses the durations of the F_1 egg stage were progressively shortened with increasing doses—i.e., 2.0, 1.0 and 0.4 d with 200, 300 and 400 Gy, respectively—and they did not develop into deutonymphs (Table 1). Based on these effects, *O. ilicis* was more radiosensitive than the other 2 species as no viable deutonymphs were produced at > 200 Gy (Table 4).

When gravid *O. ilicis* females were irradiated with 100 Gy, the viability of the F_1 eggs was closer to the control (Fig. 3) than were the viabilities of F_1 eggs of *T. urticae* (Fig. 1) and *T. desertorum* (Fig. 2) females also irradiated with 100 Gy to their controls, but the differences were not significant.

Also in the case of *O. ilicis* deutonymphs that developed from the F_1 eggs of irradiated females, the duration of the deutonymph stage was prolonged. The increased durations (d) of the deutonymphal stage in the 100 and 200 Gy treatments differed significantly from the control. Only 16.7% of F_1 deutonymphs in the 100 Gy treatment survived, yet—unexpectedly—28.8% survived in the 200 Gy treatment, while in the 300 and 400 Gy treatments all F_1 deutonymphs died (Table 4).

Discussion

GAMMA RADIATION EFFECTS ON THE BIOLOGY OF THE MITES

From the results obtained in the current study, *T. desertorum, T. urticae* and *O. ilicis* showed generally similar responses to radiation, with

Table 1. Mean (± SE) duration of the egg stage in the F_1 generation when the P generation gravid females of each of 3 species of mites were irradiated with doses ranging between 100–400 Gy of gamma radiation.

Dose (Gy)	*Tetranychus urticae* Duration (d)	*Tetranychus desertorum* Duration (d)	*Oligonychus ilicis* Duration (d)
0 (Control)	3.9 ± 0.2	3.98 ± 0.3	3.98 ± 0.3
100	5.1 ± 0.3	7.5 ± 0.7	7.5 ± 0.5
200	12.8 ± 0.4	11.4 ± 0.8	2.0 ± 0.4[b]
300	1.8 ± 0.5[b]	1.7 ± 0.4[b]	1.0 ± 0.4[b]
400	0.9 ± 0.3[b]	0.9 ± 0.2[b]	0.4 ± 0.1[b]
F[a]	63.2	58.0	53.2
P Value	< 0.001	< 0.001	< 0.001
	R^2 = 0.519	R^2 = 0.627	R^2 = 0.594

[a]Regression models: for *T. urticae*, $y = -0.0002x^2 + 0.055x + 3.583$; for *T. desertorum*, $y = -0.0002x^2 + 0.060x + 4.217$; and for *O. ilicis*, $y = -3E{-}05x^2 - 0.003x + 5.18$, where the dependent variable, *y*, is the duration of the egg stage, the independent variable, *x*, is the radiation dose and 3E is the model error term of the linear regression.

[b]Neonates that hatched from the eggs did not survive beyond first instar.

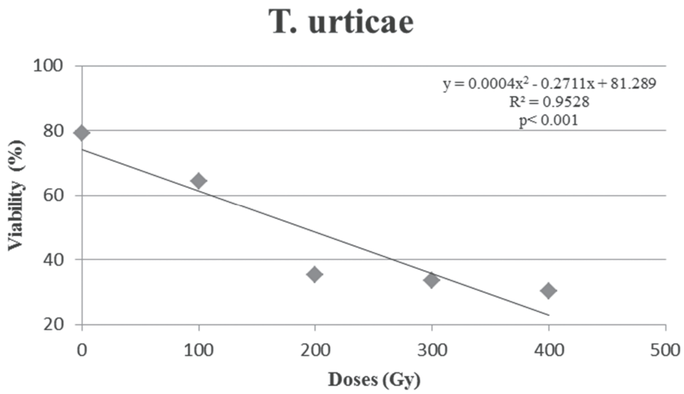

Fig. 1. Linear regression of the percent viability of *Tetranychus urticae* eggs with irradiation doses in the range of 100–400 Gy.

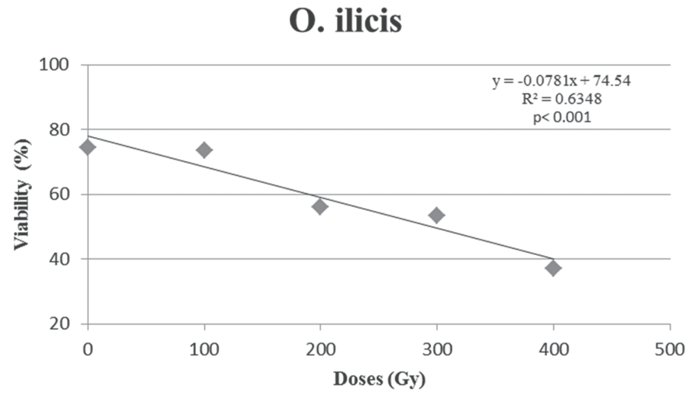

Fig. 3. Linear regression of the percent viability of *Oligonychus ilicis* eggs with irradiation doses in the range of 100–400 Gy.

a decrease in oviposition and an increase in duration of stadia of the life stages proportional to the radiation dose. Regarding viability of the 3 species, progressively increasing doses of radiation applied to gravid females were associated with progressive reductions in the viability of their F_1 eggs. Comparing egg viability of non-irradiated to irradiated mites of the 3 species, an average of 78.5% viability occurred in the non-irradiated control treatment, 69.3% in the 100 Gy treatment and 49.4% in the 200 Gy treatment. At doses greater than 200 Gy egg viability of irradiated female mites decreased to less than 50% (Figs. 1, 2 and 3).

Ignatowicz & Banasik-Solgala (1999) also observed in eggs oviposited by irradiated tetranychid females a decreased hatchability at a dose of 100 Gy and eggs not viable at doses between 200–400 Gy. Other studies on the viability of eggs oviposited by irradiated females, such as Lester & Petry (1995), showed that *T. urticae* females irradiated with 350 Gy produced only fully sterile eggs. In our case, we irradiated gravid *O. ilicis* females and found that none of the resulting F_1 neonates survived—hence complete sterility—at 200 Gy, while in the cases of irradiated *T. urticae* and *T. desertorum* females none of the resulting F_1 neonates survived—hence complete sterility—at 300 Gy. However, Majumder et al. (1996) observed that even though 5,000 Gy did not prevent egg production by irradiated *Oligonychus biharensis* (Hirst) females, 300 Gy was sufficient to cause all oviposited eggs to be sterile. In our study it was also observed that the age of the eggs was an important factor in relation to gamma radiation effects, while in older eggs there was an increase in the hatchability in comparison to eggs of younger age. In our experiment at a dose of 100 Gy in *T. urticae* the eclosion was 10.6% in 1–2 d-old eggs, 49.7% at 3–4 d-old and 99.7% in eggs older than 4 d; eggs more than 5 d-old hatched at doses up to 400 Gy. These observations are similar to Sulaiman et al. (2004), who noted in a mite from the same family (*Tetranychus piercei* McGregor) that in 1–2 d old eggs eclosion was zero at 150 Gy, while in 3–d old eggs eclosion was 41.3% and 99.0% for 4–d old eggs. Eggs older than 4 d hatched even at a dose of 600 Gy.

Goodwin & Wellham (1990) studied the effects of 300 Gy on *T. urticae* mites and noticed that after 24 h following irradiation the hatchability of eggs diminished in comparison with the control. According to Ignatowicz (1997), the sensitivity of insects and mites to irradiation varies according to the stage of development. This varies in different species, even within the same family, but in general juvenile stages have greater radiosensitivity because of intensive cell division in these stages.

Ducoff (1972) noted that ionizing radiation is responsible for breaking chemical bonds in DNA and other biomolecules such as carbohydrates, sugars, proteins and lipids that cause cell disorganization thus damaging their normal functioning in the bodies of irradiated insects.

According to Sakurai (2000) gamma irradiation also influences insect feeding, causing a breakdown in somatic cells such as the epithelial tissue present in the insect gut, preventing food absorption after irradiation resulting in starvation. Cork (1957), Riemann (1967) and Le Gall & Ardaillou (2009) reported that irradiation causes premature aging of insect cells and causes slower cellular regeneration, which could lead to enhanced oviposition in irradiated insects and mites as a response to reduced nutrition. Reproduction is an immediate response to maintain

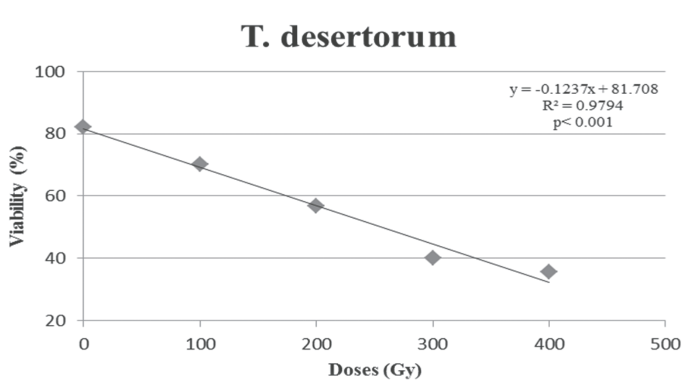

Fig. 2. Linear regression of the percent viability of *Tetranychus desertorum* eggs with irradiation doses in the range of 100–400 Gy.

Table 2. Effect of gamma irradiation of *Tetranychus urticae* gravid females on the mean (± SE) percent survival of their F_1 deutonymph progeny and the duration (d) of the deutonymph stadium. The females were irradiated with various doses in the range, 100–400 Gy.

Dose (Gy)	Survival (%)	Duration (d)
0 (Control)	76.6 ± 0.6	4.0 ± 0.3
100	37.5 ± 0.2	5.6 ± 0.2
200	0.92 ± 0.3	7.3 ± 0.1
300	0.0	—
400	0.0	—
F[a]	68.1	54.7
P Value	< 0.001	< 0.001
	R^2 = 0.953	R^2 = 0.999

[a]Regression models for irradiated *T. urticae* nymphs. The percent survival, $y = 0.0004x^2 - 0.2711x + 81.289$, where x is the radiation dose. Also the duration (d) of the deutonymphal stage, $y = -0.3786x + 76.24$, where x is the radiation dose.

Table 3. Effect of gamma irradiation of *Tetranychus desertorum* gravid females on the mean (± SE) percent survival of their F_1 deutonymph progeny and the duration (d) of the deutonymph stadium. The females were irradiated with various doses in the range, 100–400 Gy.

Dose (Gy)	Survival (%)	Duration (d)
0 (Control)	73.4 ± 0.6	3.6 ± 0.1
100	22.4 ± 0.4	4.6 ± 0.2
200	0.0	—
300	0.0	—
400	0.0	—
F[a]	63.5	54.1
P Value	< 0.001	< 0.001
	R^2 = 0.9794	R^2 = 0.975

[a]Regression models for irradiated *T. desertorum* nymphs. The percent survival, $y = -0.124x + 81.708$, where x is the radiation dose. Also the duration (d) of the deutonymphal stage, $y = 0.0083x + 5.84$, where x is the radiation dose.

Table 4. Effect of gamma irradiation of *Oligonychus ilicis* gravid females on the mean (± SE) percent survival of their F_1 deutonymph progeny and the duration (d) of the deutonymph stadium. The females were irradiated with various doses in the range, 100–400 Gy.

Dose (Gy)	Survival (%)	Duration (d)
100	16.7 ± 0.4	5.6 ± 0.2
200	28.8 ± 0.2	7.3 ± 0.1
300	0.0	—
400	0.0	—
Control	79.3 ± 0.2	4.0 ± 0.3
F[a]	64.2	54.7
P Value	< 0.001	< 0.001
	R^2 = 0.6348	R^2 = 0.987

[a]Regression models for irradiated *O. ilicis* nymphs. The percent, $y = -0.0781x + 74.54$, where x is the radiation dose. Also the duration (d) of the deutonymphal stage, $y = -0.5092x + 73.48$, where x is the radiation dose.

the species. This hypothesis is reinforced by some insect and mite species that are r-strategists, prioritizing reproduction as a way to generate offspring instead of storing the energy for its own survival (Force 1975).

The dose of 200 Gy was sterilizing for *O. ilicis* and 300 Gy for *T. urticae* and *T. desertorum*. The doses obtained in our study were lower than currently specified for phytosanitary treatment in Australia and New Zealand (Hallman et al. 2016).

Acknowledgments

This work was part of the FAO/IAEA Coordinated Research Project D62008 on Development of Generic Irradiation Doses for Quarantine Treatments. We thank the International Atomic Energy Agency for financial support through Research Contract 15201. We thank Andrew Parker (IAEA) who contributed to manuscript preparation and revision of this paper. In addition Dr. Waldemar Klassen of the University of Florida provided guidance in strengthening and vetting this report.

References Cited

Arthur V, Arthur PB, Gava MA, Franco SSH, Machi AR. 2012. Uso de técnicas nucleares em entomologia no Brasil, pp. 13-25 *In* Busoli AC, Grigolli JFJ, Souza LA, Kubata MM, Costa EN, Santos LAO, Netto JC, Viana MA [eds.], Tópicos Em Entomologia Agrícola V. Gráfica e Editora Multipress, Jaboticabal, Brasil.

Barros GSC, Spolador HFS, Bacchi MRP. 2009. Supply and demand shocks and the growth of Brazilian agriculture. Revista Brasileira de Economia 63: 35-50.

Bento JMS. 1999. Perdas por insetos na agricultura. Ação Ambiental 4: 19-21.

Cork JM. 1957. Gamma radiation and flour beetle. Radiation Research 7: 551-557.

Ducoff HS. 1972. Causes of cell death in irradiated adult insects. Biological Reviews 47: 211-240.

Force DC. 1975. Succession of R and K strategists in parasitoids, pp. 112-129 *In* Price PW [ed.], Evolutionary Strategies of Insects and Mites. Plenum, New York.

Flechtmann CHW. 1979. *Ácaros de importância agrícola.* São Paulo: Nobel. 182 pp.

Goodwin S, Wellham TM. 1990. Gamma irradiation for disinfestations of cut flowers infested by two spotted spider mite (Acarina: Tetranychidae). Journal of Economic Entomology 83: 1455-1458.

Hallman GJ. 2011. Phytosanitary applications of irradiation. Comprehensive Reviews in Food Science and Food Safety 10: 143-151.

Hallman GJ, Zhang DJ, Arthur V. 2016. Generic phytosanitary irradiation dose for phytophagous mites (Sarcoptiformes: Acaridae; Trombidiformes: Eriophyidae, Tarsonemidae, Tenuipalpidae, Tetranychidae). Florida Entomologist 99(special issue 2): 202-205.

Ignatowicz S. 1997. Post radiation mortality of the mold mite *Tyrophagus putrescentiae* (Acarida: Caridae) infesting agricultural commodities and used packagings. Annals of the Warsaw Agriculture University, Horticulture and Landscape Architecture 18: 3-11.

Ignatowicz S, Banasik-Solgala K. 1999. Gamma irradiation as a quarantine treatment for spider mites (Acarina: Tetranychidae) in horticultural products, pp. 29-47 *In* Irradiation as a quarantine treatment of arthropod pests. IAEA-TECDOC-1082, International Atomic Energy Agency, Vienna, Austria.

Khan HS, Islam MS. 2006. Efficacy of gamma radiation against housefly (*Musca domestica* L.) reproduction and survival II. Adult treatment. Journal of Bio-Sciences 14: 25-30.

Le Gall JY, Ardaillou R. 2009. The biology of aging. Bulletin de l'Academie Nationale de Medecine 193: 365-402.

Lester PJ, Petry RJ. 1995. Gamma irradiation for after harvest disinfestations of diapausing twospotted spider mite (Acari: Tetranychidae). Journal of Economic Entomology 88: 1361-1364.

Levine JM, D'Antonio CM. 2003. Forecasting biological invasions with increasing international trade. Conservation Biology 17: 322-326.

Majumder MZR, Bhuiya AD, Chowdhury N. 1996. Effects of radiation on mortality, fecundity and sterility of *Oligonychus biharensis* (Hirst) infesting common flower plants in Bangladesh. Bangladesh Journal of Zoology 24: 25-32.

Pimentel D, Zuniga R, Morrison D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52: 273-288.

Riemann JG, Flint HM. 1967. Irradiation effects on midgut and testes of the adult boll weevil, *Anthonomus grandis*, determined by histological and shielding studies. Annals of the Entomological Society of America 60: 298-308.

SEAB (Secretaria de Estado da Agricultura e do Abastecimento). 2016. DERAL - Departamento de Economia Rural. Fruticultura - Análise da Conjuntura Agropecuária. http://www.agricultura.pr.gov.br/arquivos/File/deral/Prognosticos/fruticultura_2012_13.pdf. Last accessed 29-II-2016.

Sakurai H. 2000. Eradication of weevil by sterile-insect release method (4): Physiology of reproduction in sterilized insects. Shokubutsu Boueki 54: 466- 468.

Sulaiman H, Osman MS, Othman Z, Ismail MR. 2004. Development of irradiation as a quarantine treatment of mites on cut foliage and ornamentals, pp. 133–141 *In* Irradiation as a Phytosanitary Treatment of Food and Agricultural Commodities. IAEA-TECDOC.1427. International Atomic Energy Agency, Vienna, Austria.

SAS Institute. 2016. The SAS System for windows, release 9.3. SAS Institute, Cary, N.C. http://support.sas.com/documentation/cdl/en/statug/63962/HTML/default/viewer.htm#titlepage.htm. Last accessed 29-II-2016.

UNEP (United Nations Environment Program). 2006. Handbook for the Montreal protocol on substances that deplete the ozone layer, 7th edition. http://ozone.unep.org/Publications/MP_Handbook/Section_1.1_The_Montreal_Protocol/index.shtml. Last accessed 29-II-2016.

Vilela E, Zucchi RA, Cantor F [eds.]. 2001. Histórico e impacto de pragas introduzidas no Brasil. Holos, Ribeirao Preto, Brazil.