

# Pupation, adult emergence, and F<sub>1</sub> egg hatch after irradiation of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) last instars

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## Abstract

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a quarantine pest that may be found as eggs or larvae on a number of fresh vegetable and fruit commodities. The objective of the research was to evaluate the effects of gamma radiation as a phytosanitary treatment applied to the 5th (last) instar larvae. Larvae were reared on an artificial diet. Each treatment of twenty 15–20 d-old larvae had 5 repetitions. The larvae were irradiated with doses of gamma radiation of 0 (non-irradiated control), 50, 100, 200 and 300 Gy in a Cobalt-60 source (Gammacell-220) at a dose rate of 508 Gy/h. Pupation, adult emergence and hatching of F<sub>1</sub> eggs were monitored. When the last instars were irradiated with 100 Gy the rates of pupation, adult emergence and F<sub>1</sub> egg hatch were 85, 39 and 50 percent, respectively, and the corresponding values in the non-irradiated control were 95, 85 and 90 percent, respectively. However, when the last instars were irradiated with 200 Gy, the pupation rate was reduced to 30 percent and adult emergence was reduced to 10 percent, and all of the adults that emerged were deformed, unable to fly, and died after about 2 h. Therefore, irradiation with 200 Gy is recommended as the appropriate dose for phytosanitary irradiation of *S. frugiperda* eggs and larvae. In addition this study supports the previously proposed generic dose of 250 Gy for eggs and larvae of lepidopteran pest species generally.

Key Words: prevention of adult emergence; criterion of efficacy; fall armyworm; gamma radiation; generic dose

## Resumen

El gusano cogollero, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), es una plaga de cuarentena que se puede encontrar en forma de huevos o larvas sobre un número de productos de frutas y verduras frescas. El objetivo de la investigación fue evaluar los efectos de la radiación gamma como tratamiento fitosanitario aplicado al quinto (último) estadio. Las larvas fueron criadas con una dieta artificial. Cada tratamiento de veinte larvas de edad de 15-20 días tenía 5 repeticiones. Las larvas fueron irradiadas con una dosis de radiación gamma de 0 (control no irradiado), 50, 100, 200 y 300 Gy en una fuente de cobalto-60 (Gammacell-220) a una tasa de dosis de 508 Gy/h. La pupación, emergencia de adultos y la eclosión de los huevos de F<sub>1</sub> fueron monitoreados. Cuando se irradiaron los últimos estadios con 100 Gy, la tasa de la pupación, emergencia de los adultos y eclosión de los huevos de la F<sub>1</sub> fue 85, 39 y 50 por ciento, respectivamente, y los valores correspondientes en el control no irradiado fueron 95, 85 y 90 por ciento, respectivamente. Sin embargo, cuando se irradiaron los últimos estadios con 200 Gy, la tasa de la pupación se redujo al 30 por ciento y la emergencia de adultos se redujo al 10 por ciento, y todos los adultos que emergieron estaban deformados, incapaces de volar y murieron después de aproximadamente 2 horas. Por lo tanto, se recomienda la irradiación con 200 Gy como la dosis apropiada para la irradiación fitosanitaria de huevos y larvas de *S. frugiperda*. Además, este estudio apoya la dosis genérica propuesta anteriormente de 250 Gy para los huevos y larvas de especies de plagas de lepidópteros en general.

Palabras Clave: prevención de la emergencia de los adultos; criterio de eficacia; gusano cogollero del maíz; radiación gamma; dosis genérica

## Resumo

Um dos insetos mais prejudiciais à cultura do milho é a *Spodoptera frugiperda*, conhecida vulgarmente como lagarta do cartucho, é originária das áreas tropicais e subtropicais do continente americano; sua importância econômica é devido a sua característica polífaga, atacando inúmeras gramíneas, tais como milho, sorgo, trigo, cevada, arroz e pastagens. Devido o que foi exposto o objetivo da pesquisa foi avaliar os efeitos da radiação gama sobre larvas de *S. frugiperda*. A criação massal de insetos durante o experimento foi em dieta artificial. Cada tratamento teve cinco repetições com 20 larvas com 15-20 dias de idade no total de 100 larvas por tratamento. As larvas foram irradiados com doses de radiação gama de: 0 (controle), 50, 100, 200 e 300 Gy, em fonte de Cobalto-60, tipo Gammacell-220, a uma taxa de dose de 508 Gy / hora. Pupação, emergência dos adultos e eclosão de ovos da F1 foram avaliados após a irradiação. Quando os últimos instares larvais foram irradiadas com 100 Gy as taxas de formação de pupa, emergência dos adultos e F1 eclosão dos ovos foram de 85, 39 e 50 por cento, respectivamente, e os valores correspondentes no controle não irradiado foram de 95, 85 e 90 por cento, respectivamente. No entanto, quando os últimos instares larvais foram irradiadas com 200 Gy, o estagio de pupa foi reduzida para 30 por cento e a emergência dos adultos foi reduzida para 10 por cento, todos os adultos que emergiram apresentaram deformações, incapazes de voar, e morreram depois de aproximadamente 2 h. Portanto, a irradiação com 200 Gy é recomendada como a dose apropriada para o tratamento fitossanitário de ovos e larvas de *S. frugiperda*. Além disso, este estudo apoia a dose genérica anteriormente proposta de 250 Gy para ovos e larvas de espécies de lepidópteros pragas em geral.

Palavras Chave: prevenção da emergência de adultos; critério da eficácia; lagarta do cartucho; radiação gama; dose genérica

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One of the most harmful insects attacking maize, *Zea mays* L. (Poales: Poaceae), in the tropics is *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), known commonly as the fall armyworm. This pest is native to the American tropics, but during the summer it migrates long distances into temperate regions where it may cause great economic loss to a variety of grass crops, peanut, tobacco, sugar beet, and various vegetables by defoliation and attacking reproductive structures. Cruz & Turpin (1982) and Carpenter (1983) observed that the losses caused by this insect in maize and can be as high as 35%.

The use of ionizing radiation is growing steadily as a phytosanitary treatment against insect pests on fresh commodities (Arthur 1997; Hallman 2012). Generic phytosanitary irradiation (PI) doses—where a single dose serves for a group of quarantine pests and/or commodities—are generally used commercially in the application of PI. Although some generic doses exist, doses are needed for other pest groups, such as Lepidoptera. Hallman et al. (2013) examined many studies comprising 34 species in 11 lepidopteran families, including those of significant quarantine importance and they tentatively concluded that 250 Gy would be the appropriate generic dose for the eggs and larvae of lepidopteran pest species.

The purpose of the present research was to generate data to support a generic dose for eggs and larvae of Lepidoptera.

## Materials and Methods

The experiments were conducted in the laboratory of Radiobiology and Environment of the Centro de Energia Nuclear na Agricultura - CE-NA/USP, Piracicaba, SP., Brazil. *Spodoptera frugiperda* were collected in the larval stage in maize in 1994 in the municipal district of Piracicaba and reared on artificial diet composed of bean, beer yeast, and wheat germ (Parra 1979).

Hallman et al. (2010) reviewed the literature and found that radio-tolerance of insect species—as measured by a given criterion—increases progressively as they proceed through their development stages, meaning that the most radiotolerant stage for phytosanitary irradiation would be the most developed stage that could be found in the exported commodity. Because *S. frugiperda* normally pupates off the host plant the most tolerant stage of concern is the 5th (last) instar. Therefore, this research was conducted with last instar larvae with the understanding that a dose that controls last instar will control the preceding instars and the eggs. Arthur et al. (2002) had investigated radio sterilization of pupal *S. frugiperda* as it applied to the sterile insect technique.

Hallman (2016) noted that there was negligible difference in radiosusceptibility among several lepidopteran species when each was reared on an artificial diet vs. when reared on 1 of its host plants. Therefore, to facilitate research *S. frugiperda* larvae were reared on an artificial diet, which was put in tubes (8.0 cm ht × 2.5 cm diam) up to ½ the ht and plugged with sterilized hydrophilic cotton. One recently hatched larva was placed in each tube and maintained at 25 ± 2 °C, 70 ± 5% RH and photoperiod of 14:10 h L:D. Last instar larvae 15–20 d-old were irradiated with: 0 (non-irradiated control), 50, 100, 200 and 300 Gy. The source was Cobalt-60 (Gammacell-220) with a dose rate of 508 Gy/h. Each treatment consisted of 5 repetitions with 20 larvae per repetition.

The dosimetry was done with radiochromic film (Gammachrome with a usable 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 dose measurement is maintained by comparison with measurements made by the International Dose Assurance Service, International Atomic Energy Agency in Vienna, Austria.

The last instar larvae were irradiated in petri dishes. The Petri dishes—arranged in stacks of 5—were centralized inside the irradiator in order not to disrupt the uniformity of the radiation. 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 the petri dish was ±1.6%. The dose uniformity ratio was 1.015.

The numbers of pupae and emerged adults were recorded until all larvae had emerged as adults or died. The data were analyzed with the important guideline in mind that prevention of normal adult emergence is the criterion of efficacy recommended for eggs and larvae of Lepidoptera (Hallman et al. 2013).

## Results

The means of the number of pupae and of adults that emerged after the irradiation of last instar larvae with various doses of gamma rays are shown in Table 1. The percent pupation and percent adult emergence in the 50 Gy treatment were reduced to only a small extent from the control. In the 100 Gy treatment the rates of pupation and adult emergence, and F<sub>1</sub> egg hatch were 85, 39 and 50 percent, respectively, and the corresponding values in the non-irradiated control were 95, 85 and 90 percent, respectively. The lowest dose of those tested that totally prevented normal adult emergence was 200 Gy. Indeed in the 200 Gy treatment, all of the adults that emerged were deformed, unable to fly, and died after about 2 h.

## DISCUSSION

Hallman et al. (2013) published a study in which eggs and larvae belonging 34 species of quarantine concern in 11 lepidopteran families were irradiated with the outcome that 250 Gy was tentatively supported as a generic PI dose for the Order Lepidoptera. Nevertheless 2 studies with the same 2 species suggested that doses > 250 Gy were necessary, but both of these studies were contradicted by 2 other studies that indicated that < 250 Gy was adequate. However, large-scale tests each with > 10,000 individuals have not been conducted except that such tests were done with several species belonging only to the Tortricidae. Thus it seems certain that 250 Gy is an adequate generic PI dose at least for the Tortricidae, and no data has been published to suggest that a larger dose will be needed for other lepidopteran pest species.

Based on the current study, we recommend 200 Gy as the appropriate dose for phytosanitary irradiation of *S. frugiperda* eggs and larvae. In addition this study supports the previously proposed generic dose of 250 Gy for eggs and larvae of lepidopteran pest species generally.

**Table 1.** Effect of gamma irradiation of the last (5th) larval instar larvae of *Spodoptera frugiperda* on mean (± SE) percentage pupation, adult emergence, and F<sub>1</sub> egg hatch. Each treatment consisted of 5 repetitions with 20 larvae per repetition. Thus, 100 fifth instar larvae were irradiated with each dose. Doses ranged up to 300 Gy.

Dose (Gy)	Pupation (%)	Adult Emergence (%)	Egg hatch (%)
0	95.0 ± 1.3	85 ± 1.4	90 ± 1.4
50	85.0 ± 1.3	81 ± 1.3	80 ± 1.4
100	70.0 ± 1.2	39 ± 1.3	50 ± 1.3
200	30.0 ± 1.1	*10 ± 1.2	—
300	0.0 ± 0.0	—	—

\*All adults were either deformed or could not fully emerge, and all had died after about 2 h.

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