Adult emergence and F_1 generation egg and larval production after γ-irradiation of late pupae of *Grapholita molesta* (Lepidoptera: Tortricidae)

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Abstract

The late pupal stage (pharate adult) of *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) close to adult emergence was irradiated with doses up to 350 Gy to determine a dose that could serve as a phytosanitary treatment against Lepidoptera that may pupate in shipped commodities. Adult emergence was not significantly reduced below that of the non-irradiated control until the dose reached 200 Gy. Not until the dose had been increased to 150 Gy was the number of eggs laid significantly less than the control, and oviposition was completely prevented at ≥ 300 Gy. Egg hatch was not significantly reduced compared with the non-irradiated control until the dose reached 150 Gy, and no eggs hatched at ≥ 250 Gy. This study supports a generic radiation dose for pupae of Lepidoptera of at least 250 Gy.

Key Words: egg hatch, F_1 progeny, generic phytosanitary radiation dose, oviposition, pharate adults

Resumo

A fase de pupa de *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) próximo a emergência de adultos foi irradiada com doses de até 350 Gy para se determinar a dose suficiente para o tratamento fitossanitário contra esse espécie de lepidóptero em produtos vegetais para a exportação. A emergência de adultos não foi significativamente reduzida em relação ao controle não irradiado quando as pupas foram irradiadas com doses de até 200 Gy. Foi observado também que na dose de 150 Gy o numero de ovos postos pelas fêmeas foi significativamente menor do que no controle não irradiado, mas a oviposição foi completamente inibida com uma dose de ≥ 300 Gy. Já a eclosão de larvas com doses de até 150 Gy não foi significativamente reduzida quando comparadas ao controle não irradiado, além disso, não houve eclosão de larvas na dose de ≥ 250 Gy. Este estudo concluiu que uma dose de radiação genérica para pupa deste Lepidóptero deve ser de pelo menos 250 Gy.

Palavras Chave: eclosão de ovos, progênie F_1, radiação gama, tratamento fitossanitário

In international agricultural markets the use of radiation as a method for the prevention of quarantine insects represents an important alternative postharvest disinfestation technique, which reduces the need for chemical fumigants and provides a solution to overcome quarantine barriers to trade. Although phytosanitary irradiation (PI) provides advantages in broad applicability and tolerance by fresh fruits and vegetables, a major disadvantage is that it is the only commercially applied phytosanitary treatment that does not result in significant acute mortality (Hallman 1998, 2004). Thus, the objective of PI is not acute mortality, but prevention of development or reproduction, as most commodities do not tolerate the doses required to induce acute mortality, and any quarantine treatment must be virtually 100% effective.

Insects increase in radiotolerance as they develop, and many developmental stages of the pests may be present in a shipped commodity. Much research has been conducted to determine the susceptibility of insect life stages (Bakri et al. 2005; Hallman et al. 2010).

The oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), is considered to be one of the most important pests of peach (*Prunus persica* [L.] Batsch; Rosales: Rosaceae) and other rosaceous fruits in several growing areas (Pree 1985; Rothschild & Vickers 1991; Riga 2006; Silva 2010). In Brazil this pest causes losses to peach growers of 3–5% (Farias 2003). Irradiation studies have already been done on the oriental fruit moth. Genchev (2002) determined that doses between 100 and 150 Gy caused partial sterility in males (37–43%) and the latter dose is considered as appropriate for induction of F_1 sterility for use in the sterile insect technique. Hallman (2004) reported no adult emergence from 5th instars irradiated with 200 Gy.

The objectives of the present study were to determine the rates of adult emergence and F_1 generation oviposition and hatch after the γ-irradiation of *G. molesta* pupae. This knowledge will help to determine PI generic doses required to prevent reproduction of lepidopteran quarantine pests that may pupate in the shipped commodity, such as pest species belonging to several families of Lepidoptera (Hallman et al. 2013).

Materials and Methods

ORIGIN OF INSECT COLONY AND REARING METHOD

A *G. molesta* culture was obtained from a colony established from larvae collected from infested peach fruits in Pelotas, Brazil. The larvae

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were reared on an artificial diet consisting of agar, corn flour, wheat germ, brewer's yeast, ascorbic acid, benzoic acid, formalin, and water at the following proportion: 4:28:7:7:1:0.1:0.1:900 mL, respectively.

GAMMA-IRRADIATION OF LATE PUPAE

Thirteen-d-old pupae (pharate adults) from a 3 yr-old laboratory colony reared on artificial diet were exposed to gamma radiation (Cobalt-60, type Gammacell-220; Nordion (Canada) Inc.) doses of 0 (control), 25, 50, 75, 100, 125, 150, 175, 200, 250, 300, and 350 Gy at a dose rate of 0.508 kGy/h. Pupae on d 13 of their stage were close to emerging as adults, and thus they represented the most radiotolerant age of the pupal stage (Hallman et al. 2010). Each treatment had 4 repetitions of 10 pupae each for a total of 40 pupae per treatment. After irradiation the insects were kept in a room with climatic conditions of 25 ± 5 °C and 70 ± 5% RH.

DOSIMETRY AND DOSE ASSURANCE

Dosimetry was done by the Institute for Energy and Nuclear Research with photoactive film (Gammachrome YR, Harwell, Oxfordshire, United Kingdom) read with a spectrophotometer (Genesys 20, Thermo Fisher Scientific, Waltham, Massachusetts). The traceability of measurements of doses—maintained by comparison with the service dose assurance offered by International Atomic Energy Agency, Vienna, Austria—was considered in evaluating the emergence of adults and the viability of eggs laid by females in all repetitions of the various treatments.

STATISTICAL ANALYSIS

The data was processed in the Statistical Analysis System® (SAS), version 9.0, (SAS 2002). For the adjustment of P values in all tests, a completely randomized design (CRD) was used. The data were subjected to the analysis of variance (*F* test), and the means were compared by the Tukey test at 5% probability.

Results

EFFECTS OF VARIOUS RADIATION DOSES ON ADULT EMERGENCE< FECUNDITY AND EGG HATCH

Adult emergence was not significantly reduced from the non-irradiated control until the dose had been increased to 200 Gy. At the highest dose used (350 Gy) adult emergence was 2.5%, i.e., 1 ± 0.1 (Table 1). Not until the dose had been increased to 150 Gy was the number of eggs laid significantly less than the control, and oviposition was completely prevented at ≥ 300 Gy. Egg hatch was not significantly reduced compared with the non-irradiated control until the dose had been increased to 150 Gy, and no eggs hatched at ≥ 250 Gy.

Discussion

Prevention of F_1 generation egg hatch is often used as the measurement of efficacy for PI where adults and pupae might be present in the shipped commodity (Hallman et al. 2010). This research supports a generic dose of 400 Gy against Lepidoptera in the pupal stage using this measurement of efficacy as proposed by Hallman et al. (2013).

Table 1. Mean numbers (± SE) of emerged adults, eggs laid and larvae produced after *Grapholitha molesta* pupae (pharate adults) had been irradiated with various doses of gamma radiation from cobalt-60. Each treatment had 4 repetitions of 10 pupae for a total of 40 pupae per treatment.

Doses/Gy	Number of emerged adults	Number of eggs	Number of larvae
0	9.0 ± 0.5a	141 ± 0.3a	120 ± 0.4a
25	8.2 ± 0.5a	135 ± 0.4a	113 ± 0.4a
50	8.3 ± 0.6a	147 ± 0.4a	126 ± 0.5a
75	8.6 ± 0.6a	125 ± 0.3ab	111 ± 0.4a
100	8.1 ± 0.4a	120 ± 0.3ab	115 ± 0.4a
125	8.0 ± 0.4a	113 ± 0.2ab	101 ± 0.4a
150	7.5 ± 0.2ab	84 ± 0.2bc	63 ± 0.1b
200	5.5 ± 0.1b	63 ± 0.1c	21 ± 0.1c
250	2.7 ± 0.1c	11 ± 0.1d	0.0 ± 0.0d
300	1.2 ± 0.1de	0.0 ± 0.0e	0.0 ± 0.0d
350	1.0 ± 0.1e	0.0 ± 0.0e	0.0 ± 0.0d
F	77.1	72.4	70.1
P values	< 0.001	< 0.01	< 0.001

*Means followed by the same letter are not significantly different based on Tukey's HSD test at $P < 0.05$.

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References Cited

Bakri A, Mehta K, Lance DR. 2005. Sterilizing insects with ionizing radiation, pp. 233-268 *In* Dyck VA, Hendrichs J, Robinson AS [eds.], Sterile Insect Technique, Principles and Practice in Area-Wide Integrated Pest Management. Springer, The Netherlands.

Farias RM, Nunes JLS, Martins CR, Guerra DS, Zanini C, Marodin GAB. 2003. Integrated conventional production of peach tree cv. 'Marlina' in central lowlands of Rio Grande do Sul. Brazilian Magazine of Fruit Culture. 25: 253-255. (In Portuguese)

Genchev N. 2002. Suppression of oriental fruit moth *Grapholita molesta* (Lepidoptera: Tortricidae) populations using the sterile insect technique, pp. 49-59 *In* IAEA [eds.], Proceedings of a Final Research Co-ordination Meeting, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria.

Hallman GJ. 1998. Ionizing radiation quarantine treatments. Anais da Sociedade Entomológica do Brasil. 27: 313-323.

Hallman GJ. 2004. Ionizing irradiation quarantine treatment against oriental fruit moth (Lepidoptera:Tortricidae) in ambient and hypoxic atmospheres. Journal of Economic Entomology 97: 824-827.

Hallman GJ, Levang-Brilz NM, Zettler JL, Winborne IC. 2010. Factors affecting ionizing radiation phytosanitary treatments, and implications for research and generic treatments. Journal of Economic Entomology 103: 1950-1963.

Hallman GJ, Parker AG, Blackburn CM. 2013. The case for a generic phytosanitary irradiation dose of 400 Gy for Lepidoptera that infest shipped commodities as pupae. Journal of Economic Entomology 106: 525-532.

Pree DJ. *Grapholita molesta.* 1985, pp. 307-311 *In* Singh P, Moore RFP [eds.], Handbook of Insect Rearing. Elsevier, Netherlands.

Riga, E, Lacey LA, Guerra N, Headrick HL. 2006. Control of the oriental fruit moth, *Grapholita molesta*, using entomopathogenic nematodes in laboratory and bioassays. Journal of Nematology 38: 168-171.

Rothschild GHL, Vickers RA. 1991. Biology, ecology and control of the oriental fruit moth, pp. 389-412 *In* Helle W, Van der Geest LPS, Evenhuis H.H. [eds.], Tortricidae Pests, their Biology, Natural Enemies, and Control. Elsevier, Netherlands.

SAS Institute. 2002. The SAS System for windows, release 9.0. SAS Institute, Cary NC.

Silva WD, Arthur V, Mastrangelo T. 2010. Response of oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), eggs to gamma radiation. Radiation Physics and Chemistry 79: 1063-1066.