Phytosanitary irradiation of *Diaphorina citri* (Hemiptera: Liviidae) on *Citrus* × *aurantium* (Sapindales: Rutaceae)

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Abstract

Eggs, nymphs, and adults of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae)—vector of citrus greening disease—were exposed to a series of gamma irradiation doses and examined for post irradiation development to the next stage and mortality. A dose of 150 Gy was found to prevent egg hatching and 185 Gy was sufficient to stop further development of 1st and 2nd instar nymphs. The 3rd and 4th instar nymphs were more tolerant, and an estimated dose of 204 Gy prevented their further development. A dose of 864 Gy was estimated to be required for complete adult mortality within 3 d after irradiation, although the fit of the adult mortality data to the model was very poor.

Key Words: Asian citrus psyllid; citrus greening disease; huanglongbing; *Citrus reticulata*; 'Kinnow'; prevent disease transmission; radiotolerance; acute lethal dose; sterilizing dose

Resumen

Los huevos, ninfas y adultos del psílido de los cítricos, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae)—vector de la enfermedad de enverdecimiento de los cítricos—fueron expuestos a una serie de dosis de irradiación gamma y luego examinados para su mortalidad después de la irradiación y su desarrollo al siguiente estadio. Se encontró que una dosis de 150 Gy previene la eclosión de los huevos y 185 Gy fue suficiente para detener un mayor desarrollo de la primera y segunda ninfas. Las ninfas del tercer y cuarto estadio fueron más tolerantes, y una dosis de 204 Gy se estimó para prevenir su desarrollo posterior. Una dosis de 864 Gy fue estimada para resultar en la mortalidad de todos los adultos dentro de 3 días, aunque el grado de ajuste de los datos de mortalidad de adultos en el modelo fue muy pobre.

Palabras Clave: psílido asiático de los cítricos; enverdecimiento de los cítricos; Huanglongbing; *Citrus reticulata*; 'Kinnow'; dosis letal aguda; prevenir la transmisión de enfermedades; radiotolerance; dosis de esterilización

Nature has blessed Pakistan with a wide range of tropical, sub-tropical, and temperate fruits. Today Pakistan stands among the 10 leading citrus growing countries in the world. Citrus fruits comprise ~40% of the total fruits produced in Pakistan and are cultivated over an area of 194.5 thousand ha with a mean annual production of 1,982,2 00 tonnes (Anonymous 2011). 'Kinnow', *Citrus reticulata* Blanco (Sapindales: Rutaceae), is grown primarily in the plains of Punjab province (Sharif et al. 2009). It has good demand within country and abroad, as foreign vendors generally prefer 'Kinnow' from Pakistan (Ahmad et al. 2005).

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the most destructive pest of citrus in Pakistan (Grafton-Cardwell 2013; Khan et al. 2012) and vectors the bacterium '*Candidatus* Liberibacter asiaticus Jagoueix, Bové & Garnier' ('CLa') causing citrus greening disease (Batool et al. 2007; Hall 2008; Hall et al. 2013; McClean & Schwartz 1970). Both adults and nymphs feed on young foliage. Dense psyllid populations on young flush can kill the growing tip, while moderate populations can distort shoots and leaves leading to the death of plant within 4-5 yr. *Diaphorina citri* is a quarantine pest in many parts of the word. Pakistan consequently loses exports of citrus particularly 'Kinnow' to other psyllid-free countries because the immature stages can be transmitted with fresh fruits to the importing countries.

Regulations for export of fresh commodities require disinfestations of quarantine pests before export from Pakistan. Irradiation has been adopted as a safe measure for disinfestation of quarantine pests and has gained significance in the export of fresh commodities (Hallman

Table 1. Probit analysis of data on inhibition of development (mortality) of *Diaphorina citri* after various life stages were γ-irradiated with doses in the range 0–250 Gy.

Developmental stage irradiated	Percentage inhibition at dose (Gy)					Slope	$ED_{99.99}$, Gy (95% CI)*	Heterogeneity	Chi-square	Degrees of freedom
	0	100	150	200	250					
eggs	9.3	96.1	98.5	100	100	6.59 ± 1.59	150 (132-199)	0.11	0.22	2
1st & 2nd instars	11.6	93.7	99.5	100	100	4.92 ± 0.94	185 (162–240)	1.31	0.66	2
3rd & 4th instars	31.9	95.5	98.3	99.4	100	6.56 ± 0.77	204 (184-238)	0.19	0.38	2

*ED means effective dose either for inhibition of development of nymphs, and for mortality of those that transformed into adults.
CI means confidence interval.

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Table 2. Probit analysis of mortality data of *Diaphorina citri* adults recorded 3 d after they had been γ-irradiated with doses in the range of 0–1,000 Gy.

Sex	Mortality (%) at dose (Gy)						Slope	$ED_{99.99}$, Gy (95% CI)*	Heterogeneity	Chi-square	Degrees of freedom
	0	300	400	600	800	1,000					
Male	30	72.0	80.0	95.0	100	100	5.73 ± 0.47	854 (671-1341)	2.29	6.87	3
Female	30	84	90	96.0	100	100	5.73 ± 0.45	864 (683-1367)	2.28	6.86	3

*ED means effecive lethal dose. CI means confidence interval.

2011, 2012). I am unaware of any studies on phytosanitary irradiation against psyllids, except for Hallman & Chapa (2016) also in this special issue. Pakistan Radiation Services (PARAS) Food Pvt. Lahore has adopted a radiation dose of 400 Gy for disinfestations of fruit and vegetables, but lower doses are sought to prevent possible damage and facilitate treatment.

The measure of efficacy for phytosanitary irradiation of adult pests is prevention of reproduction rather than acute mortality. In the case of insects that transmit diseases, such as *D. citri*, it is worthwhile to examine both mortality as well as growth inhibition because the growth-inhibited insects may still be able to transmit viable pathogens. The effect of various levels of radiation used in phytosanitation on disease transmission by vectors has not been studied.

The objectives of this study was to determine radiation doses that kill *D. citri* adults within several days and that inhibit the development of nymphs to the adult stage.

Materials and Methods

To determine the effect of radiation on development, 2 yr-old *Citrus x aurantium* L. plants in black polyethylene bags with 12–15 young leaves were placed in *D. citri*-infested *C. aurantium* mature trees to obtain 3 stage groupings of Asian citrus psyllids: eggs, 1st and 2nd instars, and 3rd and 4th instars. The infested plants were irradiated with doses in the range 100–250 Gy using a Co-60 gamma cell irradiator (Issledovatel GIK-7-2, Russia) with a dose rate of 0.37 Gy/min at the Nuclear Institute for Food and Agriculture (NIFA), Peshawar. The absorbed dose was measured using Fricke dosimeters attached to surfaces of infested plants at top, middle, and bottom positions. Each dose was repeated on 4 plants. After irradiation, individual plants were placed in screened plexiglass cages and maintained in the phytosanitary laboratory at 28 ± 2 °C and 60 ± 5% RH for further observations. Mortality and development inhibition were recorded at 48 h intervals under magnification. Nymphs were considered dead if they did not move after probing with a camel hair brush or grow to the next stage. In the case of eggs, hatching was recorded daily under magnification.

For assessment of adult mortality, 25 males and 25 females were placed in separate transparent plastic straws (13 cm long) that were punctured at the sides for air circulation. Both ends of the straws were plugged with dry cotton fibers. Straws containing the adults were irradiated at 300, 400, 600, 800, and 1,000 Gy. Each dose was replicated 4 times. Two year old nursery plants of *C. aurantium* were irradiated with 300 Gy to prevent any previous infestation of *D. citri*. After irradiation, individual plants were placed in screened plexiglass cages and maintained in the phytosanitary laboratory at 28 ± 2 °C and 60 ± 5 % RH. The adults irradiated in plastic straws were allowed to move to the plants. Mortality of irradiated adults was recorded every 2-3 d until all adults died. Mortality was corrected using the method of Abbott (1925). Percentage mortality and adult inhibition data were subjected to probit analysis using the log of the dose (PoloPlus, LeOra Software, Petaluma, California) to estimate the dose response of exposed eggs, nymphs, and adults.

Results

Levels of developmental inhibition of *D. citri* eggs and nymphs on plants of *C. aurantium* irradiated with various does of gamma rays are shown in Table 1. For nymphs exposed to 100 Gy, developmental inhibition ranged between 93.7 and 95.5%. Inhibition increased to between 98.3 and 99.5% when nymphs were irradiated with 150 Gy. Nymphs took longer or failed to develop to the next stage when exposed to 200 Gy. Predicted effective doses ($ED_{99.99}$) for inhibition of development were as follows: eggs, 150 Gy; 1st and 2nd instars, 185 Gy; and 3rd and 4th instars, 204 Gy.

Mortality of adults when exposed to 300–1,000 Gy showed a predicted $ED_{99.99}$ of 864 Gy for females and 854 Gy for males at 3 d after irradiation (Table 2), but the fit to the model was poor in both cases as indicated by the wide confidence 95% limits. Regardless, it is probably not feasible to propose acute mortality as an endpoint for disinfestation of *D. citri* using ionizing radiation because fresh commodities generally will not tolerate the dose required for acute mortality, especially taking into consideration the maximum dose—864 Gy in this study and greater than 1.5 kGy but less than 2 kGy in the study of Hallman & Chapa (2016)—that would be absorbed by some of the commodity when irradiated on a commercial scale.

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References Cited

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18: 265-267.

Ahmad B, Ghafoor A, Badar H. 2005. Forecasting and growth trends of production and export of kinnow from Pakistan. Journal of Agriculture and Social Sciences 1: 20-24.

Anonymous 2011. Fruit, Vegetable and Condiment, Ministry of Food, Agriculture and Livestock(MINFAL), Islamabad.

Batool A, IftikharY, Mughal SM, Khan MM, Jaskani MJ, Abbas M, Khan A. 2007. Citrus greening disease–a major cause of citrus decline in the world–a review. Horticulture Science (Prague) 34: 159-166.

Grafton-Cardwell EE, Stelinski LL, Stansly PA. 2013. Biology and management of Asian citrus psyllid, vector of the huanglongbing pathogen. Annual Review of Entomology 58: 413-432.

Hall DG. 2008. Biology, history and world status of *Diaphorina citri*, pp. 1-11 *In* 1 Taller Internacional sobre Huanglongbing de los Cítricos (*Candidatus* Liberibacter *spp*.) y el psílido asiático de los cítricos (*Diaphorina citri*). Hermosilla, Sonora, Mexico.

Hall DG, Richardson ML, Ammar ED, Halbert SE. 2013. Asian citrus psyllid, *Diaphorina citri*, vector of citrus huanglongbing disease. Entomologia Experimentalis et Applicata 146: 207-223.

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

Hallman GJ. 2012. Generic phytosanitary irradiation treatments. Radiation Physics & Chemistry 81: 861-866.

Hallman GJ, Chapa DL. 2016. Phytosanitary Irradiation of *Diaphorina citri* (Hemiptera: Liviidae). Florida Entomologist 99(special issue 2): 150-152.

Khan I, Zahid M, Khan GZ. 2012. Toxicity of botanic and synthetic pesticide residues to citrus psyllid *Diaphorina citri* Kuwayama and *Chrysoperla carnea* (Stephens). Pakistan Journal of Zoology 44: 197-201.

McClean APD, Schwartz RE. 1970. Greening of blotchy-mottle disease in citrus. Phytophylactica 2: 177-194.

Sharif M, Akmal N, Taj S. 2009. Financial viability for investing in citrus cultivation in Punjab, Pakistan. Journal of Agricultural Research 47: 79-89.