Phytosanitary Irradiation of *Diaphorina citri* (Hemiptera: Liviidae)

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Abstract

Asian citrus psyllid (*Diaphorina citri* Kuwayama; Hemiptera: Liviidae) adults reared on orange jasmine (*Murraya paniculata* (L.) Jack; Sapindales: Rutaceae) were evaluated for acute mortality and prevention of reproduction when adult insects were irradiated for phytosanitary purposes. At 2 kGy all adults (*n* = 60) were dead by 72 h post irradiation. A dose of 150 Gy did not prevent oviposition (*n* = 1,200 adults), but no nymphs were observed to emerge from the eggs. At the next lowest dose (100 Gy) some nymphs developed. These results indicate that a minimum PI treatment dose significantly greater than 1.5 kGy but less than 2 kGy is required for total mortality within 72 h and the minimum PI treatment dose to prevent egg hatch is greater than 100 Gy, but less than 150 Gy.

Key Words: citrus greening disease, huanglongbing, acute lethal dose, prevent disease transmission, radiotolerance, sterilizing dose

Resumen

Adultos del sílido asiático de los cítricos, *Diaphorina citri* Kuwayama Hemiptera: Liviidae), criados en jazmín naranja, *Murraya paniculata* (L.) Jack (Sapindales: Rutaceae), se evaluaron para mortalidad aguda y la prevención de la reproducción después de irradiación con fines fitosanitarios. A los 2 kGy, todos los adultos (*n* = 60) se murieron en 72 h. La dosis de 150 Gy no impidió la oviposición (*n* = 1.200 adultos), pero no se observó emergencia de ninfas de los huevos. En la siguiente dosis más baja (100 Gy) se desarrollaron algunas ninfas.

Palabras Clave: enverdecimiento de los cítricos, Huanglongbing, dosis letal aguda, prevenir la transmisión de enfermedades, radiotolerance, dosis de esterilización

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is a piercing-sucking insect that infests and damages citrus trees by removing plant sap, distorting leaves, and causing defoliation. The insect also serves as a disease vector of the gram negative bacterium '*Candidatus* Liberibacter asiaticus Jagoueix, Bové & Garnier' ('CLa'). This phloem-limited bacterium, which has resisted attempts at in vitro culture, is hypothesized as the causal agent of huanglongbing (HLB) also known as citrus greening disease (Halbert & Manjunath 2004). HLB is considered the most serious disease of citrus (Bové 2006) causing reduced fruit yields, lowered fruit quality, and ultimately tree death. Once in the body of *D. citri* the bacterium rapidly multiplies and is injected into citrus plants along with saliva while the insect is feeding. The bacterium can degenerate phloem channels in susceptible plant hosts, severely reducing vigor. There is no cure known for infected trees, which decline and die within a few years (Majumdar 2009).

Phytosanitary treatments are used to ensure commodities are free of viable quarantine pests so enabling trade in fresh produce; treated commodities may be shipped out of quarantined areas (Heather & Hallman 2008). Phytosanitary irradiation (PI) uses ionizing radiation (electron beams, X-rays or cobalt-60 gamma rays) to prevent development and/or reproduction of pests and is increasing in commercial use (Hallman 2011). An international cooperative research project under the auspices of the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture is developing generic PI treatments for inclusion in the International Standard for Phytosanitary Treatment (ISPM) #28, Phytosanitary Treatments for Regulated Pests (IAEA 2009; FAO 2009). Generic treatments cover groups of quarantine pests and should include data from a broad representation of the group (Hallman 2012). Reports on radiotolerance of representatives of the family Liviidae (Hemiptera) are lacking in the literature.

We are not aware of any current PI treatments that define acute mortality as a measure of efficacy because radiation doses required are generally too high (1 or more kilograys) to be tolerated by fresh horticultural commodities. Furthermore, acute mortality is not required to prevent the establishment of invasive species; that can be accomplished via prevention of development and/or reproduction with much lower doses than those required for acute mortality. Low doses of ionizing radiation (several hundred grays) are able to arrest the development of insects, inhibit reproduction or induce reproductive sterility without affecting commodity quality. Therefore PI treatments normally establish an efficacious treatment dose to prevent development and/or reproduction. However, to prevent disease transmission it may be necessary to induce acute mortality of the vectors because live but sexually sterile (irradiated) insects may still be able to act as infective agents in the short-term, even though they are not able to colonize and reproduce. The objectives of this research were to estimate PI treatment doses required to (i) provide acute mortality and (ii) prevent reproduction of *D. citri*. Irradiation will also weaken the CLa bacterium but this falls outside the scope of this study and has not been considered further.

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Materials and Methods

Diaphorina citri from citrus orchards near Weslaco, Texas were reared on orange jasmine (*Murraya paniculata* (L.) Jack: Sapindales: Rutaceae) in 0.6 m^3 nylon (60 mesh/cm) cages (BD2120F Insect Tent, MegaView Science Co. Ltd., Taichung, Taiwan) in a whitewash-coated greenhouse. Plants were kept at a height of ~ 0.45 m and grown in plastic pots (11.4 L) with a 10:1:1 mixture of potting soil (GFM Original All Purpose, Lambert, Rivière-Ouelle Québec, Canada), vermiculite (Medium Premium Grade, SUN GRO™ Horticulture, Pine Bluff, Arkansas,) and soil conditioner (Landscapers pride, New Waverly, Texas). Plants were pruned to induce new growth. *Diaphorina citri* nymphs feed exclusively on new growth and adults are known to oviposit on it only. Average daily temperature in the greenhouse was 29–33 °C. The plants were watered biweekly and fertilized monthly (Osmocote Smart Release Plant Food, Marysville, Ohio). The cages were periodically cleaned and sanitized with a dilute bleach solution (0.05% sodium hypochlorite) followed by a water rinse, or by wiping with 70% ethanol.

A ^{137}Cs (Husman model 521A, Isomedix, Inc., Whippany, New Jersey) radiation source (dose rate of ~ 33 Gy/min) at the USDA-APHIS Mexican Fruit Fly Rearing Facility in Mission, Texas was used to irradiate *D. citri*. Reference standard dosimetry was done in 1996 with the Fricke system (ASTM 2013). Routine dosimetry was done with radiochromic film (1-cm^2, Gafchromic MD-55, ISP Technologies, Inc., Wayne, New Jersey) in paper envelopes placed in the tubes with *D. citri* and read with a spectrophotometer (Genesys Model 4001, Spectronic Instruments, Rochester, New York) at 600 nm 24 h after exposure. The optical density of the exposed film becomes stable after 24 h (IAEA 2004).

Adults of *D. citri* were irradiated at doses designed either to provide acute mortality or to prevent reproduction. Tolerance to irradiation increases with increasing insect development; hence, the most developed stage can be considered the most radiotolerant (Hallman et al. 2010). All life-stages of *D. citri* could be found in shipped commodities and therefore the adult is the most radiotolerant life-stage that can occur on a shipped commodity, and the minimum absorbed dose that is effective against the adult will be effective against the other life-stages as well.

Twenty adult (10 female and 10 male) *D. citri* feeding on a branch of orange jasmine with the cut end inserted into a vial of water were placed in a transparent plastic tube (22 cm long × 4.2 cm inside diam) with both ends covered with cloth mesh. The tubes were irradiated with target doses of 1.5 and 2 kGy with a non-irradiated control (3 replicates). After irradiation, the adults were transferred to the enclosed orange jasmine branches (branches changed as needed) on whole plants in the greenhouse and mortality and oviposition were recorded at 2, 3, and 6 d after irradiation.

Actively reproducing adult *D. citri* (n = 100; ~50: 50 sex ratio) were irradiated with 50, 75, 100 and 150 Gy. Cohorts of 20 adults were held as non-irradiated controls. *Diaphorina citri* females will not oviposit unless fresh blush of host plants is available. Therefore, in order to evaluate the effect of irradiation on oviposition it is imperative that acceptable host material for oviposition is available for the bioassay. Irradiated insects were placed with plants of *M. paniculata* in the cages and oviposition and egg hatch were observed until all of the irradiated insects died by ~ 7 wk. Eggs were not counted but relative amounts were estimated and the plants were examined for the presence of nymphs on new growth. There were 6 replicates for the control, 2 each for 50 and 75 Gy, 6 for 100 Gy, and 12 for 150 Gy.

Results

Table 1 presents the dose range measured at each target dose. The lower range was close to the target dose while the upper range was ~10% higher than the target dose. To reduce confusion each treatment will continue to be referenced as the target dose with the understanding that absorbed doses varied and on average were slightly greater than the target dose.

Table 1. Absorbed dose range measured at each target dose.

Target dose (kGy)	Absorbed dose range (kGy)
0.050	0.048–0.059
0.075	0.073–0.087
0.100	0.098–0.111
0.150	0.146–0.171
1.5	1.49–1.63
2.00	1.97–2.25

Survival of *D. citri* adults 48 h after irradiation was 98.3 ± 1.7, 75.0±5.8, and 11.7 ± 1.7% for 0 (control), 1.5, and 2 kGy, respectively. None of the 60 adults irradiated with 2 kGy survived 72 h while 18.3 ± 4.4% of those irradiated with 1.5 kGy were alive 6 d after irradiation. At 6 d survival in the control was 95.0 ± 2.9%. None of the psyllids irradiated with either 1.5 or 2 kGy laid eggs.

For the studies on reproduction, abundant eggs were laid at all doses between 50–150 Gy. Nymphal development was observed at all of these doses except 150 Gy, and was fairly abundant at 50 and 75 Gy. In the 100 Gy treatment, the number of nymphs was noticeably less than with lower doses, but some survivors molted to subsequent instars. The mortality rate of adults was observed to be similar for all treatments, and all adults died by ~8 wk.

Acute mortality of all *D. citri* adults was observed for samples irradiated with 2 kGy where total mortality had occurred by 72 h post irradiation. Therefore, using total mortality as the measure of efficacy, this research suggests a minimum PI treatment dose somewhat higher than 1.5 kGy but less than 2 kGy. Thus it may not be commercially feasible to treat host material to prevent transmission of the causal agent of HLB. Reproduction of *D. citri* was observed to be prevented at a target dose of 150 Gy (range 146–171 Gy; Table 1). This research suggests that using "prevention of egg hatch" as the measure of efficacy, a minimum PI dose of significantly greater than 100 Gy, but less than 150 Gy would be necessary to prevent reproduction of *D. citri*.

Discussion

Khan (2016) found that 800 Gy killed adults within 3 d after treatment. However we found that 18.3 ± 4.4% of those irradiated with 1.5 kGy were alive 6 d after irradiation but that none survived beyond 3 d when irradiated with 2 kGy.

Radiotolerance of this psyllid as measured in the prevention of reproduction may be somewhat greater than of the related family Aphididae for which at least 2 PI studies have been reported in the literature; the asparagus aphid (*Brachycorynella asparagi* (Mordvilko); Hemiptera: Aphididae) was prevented from reproducing at 100 Gy (n = 30; Halfhill 1988), and Heather (1992) cited a personal communication where 100 Gy also was effective against the green peach aphid [*Myzus persicae* (Sulzer); Hemiptera: Aphididae].

Acknowledgments

Maura Rodriguez, Roberto Rivas, Faith Banda, Elvia Landero, Yadira Blanco and Marc Villalobos formerly of USDA-ARS, Weslaco, Texas are thanked for technical help. USDA is an equal opportunity provider and employer. Mention of trade names or commercial products in this pub-

lication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. This work was part of the FAO/IAEA Coordinated Research Project D62008 on Development of Generic Irradiation Doses for Quarantine Treatments.

References Cited

ASTM (American Society for Testing and Materials International). 2013. Standard practice for using the Fricke dosimetry system. E1026-13. ASTM International, West Conshohocken, PA.

Bové JM. 2006. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. Journal of Plant Pathology 88: 7-37.

FAO (Food and Agriculture Organization). 2009. Phytosanitary treatments for regulated pests. ISPM #28, Food and Agriculture Organization, Rome.

Halbert SE, Manjunath KL. 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: A literature review and assessment of risk in Florida. Florida Entomologist 87: 330-353.

Halfhill JE. 1988. Irradiation disinfestation of asparagus spears contaminated with *Brachycorynella asparagi* (Mordvilko) (Homoptera: Aphididae). Journal of Economic Entomology 81: 873-876.

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

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

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.

Heather NW. 1992. Review of irradiation as a quarantine treatment for insects other than fruit flies, pp. 203-218 *In* Use of Irradiation as a Quarantine Treatment of Food and Agricultural Commodities. International Atomic Energy Agency, Vienna.

Heather NW, Hallman GJ. 2008. Pest management and phytosanitary trade barriers. CABI, Wallingford, Cambridge. 257 pp.

IAEA (International Atomic Energy Agency). 2004. Dosimetry system for SIT: Manual for Gafchromic film. http://www-naweb.iaea.org/nafa/ipc/public/Dosimetry_SOP_v11.pdf.

IAEA (International Atomic Energy Agency). 2009. The development of generic irradiation doses for quarantine treatments. Report of the first research coordination meeting. http://www-naweb.iaea.org/nafa/fep/crp/generic-irradiation-1st-meeting.pdf.

Khan I. 2016. Phytosanitary irradiation of *Diaphorina citri* (Hemiptera: Liviidae) on *Citrus* × *aurantium* (Sapindales: Rutaceae). Florida Entomologist 99 (special issue #2): 153-155.

Majumdar A, Nesbitt M, Fadamiro H. 2009. Asian citrus psyllid. Extension Specialist, Agronomic Crops and Commercial Horticulture, Auburn University. http://www.aces.edu/pubs/docs/A/ANR-1341/ANR-1341.pdf