Gamma radiation phytosanitary treatment for *Hemiberlesia lataniae* (Hemiptera: Diaspididae)

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Abstract

The latania scale, *Hemiberlesia lataniae* (Signoret) (Hemiptera: Diaspididae) is a cosmopolitan and highly polyphagous species present on various hosts of economic importance. As with other scales, *H. lataniae* is quarantined by importing countries and a single viable insect can cause regulatory action. Ionizing irradiation is a promising phytosanitary treatment that is increasingly used worldwide. This study was conducted to determine a dose of radiation that would serve as a phytosanitary treatment for commodities at risk of carrying *H. lataniae*. Cobalt-60 gamma ray target doses of 50, 100, 150, and 200 Gy were used to irradiate immature and adult stages of *H. lataniae* infesting fruits of butternut squash, *Cucurbita moschata* Duschesne ex Lam (Cucurbitales: Curcubitaceae). Tolerance to irradiation in *H. lataniae* as measured by mortality was found to increase progressively as follows: first instars < second instars < adults. Adult females irradiated with a target dose of 200 Gy failed to produce offspring beyond the egg stage. Large scale tests involving a total of 31,877 female adults showed that a dose of 209 Gy (the highest dose measured when the target dose was 200 Gy) can serve as a phytosanitary treatment against *H. lataniae*. These results also support a generic dose of 250 Gy for all scale insects.

Key Words: irradiation; latania scale; quarantine treatment; phytosanitation

Resumen

La escama latania, *Hemiberlesia lataniae* (Signoret) (Hemiptera: Diaspididae) es una especie cosmopolita y altamente polífaga presente en varios huéspedes de importancia económica. Así como otras escamas, *H. lataniae* es una especie cuarentenaria por parte de los países importadores y un solo insecto vivo ocasiona restricciones cuarentenarias. La radiación ionizante es un tratamiento fitosanitario prometedor, y que actualmente es utilizado en varios países. Por ello, el presente estudio se realizó para determinar el estado *más tolerante para la* irradiación e impedir la reproducción de *H. lataniae* para garantizar un tratamiento cuarentenario seguro. Dosis de 50, 100, 150 y 200 Gy, mediante el empleo de una fuente de Cobalto 60, se utilizaron para irradiar estados inmaduros y adultos de *H. lataniae* criados sobre zapallo coreanito, *Cucurbita moschata* Duschesne ex Lam (Cucurbitales: Curcubitaceae), con la finalidad de determinar el estado *más tolerante y el* rango de dosis óptimas para garantizar un tratamiento cuarentenario seguro. El patrón de la tolerancia a la irradiación en *H. lataniae* fue primer estadio < segundo estadio < adulto. Ninguna hembra adulta viva produjo descendencia a 200 Gy. La prueba a gran escala sobre 31.877 hembras adultas irradiadas demostr*ó* que la dosis de 200 Gy es suficiente para garantizar un tratamiento fitosanitario seguro sobre la escama lataniae. Estos resultados sostienen una dosis genérica de 250 Gy para escamas.

Palabras Clave: tratamientos fitosanitarios; irradiación; estado más tolerante; escama lataniae

The latania scale, *Hemiberlesia lataniae* (Signoret) (Hemiptera: Diaspididae) is a cosmopolitan and highly polyphagous species present in various hosts of economic importance, including native and introduced ornamental and forest fruit, such as pumpkin, avocado, bamboo, banana, carambola, carob, grape, guava, mango, orange, lemon, tangerine, and sugar cane (Miller & Davidson 2005).

Argentina is a major fruit producing country exporting pome fruits, stone fruits, lemon, tangerine, and avocado among others. Europe, Brazil, United States, Russia and Chile are the main destinations for Argentine fresh fruits (Fundación ExportAr 2014). *Hemiberlesia lataniae* is quarantined by importing countries, and a single live insect can cause regulatory action. Most phytosanitary treatments in use today involve subjecting traded commodities to heat, cold, or chemical fumigants to acutely kill essentially 100% of regulated pests. Treatments involving pesticide sprays or dips, high pressure cleaning, and waxing have been used in specific cases to disinfest commodities of quarantine pests (Hallman 2007).

With increased global trade pressures and changes in chemical regulation by importing countries it is imperative that other practical treatment options be explored. Phytosanitary irradiation (PI) is a promising treatment that is increasing used worldwide (Hallman 2011). Moreover, irradiation is the ideal technology for developing generic phytosanitary treatments because it is effective at doses that do not significantly lower the quality of most fresh commodities. The generic

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phytosanitary treatment concept is that one treatment dose is used for a broad group of quarantine pests and/or commodities although not all were tested for efficacy, and a generic dose of 250 Gy is suggested for scales (Hallman 2012).

We could find no records on irradiation of *H. lataniae*. Previous irradiation studies of species in the Diaspididae have shown that in 3 other species doses of at least 150 Gy were needed for phytosanitary security (Angerilli & Fitzgibbon 1990; Follett 2006a, b).

Unlike other disinfestation techniques, PI does not cause considerable acute mortality, but renders pests incapable of completing development and/or reproducing, and therefore live, but nonviable or reproductively sterile, insects may occur with the exported commodity.

The objectives of the present study were to determine if mortality via radiation could be used to disinfest commodities of *H. lataniae* and identify a dose that could be used to prevent reproduction of the species and, thus, serve as a phytosanitary treatment. Hallman et al. (2010) found that for the measurements of efficacy used in PI radiotolerance increases as an insect develops; therefore, a PI treatment must suffice for the most tolerant stage—which is often the most developed one that may be present on a shipped commodity. All stages of *H. lataniae* may be present on a shipped commodity; therefore, a PI treatment that is efficacious against the adult will also be efficacious against all immature stages.

Materials and Methods

STUDY SITE AND INSECT REARING

The study was conducted at the Zoología Agrícola section of the Estación Experimental Agroindustrial Obispo Colombres (EEAOC), located in Las Talitas, Tucumán, Argentina. *Hemiberlesia lataniae* laboratory rearing started with an adult population collected in 2008 from olives in La Rioja, Argentina. Adults were held at 21 ± 1 °C; 60 ± 10% RH, with a photoperiod of 12:12 h L:D. Newly emerged crawlers were collected and used to infest butternut squash, C*ucurbita moschata* (Duchesne ex Lam.) (Cucurbitales: Curcubitaceae). Butternut squash was chosen as the rearing host because the picked fruit lasts longer than the life cycle of *H. lataniae*. Adult female scales live under the scale cover that is formed from secreted wax filaments cemented by anal excretions and embedded with the exuviae of the previous nymphal stages (Gullan & Kosztarab 1997). The mobile crawler stage hatches from an egg laid by the female insect beneath its scale cap and forces its way out from underneath the scale at a point near to the posterior end of the female. The crawler normally spends a few h (maximum of ~1 d) moving around looking for a suitable place to settle, before inserting its stylet into a chosen substrate and constructing its white cap (Hill & Holmes 2009). After the crawlers are fixed in place, the female nymphs pass through 3 instars until the adult stage. Parthenogenetic reproduction frequently occurs (Vargas & Rodriguez 2008). This haplotype strain of *H. lataniae* is not associated with males, but it is associated with endosymbiont bacteria (*Bacteroidetes* group). It is known that the presence of *Cardinium* bacteria are associated with the parthenogenetic lineage of the *Aspidiotus nerii* Bouché complex (Provencher et al. 2005).

EXPERIMENTAL PROCEDURE

To determine the most tolerant stage of *H. lataniae*, 1st and 3rd instars (each 4–5 d-old) and adults (6–8 d-old) were irradiated at the target dose of 0 (non-irradiated control), 50, 100, 150 and 200 Gy with 3 replications for each stage.

Approximately 200 new emerged crawlers were transferred using a small brush to each pumpkin to establish cohorts of the same age. Then, each pumpkin was carefully placed into a cylindrical plastic container (18 cm W × 25 cm H) covered with organdy cloth to allow aeration and to avoid contamination. When most individuals had matured to the desired stage pumpkins carrying scales were moved to the irradiation facility. The non-irradiated control was moved to a nearby facility with the same conditions as the irradiated samples to avoid accidental cross-contamination of irradiated insects with non-irradiated controls and vice versa. The irradiation treatment was conducted with a gamma Co-60 irradiator with an activity of 583.8 Ci (21.6 PBq) and dose rate of 40.7 Gy/ min at the National Atomic Energy Commission, Ezeiza Atomic Centre, Argentina. The pumpkin fruit surfaces with latania scales on them were placed facing the Co-60 source. The absorbed dose was measured using Fricke dosimeters attached on surfaces of host fruit (ASTM 2004).

After irradiation, the irradiated samples and control were immediately returned to the laboratory and held under standard rearing conditions. Subsequently immature and adult female scales on pumpkins were examined fortnightly under a stereomicroscope to determine their fate; i.e., if they had molted to the next stage, if adult females were able to produce offspring, or if they died. Untreated controls for each stage were examined similarly. Live scales typically have a yellowish color underneath, whereas dead scales are brown. Dead scales were counted, recorded, and removed at each review process for further analysis.

Tests were usually terminated after 150 d, at which time all scales had died or pumpkins had rotted. Survivorship at each stage of development and the reproductive status of adult females were determined at the end of the study by flipping them over onto their dorsa and examining them for evidence of eggs.

Efficacy (E), as a measure of the mortality rate due to gamma radiation, was corrected using the formula:

$$E = \left(1 - \frac{C_1 * T_2}{C_2 * T_1}\right) * 100$$

where E is the corrected adjusted percentage on mortality rate, C_1 is the latania scale number on the control butternut squash before irradiation treatment, T_2 is the latania scale number in irradiated butternut squash after irradiation treatment, C_2 is the lataniae scale number on the control butternut squash after irradiation treatment and T_1 is the latania scale number on irradiated butternut squash before irradiation treatment (Henderson & Tilton 1955). Efficacy was evaluated at 30 and 150 d after post irradiation treatment.

Due to the ability of *H. lataniae* to reproduce by parthenogenesis, the required response for an effective irradiation treatment was to prevent reproduction by the adult in the subsequent (F_1) generation. To determine most tolerant stage at least 3 replicates were made. Validation testing was done with 31,877 adults irradiated to determine the efficacy of a target dose of 200 Gy as a potential quarantine treatment.

STATISTICAL ANALYSIS

Efficacy was checked for normality using the Shapiro-Wilk test (SW). To normalize distribution of the mortality data, arcsine √% transformations were applied.

A General Linear Model (GLM) (for dose, stage, and age after irradiation treatment as fixed factors) to determine the efficacy of irradiation treatment on *H. lataniae* (i.e., most radio-tolerant stage) was used.

Moreover, offspring data were submitted to a 2-way ANOVA to determine the efficacy of radiation on different developmental stages of

H. lataniae. Means were separated by the unequal Tukey's test (HSD) at α = 0.05 (StatSoft, Inc. STATISTICA, version 10). All untransformed means (± SEM) were presented in the text.

Mortality data were also submitted to a probit analysis at the 95% confidence level to predict a radiation dose needed to cause an acute mortality ($LD_{99.99}$) after 30 and 150 d irradiation treatment (IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY, IBM Corporation). According to Postelnicu (2011) probit analysis is the preferred statistical method for analyzing dose-response relationships in biological assays.

Results

DOSIMETRY

The results of dosimetry are given in Table 1. Absorbed doses did not vary appreciably from target doses. In the large-scale confirmatory testing, 209 Gy was the highest dose measured when 200 Gy was targeted.

RADIATION-INDUCED CHRONIC MORTALITY

GLM analysis revealed significant effects of radiation-induced chronic mortality on *H. lataniae* for development stages (F = 23.921; df = 2, 49; P < 0.01), dose of radiation (F = 2.93; df = 3, 49; P < 0.04), d after irradiation (F = 271.88; df = 1, 49; P < 0.01), and the interaction of development stage*d after irradiation (F = 10.03; df = 2, 49; P < 0.01). Other interactions were not significant. Immature stages were more sensitive to irradiation than the adult. The pattern of tolerance to irradiation in *H. lataniae* scale as measured by chronic mortality was 1st instar < 3rd instar < adult (Table 2). Efficacy increased progressively with further increasing of d after irradiation.

Irradiation doses up to 200 Gy (the maximum used) did not cause 100% mortality in 3rd instar and adult life stage by 150 d after irradiation. Probit analysis predicted radiation doses of 821.10 and 586.60 Gy, respectively, to cause 99.99% mortality of female adult *H. lataniae* at 30 and 150 d (Table 2). Overall, life cycle and lifespan of *H. lataniae* increased as dosimetry increased. Adults from 200 Gy treatments survived up to 180 d, while those treated with 0, 50, 100 or 150 Gy survived up to 60, 90, 150, and 165 d, respectively. Moreover, none of the nymphs that developed from irradiated first instars developed into adults.

REPRODUCTION OF IRRADIATED FEMALE ADULT

Irradiation of adult females and third instars resulted in a significant reduction of the number of F_1 2nd instar offspring compared with the untreated control (F = 8.68; df = 4,49; P < 0.01). From 833 non-irradiated adult females and 514 3rd instars, respectively, 2,234.0 ± 376.77 and 2,287 ± 225.59 F_1 2nd instars were produced, while from 1,387 adult female scales and 334 3rd instar scales irradiated at 50 Gy, respectively, 273.33 ± 64.92 and 50.0 ± 8.74 F_1 2nd instar offspring were produced. Adults and the 3rd instars from 50 Gy treatments laid viable eggs which developed to produce one F_1 adult and subsequently one F_2 adult. The adults of only 1 replicate irradiated with 100 Gy (n = 1,415) and 150 Gy (n = 1,781) produced, respectively, 3 and 1 F_1 first instars, which died before developing further.

Efficacy was regarded as non-viability of eggs from irradiated adult females. An irradiation dose of 200 Gy was selected as a potential phytosanitary treatment because one nymph was recorded as hatching from eggs laid by 1,781 female adults irradiated with 150 Gy. Large scale tests conducted on 31,877 adults (12,310 adults irradiated on 1 d and 19,567 irradiated 15 d later) confirmed that 200 Gy produced a secure phytosanitary treatment by female sterilization as evidenced by production of only non-viable eggs.

Discussion

Acute mortality is not currently used as the end point for any PI treatment because doses required to achieve mortality by the time commodities may be inspected at ports of entry are not tolerated by fresh commodities. Hallman (2000) indicates that some arthropods may live as long as or even longer than non-irradiated ones at the doses required to prevent reproduction. A plausible explanation of irradiation-enhanced longevity may be provided by the tenet of life-history theory, which asserts that reproduction incurs a survival cost (Roff 2002).

Three other studies have been reported on the PI of diaspid scales. Angerilli & Fitzgibbon (1990) found that 300 Gy but not 150 Gy (the next lowest dose) resulted in eventual die-out of a *Quadraspidiotus perniciosus* (Comstock) (Hemiptera: Diaspididae) population infesting apples, and this indicated that sustainable reproduction was prevented by 300 Gy. However, details on stages irradiated or where reproduc-

Table 1. Results of dosimetry of the gamma Co-60 irradiator at the National Atomic Energy Commission, Ezeiza Atomic Centre, Argentina. The absorbed dose was measured using Fricke dosimeters attached to surfaces of pumpkin fruits in accordance with ASTM (2004).

Assay	Stage	Treatment (Gy)	$D_{min.}$ (Gy)	$D_{Max.}$ (Gy)	DUR	Dose rate (Gy/min)	N
Most tolerant stage	1st Instar	50	54.87 ± 3.51	58.63 ± 3.71	1.07	40.01 ± 1.28	5
		100	100.13 ± 4.01	108.09 ± 4.74	1.08	38.71 ± 1.89	5
		150	143.91 ± 4.67	155.16 ± 4.88	1.08	40.85 ± 1.39	5
		200	191.54 ± 2.95	202.66 ± 4.65	1.08	39.66 ± 1.09	5
	3rd Instar	50	48.99 ± 3.73	51.35 ± 3.98	1.08	39.33 ± 2.72	5
		100	99.03 ± 3.24	107.90 ± 3.62	1.09	34.02 ± 2.47	5
		150	146.47 ± 3.57	155.96 ± 3.48	1.06	34.05 ± 2.49	5
		200	193.45 ± 3.15	201.96 ± 4.70	1.06	33.64 ± 2.29	5
	Adult	50	48.98 ± 2.37	51.36 ± 2.49	1.04	38.66 ± 3.68	5
		100	97.44 ± 2.77	107.23 ± 3.48	1.10	38.04 ± 2.03	5
		150	146.91 ± 3.76	153.93 ± 2.89	1.08	40.08 ± 2.05	5
		200	194.39 ± 4.08	197.94 ± 4.47	1.08	40.86 ± 1.98	5
Large scale test	Adult	200	204.30	208.90	1.02	40.86	10
		200	188.75	190.98	1.02	37.35	10

D_{min}: minimum dose, D_{Max}: maximum dose, DUR: Dose uniformity ratio, N: butternut squash number per radiation treatment.

Table 2. Mortalities at 30 and 150 d of nymphs and adults of *Hemiberlesia lataniae* irradiated with 50 Gy and the predicted doses for 99.99% mortality.

	Efficacy (%)		Predicted dose for 99.99% efficacy (Gy)	
Stage	30 d	150 d	30 d	150 d
1st Instar	74.52 ± 4.74a	100 ± 0.0c	647.53	32.94
3rd Instar	45.30 ± 4.49b	96.55 ± 2.13c	767.99	374.86
Adults	45.30 ± 4.49b	90.61 ± 1.91c	821.10	586.60

Means followed by the same letter in both column and row are not significantly different [Unequal Tukey HSD ($P < 0.05$)]. Mortality data were corrected by Abbott's formula.

tion failed were not recorded. Follett (2006a) irradiated *Aspidiotus destructor* Signoret (Hemiptera: Diaspididae) females with eggs and crawlers on pumpkin with 60–200 Gy and found that the development of 2nd instars was not markedly reduced between 60 and 140 Gy (~20% throughout this dose range), but was completely prevented at the next highest dose, which was 200 Gy. The lack of reduction in a developmental parameter between 60 and 140 Gy hints at a source of contamination with non-irradiated insects that was corrected during the confirmatory testing at 200 Gy. In a third study irradiation of 35,424 adult female *Pseudaulacaspis pentagona* Targioni-Tozzetti (Hemiptera: Diaspididae) (~75% with eggs) at a target dose of 150 Gy resulted in 2,165 F_1 second instars and no F_1 adults with eggs (Follett 2006b).

Our results with 31,877 adults irradiated at a target dose of 200 Gy (maximum dose measured = 209 Gy) support a PI treatment of 209 Gy for *H. lataniae.* Also this result supports a generic PI dose of 250 Gy for scales and most insect species in general (Hallman 2012). It is possible that a target dose < 200 Gy would suffice for *H. lataniae* given that 150 Gy failed only by the presence of 1 F_1 1st instar when 1,781 adults were irradiated.

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