Phytosanitary Irradiation of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae)

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Abstract

Phytosanitary irradiation is being increasingly used as a phytosanitary treatment to disinfest fresh commodities of invasive species. Optimum irradiation treatment doses for a number of invasive species groups, such as the family Noctuidae, are required. This research was designed to provide data for developing an optimum generic irradiation dose suitable against all eggs and larvae of the Noctuidae. Last instars (the most radiotolerant stage normally found on exported commodities) of 2 noctuid species were studied, i.e., *Heliothis virescens* (F.) and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae). There was no significant difference in emergence of normal-looking adults from *H. virescens* last instars reared on either a natural or an artificial host, i.e., mature pods of *Phaseolus vulgaris* L. (Fabales: Fabaceae) or a meridic diet, respectively. Two laboratory colonies of *H. virescens* were studied, one appeared to be more radiotolerant than the other and it was used for large-scale confirmatory testing of a putative phytosanitary irradiation dose treatment. A total of 14,366 last instars were irradiated with 150 Gy (actual measured dose distribution was 145–166 Gy) with none emerging as normal-looking adults. A total of 2,197 last instar *H. zea* reared on meridic diet were irradiated with the same dose and no normal-looking adults emerged. This research indicates that a treatment dose of approximately 150 Gy is sufficient as a phytosanitary treatment for these 2 noctuid species and adds to the body of research evidence that a generic dose treatment of 250 Gy is adequate to control eggs and larvae of Lepidoptera that infest fresh commodities.

Key Words: radiation; quarantine treatment; tobacco budworm; corn earworm

Resumen

Se está utilizando la irradiación fitosanitaria cada vez más como un tratamiento fitosanitario para desinfestar productos frescos de las especies invasoras. Se requieren determinar la dosis de tratamiento de irradiación óptima para una serie de grupos de especies invasoras, tales como la familia Noctuidae. Esta investigación fue diseñada para proveer datos para el desarrollo de una dosis de irradiación genérica óptima adecuada contra todos los huevos y larvas de Noctuidae. Se estudiaron los ultimos estadios (el estadio más radiotolerante que normalmente se encuentran en los productos básicos exportados) de 2 especies nóctuidos, *Heliothis virescens* (F.) y *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae). No hubo diferencia significativa en la emergencia de adultos de aspecto normal de las ultimas estadios de *H. virescens* criados sobre un hospedero natural o artificial, como las vainas maduras de *Phaseolus vulgaris* L. (Fabales: Fabaceae) o una dieta merídica, respectivamente. Se estudiaron dos colonias de laboratorio de *H. virescens*, de estas, uno parećia ser más radiotolerante que la otra, la cual se utilizó para la prueba confirmatoria en gran escala de un tratamiento de dosis de irradiación fitosanitaria putativo. Un total de 14,366 últimos estadios fueron irradiados a 150 Gy (distribución de la dosis real medida fue 145–166 Gy) con ninguno emergentes como adultos de aspecto normal. Se irradiaron a la misma dosis un total de 2,197 larvas del último estadio de *H. zea* criadas sobre la dieta merídica y no hubo adultos de aspecto normal que emergieron. Esta investigación indica que una dosis de tratamiento de aproximadamente 150 Gy es suficiente como tratamiento fitosanitario para estas 2 especies de nóctuidos y añade al monto de evidencia de la investigación que un tratamiento de dosis genérica de 250 Gy es adecuado para el control de huevos y larvas de lepidópteros que infestan productos frescos.

Palabras Clave: radiación; tratamiento de cuarentena; gusano bellotero del tabaco; gusano del algodón; gusano elotero

Phytosanitary irradiation (PI) is increasingly being used on a commercial scale to treat fresh commodities and reduce the risk of spreading quarantine pests through trade (Hallman 2011). In virtually all of these cases generic PI treatments are used; i.e., treatments involving a specific minimum irradiation dose that applies for groups of pests and/or commodities. It is impossible to undertake detailed research on the irradiation of every insect species, however, research conducted on significant numbers of different pests and/or commodities can be used to arrive at a conservative—but not excessively upper-bound—dose that is applicable to a group of insect species even though research was not conducted on all of the pests covered by the treatment (Hallman 2012). Generic treatments are not available for all pest groups, and some of the available generic doses may be higher than needed to ensure phytosanitary security. Research presented in this special issue is part of an international effort directed toward resolving these shortcomings.

A generic dose of 150 Gy against tephritid fruit flies, arguably the most important group of quarantine pests of fresh horticultural commodities, has been accepted by both the International Plant Protection Convention (IPPC) and the United States Department of Agriculture, the latter representing the country that is the largest importer of PI-treated commodities (Hallman 2012). The second most important group of quarantine pests after the Tephritidae comprises lepidopteran larvae of the families Crambidae, Gelechiidae, Geometridae, Gracillariidae, Lycaenidae, Lymantriidae, Metarbelidae, Noctuidae, Oecophoridae, Pyralidae, Sesiidae and Tortricidae that mostly bore into fruit (Hallman et al. 2013). Two-thirds of commodities for which pest risk analyses have been done include Lepidoptera as quarantine pests for

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which phytosanitary treatments are required. A generic dose of 250 Gy has been proposed for the entire Order, but is not yet accepted by the International Plant Protection Convention because large scale testing studies have been lacking for most families. The family Tortricidae is the most phytosanitarily important of the Order, and sufficient research has now been reported to recommend a generic dose of 250 Gy to control species of the Tortricidae (Hallman et al. 2013).

After Tortricidae, the family Noctuidae is of considerable phytosanitary importance, but only small-scale PI research has been done with 7 species (Table 1). Doses of ≤ 200 Gy prevented adult emergence when applied to the last instar, but large-scale testing is required to substantiate treatment efficacy. The next lowest dose tested in the study with the highest dose (200 Gy) was 100 Gy (Arthur et al. 2016); therefore, it may be possible that a dose between 100-200 Gy would prevent adult emergence in that case.

The objective of this study was to determine a PI dose for other species of Noctuidae and add further irradiation studies of this family to the literature. *Heliothis virescens* (F.) and *Helicoverpa zea* (Boddie) were chosen because they are important quarantine pests with wide host ranges, and thus can be found on a wide variety of commodities. For example, Hallman (1980) categorized 72 host species of *H. virescens* from 19 plant families in central Colombia and EPPO (2014) mentions > 100 host species of *H. zea* in the Americas.

Materials and Methods

INSECTS

Both insect species were obtained from the USDA-ARS location at Stoneville, Mississippi. Two populations of *H. virescens* were studied, "A" and "B". Colony A insects were collected from wild hosts near Stoneville, Mississippi in 1971 and augmented with field material every year until about 7 years before this research was undertaken. Colony A insects were reared on the standard soybean-wheat germ (ARS) meridic diet. The B colony originated from a field collection in northeastern Mississippi in 2001 and was supplemented with feral insects 2 years before this research was done. Colony B insects were on a meridic diet (F9781B, Bio-Serv, Frenchtown, New Jersey).

MEASUREMENT OF EFFICACY

In this study the measure of efficacy used was the prevention of adult emergence by the irradiation of eggs or larvae. The general measurement of efficacy for PI treatments is not acute mortality, as it is for all other commercially-applied treatments, but prevention of development and/or reproduction. Because insects increase in radiotolerance as they develop, the most radiotolerant stage is invariably the most developed stage that may occur on marketed commodities, which for most Lepidoptera is the final instar (Hallman et al. 2010). Therefore, this research was conducted with last instars.

The measure of efficacy we suggest for eggs and larvae of Lepidoptera is the same level of efficacy already accepted in international standards by plant protection organizations for PI of Tephritidae; prevention of adult emergence from irradiated last instars (Hallman et al. 2013).

Irradiation System

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 late 5th instars. 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 containers with the larvae 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).

INFESTATION AND IRRADIATION TECHNIQUE

To evaluate the effect of the host species on the radiotolerance of the larvae, the latter were reared individually in 30 mL plastic cups with cardboard lids on standard soybean-wheat germ diet or mature pods of *Phaseolus vulgaris* L. (Fabales: Fabaceae) grown in a greenhouse. After the larvae reached the late last instar and before they would normally exit the plant to pupate, they were irradiated with 60 Gy. To keep the dose uniformity ratio as small as possible cups containing last instars along with their food were irradiated in single stacks in the center of the cylinder carrying the product to be irradiated. After irradiation the cups containing the insects were placed in an environmental chamber at 25 °C to observe further development until the insects emerged as adults or were obviously dead. The dose-response relationships of both colonies of *H. virescens* were analyzed via irradiation of late last instars reared on meridic diet at 50, 60, 70, 80 and 100 Gy.

STATISTICAL ANALYSES

A 2-tailed t-test was used to determine possible differences between irradiated colony A and colony B last instars after the data were adjusted for lack of adult emergence by the following formula:

$$Y_a = 100\% - [(X - Y)/X]\ (100\%)$$

where Y_a is the adjusted percentage emerged in the irradiated cohort, X is the percentage emerged in the control, and Y is the percentage emerged in the irradiated cohort.

Table 1. Noctuid species studied for phytosanitary irradiation.

Species	Lowest dose (Gy) of those tested against the last pre-imaginal instar that prevented adult emergence	Number of caterpillars tested at that dose	Reference
Agrotis ipsilon (Hufnagel)	80	19	Elnagar et al. 1985
Helicoverpa armigera (Hübner)	100	180	Kim et al. 2015b
H. armigera (Hübner)	150	176	Kim et al. 2015a
Heliothis virescens (Boddie)	100	50	El Sayad & Graves 1969
Sesamia nonagrioides (Lefebvre)	150	120	Aksoy et al. (2016)
Spodoptera exigua (Hübner)	150	177	Wit & van de Vrie 1986
S. frugiperda (J. E. Smith)	200	100	Arthur et al. 2016
S. litura (Fabricius)	100	97	Dohino et al. 1996

Probit analysis of dose-response data for *H. virescens* were analyzed with PoloPlus (Petaluma, California) using the logit probability density function and $\log_{10}$ dose.

Results

Actual absorbed doses within irradiated samples were up to 3% lower and 11% higher than the doses targeted; i.e., a targeted dose of 150 Gy resulted in an absorbed radiation dose distribution across the irradiated sample that ranged from 145 to 166 Gy.

A two-tailed t-test (t = 0.282; degrees of freedom = 4; P = 0.792) indicated no significant difference in prevention of adult emergence between last instars irradiated to 60 Gy and reared on either green beans (mean = 79.4 ± 4.5%) or the standard soybean-wheat germ diet (mean = 76.4 ± 9.8%).

The A population of *H. virescens* was observed to be more radiotolerant than the B population because ≥ 60 Gy prevented adult emergence of the latter while 100 Gy applied to the former prevented 99.81% adult emergence (Table 2). Data from the B strain could not be used in probit analysis because only the lowest dose resulted in adult emergence. Probit analysis of the A strain resulted in a χ^2 of 16.2 with a resulting heterogeneity of 5.4; degrees of freedom were 3. The mean expected dose (95% confidence limits) to achieve 99% prevention of adult emergence was calculated at 77.3 (71.6–87.8) Gy. For Probit 9 (99.9968%, the historical level of control often sought) the estimated dose was 132.5 (113.0–174.9) Gy.

Confirmatory testing was done with the A strain of *H. virescens* using a total of 14,366 last instars in 14 replicates of ~1000 larvae/replicate that were each irradiated to a target dose of 150 Gy and none emerged as normal-looking adults capable of flight. Mean emergence of normal-looking adults in the non-irradiated controls was 85.3 ± 4.4%.

A total of 2,197 last instar *H. zea* in 5 replicates of ~440 larvae/replicate were irradiated with a target dose of 150 Gy and none emerged as normal-looking adults capable of flight. Normal adult emergence in the non-irradiated controls was 87.6 ± 6.2%.

Discussion

The acceptance of research results has been questioned in the past because of a lack of comparison of the radiotolerance of lepidopteran larvae reared on laboratory diet vs. a natural plant host. For example, this was the cause for rejection of PI treatment proposals that were based on research using laboratory diet-reared larvae (Hallman et al. 2010). Including this study there are now 5 studies reported in 4 publications that compare the radiotolerances of lepidopteran larvae reared on an artificial diet to those reared on a plant host, and in all cases the difference in radiotolerance were found to be insignificant (Table 3). However, researchers should be cautious about making gen-

Table 2. Prevention of adult emergence of 2 strains of *Heliothis virescens* by irradiation of the last instars with doses of gamma radiation in the range 50–100 Gy.

	A strain		B strain	
Target dose (Gy)	*n*	Emergence prevented (%)	*n*	Emergence prevented (%)
50	107	65.4 ± 9.3	106	98.1 ± 0.95
60	366	86.1 ± 6.9	354	100
70	139	98.6 ± 0.89	139	100
80	7454	99.56 ± 0.33	1524	100
100	2161	99.81 ± 0.15	107	100

Table 3. Comparison of the effect of rearing larvae of lepidopteran species on a meridic diet-reared vs. on a host plant with regard to the efficacy of phytosanitary irradiation.

			Efficacy (%) when reared on		
Species and order	Measurement of efficacy	Dose (Gy)	Meridic diet	A host plant	Reference
Cydia pomonella (L.) (Tortricidae)	Prevent adult emergence from irradiated last instar	100	80.7 ± 2.8	81.3 ± 7.8	Mansour (2003)
Cydia pomonella (L.) (Tortricidae)	Prevent adult emergence from irradiated last instar	150	98.5 ± 1.9	95.7 ± 4.5	Mansour (2003)
Grapholita molesta (Busck) (Tortricidae)	Prevent adult emergence from irradiated last instar	100	99.2 ± 0.65*	99.2 ± 0.65*	Hallman (2004)
Heliothis virescens (Boddie) (Noctuidae)	Prevent adult emergence from irradiated last instar	60	76.4 ± 9.8	79.4 ± 4.5	Current study
Ostrinia nubilalis (Hübner) (Crambidae)	Prevent F_1 egg hatch from irradiated late pupa	50	89.0 ± 2.4	88.4 ± 2.9	Hallman & Hellmich (2009)

*Combined mean reported because there were no statistical differences (P = 0.64) eral conclusions that a laboratory diet can substitute for a host plant when developing phytosanitary treatments because relatively little relevant research has been reported. The indications until now are that it is not significant consideration for lepidopteran species, but some research findings involving tephritid species did reveal differences (Hallman 2014).

The noticeable difference in radiotolerance between the A and B strains of *H. virescens* demonstrates that populations of the same species may vary based on measures of efficacy relevant to PI with implications for the broad applicability of treatments. Consequently, further research on relative radiotolerance among populations within a species is warranted because current PI treatments are applied assuming no difference among populations of the same species.

A total of 14,366 last instars of *H. virescens* were irradiated in large scale testing at a target dose of 150 Gy. The initial objective of this research was to irradiate ~30,000 insects, but these plans were truncated because a funding rescission resulted in the decision of the USDA-ARS to close its research laboratory at Weslaco, Texas. Further research studies with *H. zea* and other lepidopteran species reared on various plant hosts, and strains of *H. virescens* were prevented by this action. Because a target dose of 150 Gy resulted in doses up to 166 Gy being recorded by dosimeters it would be prudent to recommend 166 Gy as the dose for a phytosanitary treatment against *H. virescens* that may be deemed sufficiently supported by this research.

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