Phytosanitary irradiation of *Diatraea saccharalis, D. grandiosella,* and *Eoreuma loftini* (Lepidoptera: Crambidae)

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Abstract

Phytosanitary irradiation (PI) is increasingly being used to disinfest horticultural commodities of invasive quarantine pests. Most disinfestation is done with generic treatments, where the same dose is applied against a group of pest species and/or commodities. The current generic treatment used by the Animal and Plant Health Inspection Service of the United States Department of Agriculture (USDA-APHIS) against Class Insecta—i.e., 400 Gy—does not include pupae of any species of Lepidoptera. More data are needed to develop a generic dose for those Lepidoptera that pupate on or within shipped commodities, such as Crambidae species. Tests were done with the crambids *Diatraea grandiosella* Dyar, *D. saccharalis* (F.), and *Eoreuma loftini* (Dyar) (Lepidoptera: Crambidae) to seek doses that would provide quarantine security no only against late pupae but also against last instar larvae, and that would possibly contribute to the development of a generic PI dose that is less than the current 400 Gy. The results of this research support proposed generic doses of 250 and 400 Gy for larvae and pupae of Lepidoptera species, respectively, and suggest lower specific doses for the individual insect species.

Key Words: generic treatment dose; ionizing radiation; quarantine treatment; invasive species; phytosanitation; *Diaprepes abbreviatus*

Resumen

La irradiación fitosanitaria (IF) es cada vez más utilizada para desinfectar productos hortícolas de plagas invasivas de importancia cuarentenaria. Se realiza la mayoría de desinfestación con tratamientos genéricos, donde se aplica la misma dosis en contra de un grupo de especies de plagas y/o productos. El tratamiento genérico utilizado actualmente por el Servicio de Inspección Sanitaria de Animales y Plantas del Departamento de Agricultura de Estados Unidos (USDA-APHIS) en contra de la clase Insecta—400 Gy— no incluye pupas de ningun especie del Orden Lepidóptera. Se necesitan más datos para desarrollar una dosis genérica para los lepidópteros que se transforman en crisálidas en o dentro de productos transportados, como las especies de la familia Crambidae. Se realizaron las pruebas con los crambids *Diatraea grandiosella* Dyar, *D. saccharalis* (Fabricius), y *Eoreuma loftini* (Dyar) (Lepidoptera: Crambidae) para buscar la dosis que proveerá la seguridad de cuarentena no sólo contra las pupas mas maduras, asi como contra larvas de último estadio, y que posiblemente poder contribuir al desarrollo de una dosis IF genérica que es menor que la 400 Gy actual. Los resultados de esta investigación apoyan las dosis genéricas de 250 y 400 Gy propuestas para las larvas y pupas de especies de lepidópteros, respectivamente, y sugieren una dosis más baja específica para las especies de insectos individuales.

Palabras Clave: dosis de tratamiento genérico; radiación ionizante; tratamiento cuarentenario; especies invasoras; fitosanidad; *Diaprepes abbreviatus*

Stems of sugarcane, *Saccharum officinarum* L. (Poales: Poaceae), are cut in various lengths from whole canes and marketed as pieces ~2 m-long to much less than 1 m. They are used in a variety of ways, such as split swizzle sticks in drinks, as smoke flavoring in outdoor grill cooking, in a diversity of food recipes, or simply chewed for the sweet taste.

Several insect species may bore inside sugarcane stems and could be present as larvae or pupae inside cut stems that are marketed. Quarantines against any one of these insect species in market destinations would require the use of phytosanitary treatments. These insect species also bore into other commodities, so treatment doses against them would be useful for a variety of commodities.

The sugarcane borer, *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae), attacks plants of the Poaceae family, mainly sugarcane. Eggs are laid on leaves and the larvae initially feed on foliage then tunnel into leaf midribs and when they are approximately half-grown larvae they tunnel into the stem. After completing larval development they pupate within the stem leaving a thin window of plant material over the hole through which the adult will emerge. The pest is found throughout the Neotropics and subtropics, i.e., throughout the Caribbean, Central America, and the warmer portions of South America south to northern Argentina. It occurs in the southern USA but not in California and neighboring territories.

The Mexican rice borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Crambidae), bores into sugarcane stems in superficially the same way as *D. saccharalis*, pupating under a thin window in the stem, and is a pest of other Poaceae, such as rice (*Oryza* spp.), corn (*Zea mays* L.), and grain sorghum (*Sorghum bicolor* [L.] Mensch) (Legaspi et al. 1997a, b). In southern Texas it has replaced *D. saccharalis* as the major pest of

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sugarcane. It is thought that the pest originated in southwestern Mexico and spread north to Arizona and California and later into eastern Mexico and then to Texas by about 1980 (Johnson 1984). From Texas it migrated along the coast of the Gulf of Mexico and is currently spreading into Florida.

The southwestern corn borer, *Diatraea grandiosella* Dyar (Lepidoptera: Crambidae), infests corn, sorghum, sugarcane, and some other grasses. It occurs throughout the Mississippi River valley as far north as Nebraska and Indiana, down into Mexico and as far west as Arizona.

Because the species of *Diatraea* are found in Florida but not in California sugarcane shipped from the former to the latter must be disinfested to prevent establishment of the pests in California. Hallman & Chalot (1993) list a cold, a heat, and a drowning treatment which may be used to disinfest sugarcane of *D. saccharalis*, but the treatments have not been evaluated for efficacy against the other species.

The root weevil *Diaprepes abbreviatus* (Linnaeus) (Coleoptera: Curculionidae) is also a quarantine pest for shipping sugarcane from Florida to California. and preliminary research suggests that ionizing radiation at 50 Gy prevents eclosion of eggs laid by irradiated adults (Gould & Hallman 2004).

Phytosanitary irradiation (PI) is increasingly being used commercially to reduce the risk of spreading invasive quarantine pests through commerce by irradiating fresh produce (Hallman 2011). The objective of this study was to determine PI doses for controlling *D. saccharalis, E. loftini,* and *D. grandiosella* for use as a phytosanitary treatment for sugarcane and to contribute to generic PI doses for Lepidoptera immatures (Hallman 2013 a, b). Preliminary research is reported in Darmawi et al. (1998).

Materials and Methods

Insects

Diatraea saccharalis and *E. loftini* were obtained from sugarcane at the Texas Agricultural Experiment Station in Weslaco, Texas, and *Diatraea grandiosella* was obtained from a laboratory colony at the USDA-ARS facility in Mississippi State, Mississippi. All were raised on diet using the methods of Martinez et al. (1988) in the laboratory.

Measurement of Efficacy

The 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 (IPPC 2003). Because insects increase in radiotolerance as they develop, the most radiotolerant stage is invariably the most developed stage that may occur on marketed commodity, which for Lepidoptera that may pupate in the commodity, such as many Pyraloidea, is the late pupal stage (= pharate adult stage) (Hallman et al. 2010). Therefore, this research was conducted with the late pupal stage. However, as support for a generic PI dose against larvae of Lepidoptera, data were also generated on doses required to prevent adult emergence from irradiated last instars, which is the measurement of efficacy against eggs and larvae of Lepidoptera (Hallman et al. 2013a).

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 insects. Reference standard dosimetry was done in 1996 with the Fricke system (ASTM 2013), and the research was conducted soon after. Routine dosimetry was done with radiochromic film (1-cm^2, Gafchromic HD-810, 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). Uncertainty in the dosimetry system was approximately ± 3%.

Infestation and Irradiation Technique

The three crambid species were reared on semi-artificial diet (Martinez et al. 1988) because previous research with Lepidoptera has not found a difference in response to PI between rearing on diet and plant hosts (Hallman 2016). The insects were reared to the pupal stage within 1-2 d of adult emergence and then placed in cylinders in the irradiation chamber and subjected to doses ranging from 50 to 400 Gy; lower doses were used for larvae than pupae.

Statistical Analyses

Analysis of dose-response data from phytosanitary treatment research usually requires a binary response; e.g., insect dead or alive; developed to adult or not. Dose-response data were analyzed with PoloPlus (Petaluma, California).

Results

Measured dose ranges are shown in Table 1 for target doses from 50–400 Gy. Variation around the target dose was approximately 3% below to approximately 6% above each target dose.

DOSE RESPONSES FOR LAST INSTARS

Diatraea saccharalis

Cumulative percentage of normal adult emergence of irradiated last instar *Diatraea saccharalis* was 64, 84, 83, 99, 0, and 0 %, respectively, for 0, 20, 30, 50, 75, and 100 Gy. At 75 Gy 7.4% (n = 108) of last instars pupated and 1 adult (0.93%) emerged with bent wings and this adult was unable to fly. At 100 Gy 4.9% pupated and one adult (0.82%) emerged deformed and unable to either walk or fly.

The data fit the probit model (probability value = 0.26; degrees of freedom = 11; X^2 = 13.6; Y-intercept = 0.55 ± 0.060; slope = −1.34 ± 0.59), and the projected dose to achieve 99.99683% (probit 9) efficacy based on prevention of the emergence of normal-looking adults was 85 Gy (95% fiducial limits 64–186 Gy).

Table 1. Results of routine dosimetry with radiochromic film (Gafchromic HD-810, ISP Technologies, Inc., Wayne, New Jersey) read with a spectrophotometer at 600 nm 24 h after exposure.

Target dose (Gy)	Dose range measured (Gy)
20	19–22
30	28–33
50	48–53
75	72–80
100	97–105
150	145–161
200	194–212
250	244–266
300	293–317
350	339–361
400	387–426

Eoreuma loftini

A higher dose was needed to prevent adult emergence of *E. loftini* compared with *D. saccharalis*. At 75 Gy (*n* = 98) 5.1 and 4.1%, respectively, of *E. loftini* last instars emerged as normal-looking and bent-winged adults. At 100 Gy (*n* = 101) 2.0% emerged as normal-looking adults. At the next highest dose studied (150 Gy, range 145-161), no adults, neither normal-looking nor otherwise, emerged (*n* = 59), while 6.8% of irradiated last instars pupated.

The data for *E. loftini* did not fit the probit model (X^2 = 30.5; probability value at 95% = 0.033). Nevertheless, if it is accepted that the model is useful in representing the data, the projected dose to achieve 99.99683% efficacy based on prevention of the emergence of normal-looking adults is 109 Gy (95% fiducial limits 91-141 Gy).

DOSE RESPONSES FOR LATE PUPAE

Diatraea saccharalis

A reduction in percentage emergence of normal-looking adults from irradiated late pupae (pharate adults) of *D. saccharalis* started to become apparent at doses > 150 Gy (Table 2). But even at 300 Gy emergence was still 65% compared with 88% for non-irradiated pupae.

A total of 5,350 late pupal *D. saccharalis* were irradiated at 300 Gy; adult females laid 37.3% of the number of eggs laid by non-irradiated females, 0.032% hatched, and none developed to the 2nd instar. By 12 d after irradiation with 300 Gy females stopped laying eggs although 43.4% were still alive. Eighteen d after irradiation with 300 Gy the last of the adults that had emerged from irradiated pupae had died, while 20.6% of non-irradiated adults were still alive. Non-irradiated adults continued laying eggs until they died.

Irradiation of a total of 2,000 late pupae at 350 Gy did not prevent oviposition by emerged adults; however, no eggs hatched. Oviposition by the non-irradiated controls was abundant, and 75.2% of the eggs hatched and developed beyond the 1st instar. Irradiation of a total of 5,350 late pupae of *D. saccharalis* at 300 Gy resulted in some egg hatch but none developed to the 2nd instar.

Eoreuma loftini

Adult emergence was not affected by irradiation of late *E. loftini* pupae with 300 Gy as the mean (± SEM) emergence of normal-looking adults was 82.5 ± 3.7 (*n* = 8 replicates) and 83.9 ± 5.3 (*n* = 4 replicates), respectively, for non-irradiated and irradiated pupae. Longevity was shorter for adults emerging from pupae irradiated at 300 Gy, and they laid approximately 1/4th of the number of eggs laid by non-irradiated females; while egg hatch for the latter was 97.5%, no eggs hatched of those laid by adults emerging from a total of 474 late pupae irradiated with 300 Gy.

F_1 egg hatch at 150, 200, and 250 Gy, respectively, was 5.4, 2.7, and 0.40%. When 217 late pupae were irradiated at 250 Gy none of the F_1 1st instars that emerged survived to the 2nd instar.

Table 2. Emergence of normal-looking adults from irradiated late pupae of *Diatraea saccharalis* versus dose.

Dose	No. replicates	Emergence ± SEM (%)
0	9	87.6 ± 2.5
150	4	87.0 ± 10.0
200	4	77.4 ± 6.8
250	4	75.0 ± 6.7
300	4	64.6 ± 6.8

Diatraea grandiosella

A total of 6,075 late pupae of *D. grandiosella* were irradiated at 300 Gy and 54 F_1 eggs hatched although none of these 1st instars developed to the 2nd instar. Total egg counts were not made, although it was observed that adults emerging from irradiated pupae laid considerably fewer eggs than adults from non-irradiated pupae and that F_1 egg hatch rates of the non-irradiated insects was very high.

Discussion

This research originally began as support to transport sugarcane cuttings from Florida to California. Research on *E. loftini* was conducted because it was invading new territory, becoming a quarantine pest for an increasing geographic and host range.

The results of this research support generic doses of 250 and 400 Gy for Lepidoptera larvae and pupae, respectively (Hallman et al. 2013a, b). Table 3 summarizes doses that could be suggested for specific quarantine security against the last instars and late pupae of the 3 Crambidae species in this study. Usually considerably larger numbers of insects than those cited in Table 3 must be tested to confirm that a target dose of irradiation meet the probit 9 or related criterion of a phytosanitary treatment.

The greater radiotolerance found in last instar *E. loftini* compared to *D. saccharalis* (Table 3) as measured in the prevention of emergence of normal-looking adults does not seem to be manifested when the target dose is applied to late pupae and the effect is measured as the prevention of development of the F_1 generation. However, the number of *E. loftini* pupae tested for the prevention of hatch of F_1 eggs was small compared with the number of *D. saccharalis* pupae.

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Table 3. Doses (Gy) that might suffice as phytosanitary irradiation treatments applied in the last instar and late pupal stage of *Diatraea saccharalis*, *D. grandiosella* and *Eoreuma loftini*. The numbers irradiated with each target dose are shown in parenthesis.

	Dose and (numbers tested) per irradiated stage		
		Late pupa to prevent	
Crambid species	Last instar to prevent normal adult emergence	F_1 egg hatch	Development of 2nd instars in the F_1 generation
Diatraea grandiosella	—	350 Gy (2,000)	300 Gy (6,075)
Diatraea saccharalis	75 Gy (108)	350 Gy (2,000)	300 Gy (5,350)
Eoreuma loftini	150 Gy (59)	300 Gy (474)	250 Gy (217)

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