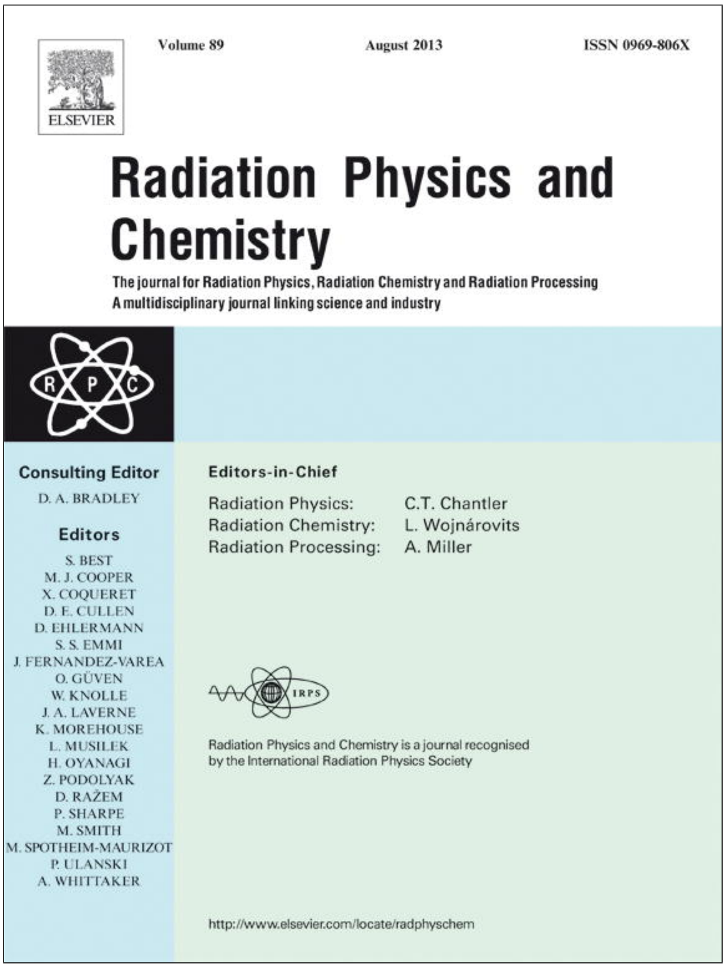
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The case for a generic phytosanitary irradiation dose of 250 Gy for Lepidoptera eggs and larvae

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HIGHLIGHTS

- A radiation dose of 250 Gy is suggested as a generic phytosanitary treatment for Lepidoptera larvae.
- The dose of 250 Gy is especially supported for the family Tortricidae.
- The endpoint is prevention of normal-looking adults, assumed not able to fly or reproduce.

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ABSTRACT

The literature on ionizing irradiation of Lepidoptera is critically examined for a dose that could serve as a generic phytosanitary treatment for all eggs and larvae of that order, which contains many quarantine pests that inhibit trade in fresh agricultural commodities. The measure of efficacy used in deriving this dose is the prevention of emergence of normal-looking adults that are assumed not able to fly. A dose of 250 Gy is supported by many studies comprising 34 species in 11 lepidopteran families, including those of significant quarantine importance. Two studies with two different species found that doses >250 Gy were necessary, but both of these are contradicted by other studies showing that <250 Gy is adequate. There is a lack of large-scale ($>10,000$ individuals) testing for families other than Tortricidae (the most important quarantine family in the Lepidoptera). Because several large-scale studies have been done with tortricids a dose of 250 Gy could be justifiable for Tortricidae if it is not acceptable for the entire Lepidoptera at this time.

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1. Introduction

Phytosanitary irradiation (PI) uses ionizing irradiation to disinfest quarantined agricultural commodities (e.g., fruits and vegetables) of quarantine pests that could be present in the commodities (Hallman, 2011). Generic PI treatments are extremely versatile in that they may be applied to broad categories of commodities and quarantine pests at different stages of packing (Hallman, 2012). Five PI treatments generic for pest groups are currently approved and being used for trade (Table 1). They range in coverage from two treatments that are for all pests of one fruit shipped from one country to another to one treatment that is for almost all insects on all hosts shipped from most countries to one country. Essentially all other PI treatments approved for individual species are generic for host as they can be used on any fresh commodity (APHIS (Animal and Plant Health Inspection Service), 2013; IPPC (International Plant Protection Convention), 2011b). Efforts to develop additional generic PI treatments are ongoing with a broad-based 5-year, 12-nation cooperative research project coordinated by the Joint Food and Agricultural Organization/International Atomic Energy Agency Programme of Nuclear Techniques in Food and Agriculture (IAEA (International Atomic Energy Agency), 2009).

The International Plant Protection Convention (IPPC) is developing a manual of phytosanitary treatments (International Standard on Phytosanitary Treatments #28) (IPPC (International Plant Protection Convention), 2011b). This paper presents the case for a generic treatment for all eggs and larvae of the important quarantine pest insect order Lepidoptera and has been submitted as supporting evidence for such a dose. Lepidoptera eggs and larvae comprise the key group of quarantine pests of fruits after Tephritidae fruit flies (Hallman, 2011). As PI expands to vegetables from its current use mostly with fruits Lepidoptera will become relatively even more important because many vegetables are attacked by Lepidoptera

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Table 1
Generic phytosanitary irradiation treatments currently approved; all are being commercially used.

Commodities	Pests	Dose (Gy)	Importing and exporting countries
All hosts	Tephritidae	150	IPPC members
Mango, papaya	All arthropods	250	New Zealand from Australia
Mango	All arthropods	300	Malaysia from Australia
Lychee	All arthropods	350	New Zealand from Australia
All hosts	Insects except pupae and adult Lepidoptera	400	US from trading partners

while few tephritids are quarantine pests of vegetables. Families of quarantine concern in the Lepidoptera include Crambidae, Gelechiidae, Geometridae, Gracillaridae, Lycaenidae, Lymantriidae, Metarbilidae, Noctuidae, Oecophoridae, Pyralidae, Sesiidae, and Tortricidae. These families all contain quarantine pests of commodities for which PI has been proposed or is currently being used at a generic dose of 400 Gy.

The objective of this paper is to critically examine the literature on ionizing irradiation of Lepidoptera to determine a dose that could serve as a phytosanitary treatment for all eggs and larvae of that order or families within that order.

2. Criteria for analysis of literature

The generic dose investigated in this study is for eggs and larvae of Lepidoptera and may not be sufficient for Lepidoptera that may pupate in the part of a commodity that is shipped (e.g., Crambidae and Pyralidae). Insects generally increase in radiotolerance as they develop (Hallman et al., 2010) with pupae being generally more radiotolerant than eggs or larvae. Nevertheless, the vast majority of quarantine pests of the Lepidoptera occur only as eggs or larvae in shipped commodities and, thus, a generic dose against these stages of Lepidoptera would have broad application.

The objective of a phytosanitary treatment is to prevent invasive species from becoming established, and this is usually accomplished by ensuring essentially 100% mortality of any quarantine pests present in shipped commodities (Heather and Hallman, 2008). PI is unique among phytosanitary treatments in that acute mortality is not the usual measure of efficacy because acute mortality is not required to prevent establishment of a pest and doses for 100% acute mortality would be higher than most fresh commodities can tolerate. PI is effective at arresting insect development and preventing their reproduction using doses that do not significantly alter the quality of most fresh commodities. In fact, one of the advantages of PI compared with other phytosanitary treatments is that it is tolerated by more fresh commodities than any other commercial treatment (Heather and Hallman, 2008).

The measure of efficacy of phytosanitary treatments must be specifically defined; it is insufficient to use general terms such as "mortality" and "sterility" without defining precisely at what threshold efficacy is no longer achieved. The measure of efficacy for Lepidoptera eggs and larvae could be any of a progression of thresholds ranging from highest to lowest dose: acute mortality, prevention of development to the next metamorphic stage, prevention of any adult emergence from pupa, prevention of emergence of normal-looking adults from pupa, prevention of oviposition from surviving females, prevention of F1 egg hatch, and so on. Hallman et al. (2010) argue that measures of efficacy for PI should ideally prevent significant F1 development to provide a margin of security because there is no independent verification of efficacy for PI like there is for all other commercial treatments: dead insects soon after treatment. Therefore, there is arguably a greater need for a margin of security in the treatment dose to cover unknown variations in response not covered by the research supporting the treatment.

The measure of efficacy for eggs and larvae of Lepidoptera is suggested as prevention of the emergence of normal-looking adults, i.e., adults that do not have deformed wings and would not be readily distinguished from untreated individuals. Only data concerning late instar larvae need be considered because they are more radiotolerant than eggs and early instars (Hallman et al., 2010). Therefore, studies that report a series of radiation doses applied to a number of late-instar Lepidoptera with at least one dose resulting in no emergence of normal-looking adults are relevant to the purposes of this analysis. There are many studies that fail these criteria for various reasons; we do not discuss those studies here. Two anonymous reviewers suggested a number of references, and all except one did not satisfy these basic criteria. Suffice it to say that we have striven to find all available studies, published or not, that would illuminate a generic dose for Lepidoptera eggs and larvae through our files, contacts, web searches, and the International Database on Insect Disinfestation and Sterilization (Bakri et al., 2005).

A generic dose should be higher than the maximum specific dose found for any insect it covers. In practice doses reported in some research may be higher than seems reasonable, and if good reason exists for suspecting a dose (such as contradiction by other studies) it may be justifiable to not use those results in determining the generic dose. This strategy was used in developing a generic dose for tephritid fruit flies of 150 Gy when some studies suggested that the dose could be as high as 1 kGy (Hallman and Loaharanu, 2002; Hallman, 2012).

Hallman et al. (2010) note that some proposals were rejected by the IPPC because the research was done entirely with insects reared on diet without comparing efficacy with insects reared on a plant host, but concluded that, "Host does not seem to affect efficacy for the measures of efficacy used for PI treatments". Studies with four species of Lepidoptera directly compared insects reared on diet and a plant host and found no difference in radiotolerance between them (Mansour, 2003; Hallman, 2004; Hallman and Hellmich, 2009; Hallman, unpublished data).

3. Results and discussion

Table 2 presents all of the studies that could be located that give a dose that prevents emergence of normal-looking adults when last instars are irradiated (33 species from 11 families). In all but two studies with two different species 250 Gy or less prevented emergence of normal-looking adults. Two studies indicate that 250 Gy is insufficient to prevent adult emergence of *Plodia interpunctella* (Hübner) but they are contradicted by six studies which find that 100–200 Gy is adequate for this species (Table 2). Although *P. interpunctella* (Indianmeal moth) is not a quarantine pest and does not attack fresh commodities it can be considered as representative for Lepidoptera from the standpoint of a generic dose. A study with *Opogona sacchari* (Bojer) found that 300 Gy was necessary to prevent adult emergence, but that study is contradicted by another that found that 240 Gy is adequate (Table 2). Additionally in both studies few adults survived lower doses and the distinction between normal and abnormal adults was not

Table 2
Radiation doses absorbed by late-instar Lepidoptera to prevent emergence of normal-looking adults.

Species	Family	Dose (Gy)	No. of insects treated at that dose	Reference
Diacrisia obliqua	Arctiidae	100	50	Ahmed et al. (1978)
Carposina sasakii	Carposinidae	208	7421	Zhan (unpublished)
Neoleucinodes elegantalis	Crambidae	225	100	Pereira et al. (1997)
N. elegantalis	Crambidae	200	50	Arthur (2004)
N. elegantalis	Crambidae	200	100	Costa et al. (2009)
Stenoma catenifer	Elachistidae	200	50	Silva et al. (2007)
Phthorimaea operculella	Gelechiidae	120	150	Al-Taweel et al. (2007)
P. operculella	Gelechiidae	125	450	Saour and Makee (2004)
Sitotroga cerealella	Gelechiidae	175	150	Cogburn et al. (1966)
S. cerealella	Gelechiidae	200	42	Qureshi and Wilbur (1966)
S. cerealella	Gelechiidae	200	100	Arthur (1985)
Tuta absoluta	Gelechiidae	200	50	Arthur (2004)
Conopomorpha sinensis	Gracillaridae	250	1878	Hu et al. (1999)
Lymantria dispar	Lymantriidae	100	30	Godwin et al. (1964)
Agrotis ipsilon	Noctuidae	80	19	Elnagar et al. (1985)
Helicoverpa zea	Noctuidae	175	2197	Hallman (unpublished)
Heliothis virescens	Noctuidae	100	50	El Sayed and Graves (1969)
H. virescens	Noctuidae	175	14,366	Hallman (unpublished)
Spodoptera exigua	Noctuidae	150	177	Wit and van de Vrie (1986)
Spodoptera frugiperda	Noctuidae	94	568	Horak (unpublished)
Spodoptera litura	Noctuidae	100	97	Dohino et al. (1996)
Cadra cautella	Pyralidae	200	90	Cogburn et al. (1973)
Corcyra cephalonica	Pyralidae	200	190	Huque (1971)
C. cephalonica	Pyralidae	200	50	Duarte Aguilar and Arthur (1993)
Diataea saccharilis	Pyralidae	75	108	Darmawi et al. (1998)
Eoreuma loftini	Pyralidae	150	59	Darmawi et al. (1998)
Ephestia kuehniella	Pyralidae	200	120	Ayvaz and Tunçbilek (2006)
Plodia interpunctella	Pyralidae	132	150	Cogburn et al. (1966)
P. interpunctella	Pyralidae	175	60	Beczner and Farkas (1974)
P. interpunctella	Pyralidae	100	100	Brower (1980)
P. interpunctella	Pyralidae	150	50	Arthur (1988)
P. interpunctella	Pyralidae	200	50	Tamborlin (1988)
P. interpunctella	Pyralidae	125	50	Barbieri et al. (1994)
P. interpunctella	Pyralidae	350	90	Ayvaz et al. (2008)
P. interpunctella	Pyralidae	300	80	Abbas et al. (2011)
Tryporyza incertulas	Pyralidae	150	248	TRGEP [Teaching and Research Group of Entomology and Pesticides] (1974)
Opogona sacchari	Tineidae	240	100	Potenza et al. (2000)
O. sacchari	Tineidae	300	200	Potenza et al. (2006)
Clepsis spectrana	Tortricidae	200	73	Wit and van de Vrie (1986)
Cryptophlebia illepida	Tortricidae	289	11,910	Follett and Lower (2000)
Cydia pomonella	Tortricidae	100	332	Burditt and Moffitt (1985)
C. pomonella	Tortricidae	156	237	Burditt (1986)
C. pomonella	Tortricidae	153	4230	Burditt and Hungate (1989)
C. pomonella	Tortricidae	200	> 133,953	Mansour (2003)
Ecdytolopha aurantiana	Tortricidae	200	50	Faria et al. (1998)
E. aurantiana	Tortricidae	200*	50	Arthur (2004)
Epichoristodes acerbella	Tortricidae	150	42	Bestagno et al. (1973)
Epiphyas postvittana	Tortricidae	200	56	Batchelor et al. (1984)
E. postvittana	Tortricidae	199	600	Dentener et al. (1990)
E. postvittana	Tortricidae	150	38,202	Follett and Snook (2012)
Eucosma notanthes	Tortricidae	150	60	Lin et al. (2003)
Grapholita molesta	Tortricidae	232	58,779	Hallman (2004)
Lobesia botrana	Tortricidae	200	250	Mansour (unpublished)
Thaumatotibia leucotreta	Tortricidae	163	6000	Hofmeyr (unpublished)

* In the publication the data between Tables 2 and 3 were switched.

made; often when only few adult Lepidoptera survive irradiation as late instar they are abnormal (Hallman, unpublished studies with noctuids and pyralids). Hollingsworth and Follett (2007) determined that tolerance of the immature stages of *O. sacchari* increased as they developed and that 150 Gy is sufficient to prevent successful reproduction of late pupae based on prevention of F1 egg hatch from eggs laid by adults emerging from 4031 late pupae irradiated with 150 Gy. Although that study did not determine a dose to prevent adult emergence from irradiated late larvae it does show that even for an insect that seems marginal for a 250 Gy dose applied to late larvae (Table 2), the margin of security in the 250 Gy dose is sufficient so that reproduction from late pupae (more tolerant than larvae) is stopped at the F1 egg stage when these pupae are irradiated with 150 Gy.

Research with *Cryptophlebia illepida* (Butler) reports a dose of 250 Gy to prevent adult emergence from irradiated late instars (Follett and Lower, 2000). For the first five of 11 replicates at the target dose of 250 Gy the actual absorbed dose ranged from 150 Gy to 289 Gy and no adults emerged. Normally the maximum dose recorded in large-scale confirmatory testing becomes the minimum dose for commercial application; hence, 289 Gy would be recommended for *C. illepida*. But two factors allow room for arguing that a lower dose would prevent adult emergence with a high degree of security. One, because of the broad but skewed dose distribution in the irradiation chamber, many of the insects were irradiated at doses well under 250 Gy with no adults emerging which indicates that a dose <250 Gy might suffice. Two, the next lowest dose targeted in the study was 125 Gy; assuming a dose uniformity ratio of 1.9 (as was observed at the target dose of 250 Gy) the actual dose absorbed by the larvae when 125 Gy was targeted probably spanned a similar broad, but skewed, dose distribution that ranged from approximately 75 to 145 Gy at which adult emergence was 1.4%. Therefore, at a target dose of 125 Gy many insects were irradiated with well under that dose. It is quite possible that a dose intermediate to the doses targeted (125 and 250 Gy) would achieve efficacy. Also in that study another species, *Cryptophlebia ombrodelta* (Lower), was found to be more radiosusceptible than *C. illepida*; therefore, the generic dose of 250 Gy would suffice for that species, making a total of 34 that have been studied.

The number of species studied (34) to support a generic dose of 250 Gy is a fraction of potential quarantine pests of the Lepidoptera but it includes many key families and species and contains only two outliers, which are contradicted by other studies with the same species. Contrast that with the generic dose of 150 Gy accepted for tephritid fruit flies (IPPC (International Plant Protection Convention), 2011a) which was supported by studies with 14 species with several of the studies indicating that 150 Gy was too low (Hallman and Loaharanu, 2002; Hallman, 2012). The data for Lepidoptera are more harmonious in supporting a dose of 250 Gy. The data for Tephritidae included a number of large-scale confirmatory tests; however, some of these large-scale, as well as small-scale, tests did not support a dose of 150 Gy. For example, Seo et al. (1973) found two adults emerged from an estimated total of 110,800 *Ceratitis capitata* larvae in papaya irradiated with a minimum absorbed dose of 225 Gy. In three other tests the authors did with a total of 280,624 *Bactrocera dorsalis* larvae in irradiated papaya, 21 adults emerged. A study discussed by Hallman (1999) found that 840 Gy resulted in four adult *C. capitata* emerging when an unknown but modest amount of larvae in apples were irradiated. The data for Lepidoptera do not show this large degree of variation in reported doses, and what they may lack in depth compared with Tephritidae (regardless of the large variation reported in that family) they exceed in number of total studies conducted and number of species studied.

There are uncertainties that may increase or decrease the dose for each study. In most studies dosimetry is not reported in detail and only target doses are given. Sometimes target doses may be in the low part of the absorbed dose range (Hallman, 2004) and sometimes they may be high (Follett and Lower, 2000); therefore, it is not possible to decide in general what impact lack of dosimetry reporting has on the efficacy of reported doses. Sufficient information should be provided to adequately characterize studies so that the absorbed doses may be reported properly and are suitable to establish a quantitative relationship between absorbed dose and the relevant effect (ISO/ASTM (International Organization for Standardization/American Society for Testing and Materials), 2002). An ideal experimental design would result in samples irradiated uniformly with each spatial element within the sample receiving an identical dose, but in practice a variation in dose will be found through an irradiated sample. Experimental procedures should minimize this variation insofar as is possible and ensure that all samples are presented for irradiation in a similar fashion to further minimize inter-sample variation.

Another source of uncertainty is the measurement of efficacy: prevention of normal-looking adults. In most cases researchers did not make the distinction between normal or not and simply report adult emergence. This makes those treatments more conservative, thus increasing the probability that the generic dose of 250 Gy is adequate.

A generic dose of 250 Gy for eggs and larvae of Lepidoptera is conservative; it is higher than needed for almost all of the species studied. Also, when it is applied commercially, the radiation processing procedure involves establishing that the minimum dose imparted to the consignment in the irradiation chamber (process load) is at least 250 Gy. This is standard practice, as the commodities being irradiated will receive a distribution of dose due in part to natural variation in the commodity (e.g., density) and also due to the design of the irradiation facility, which will have mechanical and operational limitations that give rise to a variation in dose. The correct application of commercial irradiation technology involves first establishing the typical dose distribution within the process load and then applying that treatment in routine operation and continually monitoring the process using established dosimetry methods to ensure the minimum dose (250 Gy) received is sufficient to result in the desired outcome while at the same time the maximum dose is less than that which can be tolerated by the commodity. Essentially all insects present will receive more than 250 Gy because the lowest dose that commercial applicators target will marginally exceed 250 Gy to ensure that no area of the load receives less than 250 Gy. Furthermore the dose uniformity ratio (DUR) (maximum dose/minimum dose) for commercial applications is greater than 1.15 meaning that most insects in a process load will absorb closer to 300 Gy or often more, depending on the DUR of the irradiation system. In this way, therefore, a generic PI dose of 250 Gy could be efficacious against all eggs and larvae of Lepidoptera while providing a liberal margin of security.

The major weakness in support of a generic dose of 250 Gy for all Lepidoptera is lack of large-scale tests for species other than tortricids (Table 2). The largest non-tortricid test comprises 14,366 larvae for a noctuid (*Heliothis virescens*) and is not yet published. Therefore, if a generic dose for Lepidoptera is not yet sufficiently supported, one for Tortricidae may be.

4. Tortricidae

The most important of the lepidopteran families of quarantine significance is the Tortricidae. Follett and Snook (2012) suggest that a generic PI dose for the family Tortricidae "might be 250 Gy" based on studies with nine species (*Conopomorpha sinensis* Bradley is included but is not a tortricid). Other discrepancies between that and our study are pointed out for clarity in support of a dose of 250 Gy for Tortricidae:

1. Follett and Snook (2012) cite Wit and van de Vrie (1985) as having treated 150 *Clepsis spectrana* larvae at 200 Gy when the latter tested only 80 larvae at that dose. We cite Wit and van de Vrie (1986) because their 1985 study combined the last three instars while the 1986 study separated instars.
2. Although a dose of 250 Gy would suffice for *C. ombrodelta* because it was shown to be less radiotolerant than *C. illepida* we do not consider it accurate to include it in the table showing that 11,256 larvae were irradiated at 250 Gy because that species was not tested at 250 Gy, only shown to be more radiosusceptible than *C. illepida* at 62.5 Gy.
3. Although the study of Lester and Barrington (1997) does not contradict a generic dose of 250 Gy for Tortricidae no data are provided in that paper for the number of 5th instar *Ctenopseustis obliquana* (Walker) irradiated with no adult emergence.
4. In Table 2 we show higher numbers of larvae treated for *C. illepida*, *Cydia pomonella*, and *Epiphyas postvittana* because we include all last instars treated at the dose given, not only those done in the final large-scale confirmatory testing.
5. Arthur (2004) treated 50 not 100 larvae of *Ecdytolopha aurantiana*.
6. The dose and number treated for *Thaumatotibia leucotreta* are greater in Table 2 than those given by Follett and Snook (2012) because we probably have more recent information from Hofmeyr (unpublished).

Table 3
Phytosanitary irradiation doses approved for Tortricidae (APHIS (Animal and Plant Health Inspection Service), 2013; IPPC (International Plant Protection Convention), 2011b).

Species	Doses (Gy) approved by two plant protection organizations	
	IPPC	APHIS
Cydia pomonella	200	200
Cryptophlebia illepida	–	250
Cryptophlebia ombrodelta	–	250
Grapholita molesta	232	200

Twelve of the 34 species for which data are presented in our study are tortricids (11 in Table 1 plus *Cryptophlebia ombrodelta*). More significantly, Tortricidae includes four of the five studies confirmed with more than 10,000 individuals irradiated, with one study confirmed by more than 130,000 individuals, and there are no contradicting studies. Furthermore PI doses are already approved for some tortricids (Table 3). As was discussed above, it is possible that the doses for the two *Cryptophlebia* spp. could be less than 250 Gy.

Other available studies do not contradict a dose of 250 Gy for Tortricidae eggs and larvae although they fall short of satisfying all of the criteria for inclusion used by the current analysis. Adult emergence of a combination of 4th and 5th instar *Amorbia emigratella* Busck irradiated at 150 Gy resulted in 3.2% adult emergence; no higher dose was attempted (Follett, 2008). A dose of 117 (95% confidence interval 106–128) Gy was estimated by probit analysis to prevent 99% adult emergence of irradiated 5th instar *Ctenopseustis obliquana*; raw data are not presented (Lester and Barrington, 1997). Two percent of late instar *Epichoristodes acerbella* (Walker) emerged as adults, but did not lay eggs, after irradiation with 150 Gy; no higher dose was attempted (Köllner, 1977). Including these four species makes 15 tortricid species that do not contradict a dose of 250 Gy as preventing emergence of normal-looking adults when applied to late instars.

5. Conclusions

A generic dose of 250 Gy is suggested for eggs and larvae of the family Tortricidae because several large-scale confirmatory studies have been done in that family. A generic dose of 250 Gy for eggs and larvae of Lepidoptera is supported by results from 34 species in 11 families. The measure of efficacy of the treatment is prevention of the emergence of normal-looking adults, which are assumed not able to fly or reproduce. This paper is part of a larger process of analyzing available studies with the objective of lowering for some pest groups the generic dose of 400 Gy for all insects (except pupae and adults of Lepidoptera) that is accepted by the USDA-Animal and Plant Health Inspection Service but not by the IPPC (International Plant Protection Convention) (2011b) and for establishing these doses in the IPPC. As the literature in this area grows it is likely that the 400 Gy generic dose could be lowered for many insect groups (Hallman, 2012). A lower dose will reduce cost and duration of treatment and lower the risk of adversely affecting commodity quality.

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