Ionizing Irradiation of Adults of Angoumois Grain Moth (Lepidoptera: Gelechiidae) and Indianmeal Moth (Lepidoptera: Pyralidae) to Prevent Reproduction, and Implications for a Generic Irradiation Treatment for Insects

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ABSTRACT Ionizing irradiation is used as a phytosanitary treatment against quarantine pests. A generic treatment of 400 Gy has been approved for commodities entering the United States against all insects except pupae and adults of Lepidoptera because some literature citations indicate that a few insects, namely, the Angoumois grain moth, Sitotroga cerealella (Olivier) (Lepidoptera: Gelechidae), and the Indianmeal moth, Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae), are not completely controlled at that dose. Radiotolerance in insects increases as the insects develop, so the minimum absorbed dose to prevent F_1 egg hatch for these two species when irradiated as adults was examined. Also, because hypoxia is known to increase radiotolerance in insects, Angoumois grain moth radiotolerance was tested in a hypoxic atmosphere. A dose range of 336–388 Gy prevented F_1 egg hatch from a total of 22,083 adult Indianmeal moths. Dose ranges of 443–505 and 590–674 Gy, respectively, prevented F_1 egg hatch from a total of 15,264 and 13,677 adult Angoumois grain moths irradiated in ambient and hypoxic atmospheres. A generic dose of 600 Gy for all insects in ambient atmospheres might be efficacious, although many fresh commodities may not tolerate it when applied on a commercial scale.

KEY WORDS Sitotroga cerealella, Plodia interpunctella, quarantine, commodity treatment, radiation

Ionizing irradiation has many uses in entomology, including sterilizing insects for population management and eradication programs, as a phytosanitary treatment, and for general disinfestation of commodities (Bakri et al. 2005). The International Consultative Group on Food Irradiation (ICGFI) suggested that 0.3 kGy be used as a generic, default phytosanitary dose of ionizing radiation for all insects and mites (ICGFI 1991). In 1994, this issue was taken up at a workshop, and a review of the literature showed that 0.3 kGy would probably be insufficient, as some species and life stages of insects and phytophagous mites are reported to reproduce to some extent after an exposure to that dose (Hallman 1994). Questions about methodology and radiation dosimetry in some of the research lead to a call for closer examination of the literature, research methodology, and doses required to achieve quarantine security for a variety of quarantine pests.

A generic, default dose of 0.4 kGy was recently approved as a phytosanitary treatment for all insects other than pupae and adults of Lepidoptera for com-

modities entering the United States (APHIS 2006). Pupae and adults of Lepidoptera were excluded from that recommendation because available information suggested that doses to prevent reproduction of some species when irradiated at these stages might be >0.4 kGy.

Data for the most radiotolerant insects set the minimum limit for a generic dose for the entire class. A review of the literature revealed that the Angoumois grain moth, Sitotroga cerealella (Olivier) (Lepidoptera: Gelechiidae), and the Indianmeal moth, Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae), seemed to be the most radiotolerant arthropods known, with doses to prevent reproduction estimated at >1 kGv (Hallman 1998). A few female adult Indianmeal moths reportedly reproduced after irradiation with 1 kGy (Cogburn et al. 1966). The only reference to dosimetry reported by Cogburn et al. (1966) was "all dosages [except one noted to be \pm 10%] were accurate within a very small tolerance." Brower (1975) found that reproduction in Indianmeal moth was stopped with 450 Gy but not 400 Gy when a total of 20 irradiated 1-d-old adult female were paired with nonirradiated males. Although absorbed doses were not given, doses were "confirmed" with a lithium fluoride dosimetry

A dose of 250 Gy did not completely prevent pairs of irradiated Angoumois grain moths from reproduc-

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ing (Qureshi et al. 1968). When Angoumois grain moth adult pairs were irradiated at 220 Gy, but not 200 Gy, hatching of eggs laid posttreatment was stopped (Ismail et al. 1987). Both studies did not report dosimetry.

When only the female Angoumois grain moth was irradiated at 240 Gy there was a 1.75% eclosion rate versus 94% for the nonirradiated control; no higher dose was tried by Ismail et al. (1987). Johnson and Vail (1987) found that \approx 300 Gy prevented F_1 egg hatch when female Indianmeal moths were irradiated as pupae (day 9 after infestation); dosimetry is reported.

A brief report in a proceedings found that 1% of eggs laid by Indianmeal moth adults irradiated with a target absorbed dose of 400 Gy hatched (Hallman 2004). Subsequent observations questioned the validity of that preliminary conclusion, suggesting that untreated control insects were contaminating petri dishes holding irradiated eggs. After controls and irradiated insects were kept in separate rooms and petri dishes taped shut, no more first instars were found after 6,238 additional adults were irradiated with a target dose of 400 Gy (absorbed dose range was 395–448 Gy; G.J.H., unpublished data).

In the same proceedings, Zolfagharieh (2004) briefly reported that 350 Gy sterilized 200 adult Indianmeal moths. Sterilization was not defined, nor was dosimetry measurement or absorbed dose range given. Also in the same proceedings, Ignatowicz (2004) found that a target dose of 600 Gy applied to a mixture of an estimated 57,013 Angoumois grain moth of all stages, including adults prevented development of F_1 adults. At a target dose of 500 Gy applied to 1–2-d-old adults, 7.7% of F_1 eggs hatched. A target dose of 400 Gy applied to a mixture of an estimated 57,013 Angoumois grain moth of all stages, including adults, resulted in 0.77% reaching the F_1 adult stage. The dosimetry system used and dose range measurements were not given.

A phytosanitary treatment must be effective against the most tolerant life stage of quarantine pests that could normally be present in the shipped commodity, regardless of whether that stage is less commonly found than others. Insects increase in radiotolerance as they develop, when using a common measure of efficacy (Hallman 2000). Therefore, actively reproducing adults would require the highest dose to prevent successful reproduction.

Prevention of insect reproduction can be defined in various ways that could be subdivided by life stage and age within life stage as long as each subdivision could be defined. Development of phytosanitary treatments should include a precise description of the response that achieves efficacy. It is insufficient to state that a phytosanitary treatment prevents reproduction; prevention of reproduction should be defined as precisely as practical. Regulatory agencies must know when the threshold has been crossed and the treatment may be considered unsuccessful.

An insect can be quickly killed with radiation, although acute mortality requires doses so high (>3 kGy) that they are not tolerated by most fresh com-

modities. Doses to achieve acute mortality are much higher than needed to prevent the establishment of exotic invasive species (Hallman 1998). Prevention of the development of reproductive organs would be an early step to preventing reproduction when acute mortality is not the goal. This would only suffice for those species not present as actively reproducing adults; most quarantined insect species could be present as adults. Prevention of the production of gametes is a further step that could define treatment efficacy. That step could be further refined by restricting it to the prevention of egg production, which is usually accomplished with a lower dose than sperm production in insects (Hallman 1998). Further steps could be prevention of oviposition, then oviposition but no emergence of first instar, or prevention of successive F₁ life stages. Some studies base efficacy on prevention of the F₂ generation. For example, Follett (2006) based efficacy of an irradiation treatment of coconut scale, Aspidiotus destructor Signoret, on prevention of F₁ adults with F₂ eggs when parent generation adults with eggs and newly hatched crawlers were irradiated. Prevention of the F₂ generation might be especially relevant to Lepidoptera, which may suffer greater sterility in subsequent generations than in the one irradiated (Bloem et al. 2003, Wee et al. 2005).

When the same life stage is irradiated, prevention of each successive stage is usually accomplished by a lower irradiation dose than needed to prevent the previous life stage because more total developmental change must be achieved. However, each successive step in lowering the dose by accepting more development is probably accompanied by increased risk of treatment failure, because as development progresses the number of developmental stages until reproduction is successful logically decreases.

A major disadvantage with phytosanitary irradiation treatments compared with other quarantine treatments is that because irradiation is the only commercially applied treatment that does not result in significant acute mortality, phytosanitary inspectors have no independent verification of treatment efficacy. Because of this, any deficiency in the research or application of irradiation may lead to a greater risk of establishment of invasive species compared with other treatments for which discovery of live insects posttreatment denotes treatment failure. Research and application of phytosanitary irradiation treatments should be held to higher standards than other treatments. It may be prudent, therefore, to leave a few developmental steps between the phytosanitary treatment effect and successful reproduction so that unexpected variations in efficacy will have a lower risk of leading to successful reproduction and establishment of invasive species.

The objective of this research was to determine minimum absorbed doses required to prevent F_1 egg hatch when the two moths are irradiated. Because the Angoumois grain moth seems to be more radiotolerant than the Indianmeal moth, egg hatch of the former was tested under hypoxic conditions also. Hypoxia is used to prolong shelf life of stored commodities and may

reduce the efficacy of phytosanitary irradiation (Hallman and Hellmich 2008).

Materials and Methods

Source of Insects. The Indianmeal moth colony was started with insects collected in Stillwater, OK, and maintained for $10~\rm yr$ ($\approx 120~\rm generations$) at Oklahoma State University on a diet of cornmeal, chick starter mash, egg crumbles, and glycerol (4:2:2:1 by volume) in glass jars in a growth chamber at 28°C, $60~\rm -70\%$ RH, and a photoperiod of 16:8 (L:D) h. Pupae were collected from the laboratory colony by placing rolls of 1-cm corrugated cardboard strips in the culture jars with wandering stage fifth instars for 3–5 d. Larvae would seek refuge and pupate within the corrugations of the rolled cardboard.

Angoumois grain moths originated from a colony (>10 yr; >120 generations) of unknown origin provided by the Biological Research Unit of the USDA-ARS Grain Marketing and Production Research Center, Manhattan, KS, and maintained at Oklahoma State University for 3 yr during the time of these studies. Angoumois grain moths were reared on whole kernels of hard red winter wheat, *Tritichum aestivum* L., in glass jars maintained at 28°C, 60–70% RH, and a photoperiod of 16:8 (L:D) h. Newly emerged adults were from colony jars that had been set up 21 d or more before use.

Irradiation Source and Dosimetry. The radiation source used was ¹³⁷Cs (Husman model 521A, Isomedix, Inc., Whippany, NJ) located at the USDA-APHIS Mexican Fruit Fly Rearing Facility at Mission, TX. It delivered a gamma ray dose rate of ≈40 Gy min⁻¹. Reference standard dosimetry was done in 1996 with the Fricke system (ASTM 2008). Routine dosimetry was done with radiochromic film (Gafchromic MD-55, ISP Technologies, Inc., Wayne, NJ) read with a spectrophotometer (Milton Roy Spectronic 401, Ivyland, PA) at 580 nm.

Insect Irradiation. Adults (0-4 d old) were collected from liter glass jars by placing a plastic cylinder (27 cm in length, 4 cm i.d.) over an opening in the jar lid and gently tapping to entice adults to fly up into the cylinder. Dosimeters were placed in the inside center wall and both ends of the cylinder, areas with the most extreme dose readings. The cylinders were placed along the center line of the irradiation chamber to minimize the dose uniformity ratio. Control insects in the cylinders went to the irradiation facility but were not passed through the irradiator.

A batch of several hundred to a few thousand Indianmeal moth adults were initially irradiated each week with a target absorbed dose of 200 Gy, and the dose was raised each time reproduction occurred until a dose was achieved that prevented reproduction after $\approx 20,000$ adults were tested.

Angoumois grain moth adults were tested under both ambient and hypoxic atmospheres. The initial target absorbed dose for this insect was 300 Gy, and several hundred to a few thousand adults were irradiated weekly until ≈15,000 were irradiated in each atmosphere at a dose that prevented reproduction. Each time a dose failed, it was raised. Hypoxia was achieved by placing the 4-cm-i.d. cylinder inside a larger cylinder (polyvinyl chloride, 37.5 cm inside length, 10 cm i.d.) fitted on one end with a screw cap sealed with vacuum grease. On either end was one brass barbed nipple compression hose fitting (25 mm in length, 4 mm i.d.). The atmosphere inside the cylinders was purged with nitrogen at a pressure of ≈ 3 kPa for 2 min \approx 3.5 h before irradiation. After purging, the fittings were sealed with rubber septa and held at ≈ 24 °C until irradiation. ≈ 1.5 h after irradiation the insects were removed from the cylinders. Angoumois grain moth adults irradiated under ambient atmospheres also were placed in the larger cylinders; however, they were not flushed nor were the fittings sealed with rubber septa. The oxygen level inside the cylinders was measured by running the atmosphere in the cylinder through a meter (CheckMate II, PBI Dansensor, Ringsted, Denmark) via a loop of tubing connected to both fittings.

After irradiation insects were placed in transparent plastic cylinders (8.5 cm length, 4 cm i.d.) fitted with plastic screen on one end. Every 2–3 d until the adults died, eggs were gently shaken out of the cylinder through the screen end (≈1.3 mesh per mm) and placed on black filter paper (catalog no. 4740C10, Thomas Scientific, Swedesboro, NJ) in plastic petri dishes (catalog no. 4488C01, Thomas Scientific) that were taped shut to observe hatch. Irradiated and control insects were kept in nonadjoining rooms to prevent accidental contamination from control individuals into irradiated containers. Workers examined controls and irradiated lots on separate days, and they did not remove them from their respective rooms until the insects were dead. Accidental contamination could be responsible for some of the variation in responses to irradiation observed in the literature (Hallman and Loaharanu 2002).

Resulting data were subjected to probit analysis (PROC PROBIT, SAS Institute 2007).

Results and Discussion

Indianmeal moth. Small numbers of eggs of Indianmeal moth hatched when adults were irradiated with 200–300 Gy (Table 1). Analysis of numbers of eggs failing to hatch did not fit the probit model by using the normal or Gompertz probability density functions, with or without \log_{10} of dose. Chi-square (χ^2) values were 31.7-43.5 (df = 2), and the probabilities of the data fitting any of the four models were near zero. Eggs that hatched were not randomly distributed among treated cohorts of adults. For example, of 21 cohorts of a mean of 702 adults irradiated at 300 Gy, a total of 96 eggs hatched. Fourteen cohorts had no eggs hatching, whereas four cohorts had 15–32 eggs hatching. In the other three cohorts, one, one, and three eggs hatched. This may indicate that fertile eggs from irradiated adults may occur in a negative binomial distribution. Indianmeal moth eggs are laid singly or in clusters. Perhaps when one egg from a cluster is

Table 1. Failure of hatch of eggs laid by irradiated adult Indianneal and Angoumois grain moths at various doses for probit analysis

Dose (Gy)			Angoumois grain moth ^a in					
	Indianmea	ll moth	Ambier atmosph		Hypoxic atmosphere			
	Egg hatch failure (%)	No. eggs	Egg hatch failure (%)	No. eggs	Egg hatch failure (%)	No. eggs		
200	99.26	9,923						
250	99.80	100,179						
300	99.93	145,907	99.25	2,803				
320	100	16,675						
350			99.84	3,155				
400			99.91	6,608	98.03	4,718		
450			100	3,834	99.73	2,213		
500					99.93	2,851		
600					100	5,000		

[&]quot;Angoumois grain moths were irradiated in ambient and hypoxic atmospheres.

fertile the likelihood increases that other eggs in that cluster are fertile. We did not test this hypothesis nor could we find an answer in the literature. Although Brower (1975) held irradiated Indianmeal moths in sexual pairs, which could have addressed the question of independence of hatch among eggs, data for each dose were combined before being reported.

Data for egg hatching versus irradiation of adults were transformed by dividing numbers of eggs at each dose by an assumed number of eggs per cluster, assuming that although egg hatching was not a random factor, cluster hatching might be. Cluster size was set as the mean, rounded to the nearest whole number, of the eggs hatching in the four cohorts with 15–32 eggs hatching, which was a mean of 23. Raw data for total eggs and eggs hatching were divided by 23 and rounded to the nearest whole number. When these data were subjected to probit analysis they fit all four models (Table 2). Probability of fit and effective doses (EDs) to prevent egg hatch at the 99 percentile were very close among all models. Models that did not use log₁₀ of dose had lower ED₉₀ values and wider confidence limits than models that did use \log_{10} of dose.

In subsequent large-scale testing at a target dose of 350 Gy (routine dosimetry readings were 336–388 Gy), no eggs from a total of 22,083 adults hatched. Egg hatch in controls was generally >90%.

Angoumois Grain Moth. F_1 egg hatch from irradiated Angoumois grain moth adults versus dose is presented in Table 1. Analysis of numbers of eggs failing to hatch fit the probit model using the normal or

Gompertz probability density functions with or without \log_{10} conversion of dose (Table 3). Unlike Indianmeal moth, egg hatch of Angoumois grain moth did not seem to be clumped in distribution, but more random. For example, in the six replicates done at 400 Gy the number of eggs hatched was zero, zero, one, one, one, and three.

In subsequent large-scale testing, no eggs laid from totals of 15,264 and 13,677 adults irradiated with 450 Gy (routine dosimetry readings were 443–505) in ambient atmosphere and 600 Gy (routine dosimetry readings were 590–674) in hypoxic atmosphere, respectively, hatched. The oxygen level in the hypoxic tubes was always <0.5%.

Generic Phytosanitary Dose for Insects. The research reported herein indicates that both the Indianmeal moth and the Angoumois grain moth are less radiotolerant than suggested by previous research (Cogburn et al. 1966) and subsequent reviews (Brower and Tilton 1985, Hallman 1998). The Angoumois grain moth is the most radiotolerant insect that has been tested, with a dose to prevent hatch of eggs, when actively reproducing adults are irradiated, being ≈500 Gy in this study (when the upper dosimetry readings are considered) and ≈ 600 Gy in Ignatowicz (2004). The latter study does not report dosimetry, so it is not known what the dose range was. When dosimetry is not reported, the centerline dose estimate is often used. This estimate often tends to be near the lower end of the absorbed dose range. It could be, therefore, assumed that the doses presented by Ignatowicz (2004) were actually somewhat lower than the maximum doses absorbed by the irradiated insects, although there is no way of knowing the absorbed dose range in that study without data on dosimetry.

Results of four different studies with Angoumois grain moth show very different results (Table 4). The four studies did not use the same methodology nor report all of the details, such as dosimetry, making direct comparisons tenuous. For example, sample size was not given in Ismail et al. (1987), was 10 moths in Qureshi et al. (1968) and Ignatowicz (2004) and varied from 100 to 2300 moths in the current study. It may seem intuitive that when levels of reproductive sterility are high, having larger sample sizes will increase the probability that fertile insects of both sexes will be in the same group and, thus, could mate. However, this does not seem to be responsible for differences found in various studies with this insect as Ignatowicz (2004) found high levels of reproduction at higher doses than

 ${\bf Table\ 2.} \quad {\bf Results}^a \ {\bf of\ probit\ analysis\ of\ nonhatching\ of\ eggs\ of\ irradiated\ Indian meal\ moth\ adults$

Probability density function	Log ₁₀ of dose	χ^2	Prob. $> \chi^2$	Slope ± SE	ED ₉₀ (Gy) (95% CL)	ED ₉₉ (Gy) (95% CL)
Gompertz	Yes	1.60	0.66	2.78 ± 0.60	110 (58-143)	195 (154–216)
Gompertz	No	1.19	0.75	0.0046 ± 0.0010	37 (0-108)	187 (123-215)
Normal	Yes	1.84	0.60	5.46 ± 1.15	126 (76–157)	197 (160–217)
Normal	No	1.36	0.71	0.0092 ± 0.0020	78 (0–134)	191 (134–216)

[&]quot;Effective dose (ED) is the dose that prevents egg hatch. Degrees of freedom is 2. Data for total eggs and eggs hatching were divided by 23 (assumed mean number of eggs in a cluster) and rounded to the nearest whole numver.

Table 3. Results" of probit analysis of nonhatching of eggs of Angoumois moth adults irradiated in ambient or hypoxic atmospheres

Atmosphere	Probability density function	Log ₁₀ of dose	χ^2	Prob. $> \chi^2$	Slope ± SE	ED ₉₀ (Gy) (95% CL)	ED ₉₉ (Gy) (95% CL)
Ambient	Gompertz	Yes	2.08	0.35	3.24 ± 0.55	175 (125–207)	286 (259–303)
Ambient	Gompertz	No	2.22	0.33	0.0040 ± 0.00069	108 (0–169)	282 (247-302)
Ambient	Normal	Yes	2.02	0.36	3.32 ± 1.08	197 (150-225)	288 (263-303)
Ambient	Normal	No	2.13	0.34	0.0078 ± 0.0014	151 (53-200)	285 (253-303)
Hypoxic	Gompertz	Yes	0.98	0.61	6.98 ± 0.92	335 (311-350)	421 (415-429)
Hypoxic	Gompertz	No	1.47	0.48	0.0069 ± 0.00093	322 (289-341)	422 (416-431)
Hypoxic	Normal	Yes	0.57	0.75	12.83 ± 0.77	348 (327-360)	419 (413-427)
Hypoxic	Normal	No	0.88	0.64	0.013 ± 0.0018	338 (310–354)	421 (414–429)

^a Effective dose (ED) is the dose that prevents egg hatch. Degrees of freedom is 2.

the other three studies by using small numbers of insects per test.

Ignatowicz (2004) used a different measure of efficacy (prevention of F_1 adult emergence) than we did (prevention of F_1 egg hatch). It is reasonable to hypothesize that if Ignatowicz (2004) had used prevention of egg hatch as the measure of efficacy in the large-scale testing, the dose to prevent reproduction of Angoumois grain moth using his methodology and dosimetry would be >600 Gy.

Also, we may speculate on the reasons for the discrepancy between the high radiation doses reported by Cogburn et al. (1966) to prevent grain moth reproduction and other studies. Preliminary research with Indianmeal moth as discussed above indicates that stored grain moths are adept at infesting seemingly protected stored products, which may result in them contaminating insufficiently protected experimental units. A few misplaced individuals may result in higher doses than needed in research that is designed to develop phytosanitary methods. Another reason for discrepancies is insufficient methodology given from earlier research, especially concerning dosimetry. Actual absorbed doses may be significantly different from what was reported. Both of these problems have apparently happened with phytosanitary research on tephritids (Hallman and Loaharanu 2002).

Last, we cannot discount the possibility that there might be differences in radiotolerance among different populations. For example, Hallman (2003) found that the northern strain of plum curculio, *Conotrachelus nenuphar* (Herbst), was more radiosusceptible than the southern strain.

Table 4. F_1 egg hatch (%) when both sexes of Angoumois grain moth adults irradiated and held together

Dose (Gy)	Qureshi et al. (1968)	Ismail et al. (1987)	Ignatowicz (2004)	Current study
0	96	93.0	92.3	91.6
100	56	82.0	91.3	
200	13	$1.5, 2.6^a$	87.3	
220		0		
250	5			
300			60.3	0.75
400			29.7	0.09
500			7.7	0

^a Results of two different tests.

Although the Indianmeal moth may be the most radiotolerant insect identified in the literature other lepidopterans may approach the same level of radiotolerance. Suckling et al. (2006) irradiated 15 painted apple moth, *Teia anartoides* Walker, female pupae with 400 Gy during the last third of their pupal development, with 0.07% of F_1 eggs hatching after mating the resulting adults with nonirradiated males. Because insects generally tend to increase in radiotolerance as they develop (Hallman 2000), perhaps T. anartoides irradiated as an adult female would be more radiotolerant than the pupa.

Regarding a generic phytosanitary irradiation dose for all insects, a very conservative objective for the most tolerant stage (adult) of the most tolerant individuals known from the most tolerant group of insects (Lepidoptera) seems to be accomplished with a dose of ≈500–600 Gy when low oxygen storage is not used. If the objective were to allow egg hatch but prevent a subsequent stage of development or reproduction. possibly somewhat lower doses could be achieved. However, we feel that a generic dose that will be broadly applied to a large group of organisms, such as the Insecta, when only a small fraction of that group has been tested and that has no independent means of verification, such as dead insects soon after treatment, should be conservative and leave a margin of security between the measure of efficacy sought and the most risky measure of efficacy possible. At this point that generic dose for all Insecta seems to be ≈600 Gy, although when applied on a commercial scale (resulting in absorbed doses of at least 1.5 times 600 Gy) many fresh commodities may not tolerate it. This dose may not be effective for commodities in low-oxygen storage at the time of treatment.

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