

Ionizing Radiation as a Phytosanitary Treatment Against European Corn Borer (*Lepidoptera: Crambidae*) in Ambient, Low Oxygen, and Cold Conditions

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ABSTRACT The European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), is a quarantine pest for several fresh commodities, including corn-on-the-cob, bell peppers, and green beans. Methyl bromide fumigation is the usual phytosanitary treatment, but the fumigant is under increasing regulation as a stratospheric ozone-depleting substance. Ionizing radiation is a relatively new commercial alternative that is currently used in several countries. The present research explored radiation doses that would provide quarantine security for commodities at risk of being infested by *O. nubilalis*. Radiotolerance of late pupae (the most tolerant stage infesting commodities) as determined by hatch of F₁ eggs was not affected by host (meridic diet versus ear corn) or temperature (1 versus 13°C) but was positively affected by low oxygen. Longevity was shorter for adults of irradiated than nonirradiated pupae. The minimum absorbed dose for phytosanitary irradiation against *O. nubilalis* could vary from 233 Gy for prevention of F₁ pupation to 343 Gy for prevention of F₁ egg hatch. Lower doses might be possible if greater risk of treatment failure was acceptable.

KEY WORDS *Ostrinia nubilalis*, quarantine, commodity treatment, irradiation, disinfestations

The European corn borer, *Ostrinia nubilalis* (Hübner), (Lepidoptera: Crambidae), infests many crops grown in temperate North America east of the Rocky Mountains and from the Mediterranean basin north to Sweden and east to Kazakhstan. Countries and regions that do not have *O. nubilalis* have placed quarantines against agricultural products from infested areas that might harbor it. For example, corn, broom corn, sorghum, sudangrass, green beans, bell peppers, and several flowers cannot be shipped to California from infested areas unless they have been certified as free of *O. nubilalis* or treated to eliminate or kill all individuals that might be present. Treatment is usually done with methyl bromide fumigation, which is under regulation as a stratospheric ozone-depleting substance under the Montreal Protocol of Substances that Deplete the Ozone Layer (UNEP 2006). Research is ongoing to develop alternatives to methyl bromide.

Ionizing radiation phytosanitary treatment shows promise for use on a variety of commodities and is used commercially in the United States, Australia, India, and Thailand to disinfest several fresh commodities (Heather and Hallman 2008). Irradiation could be an alternative to methyl bromide for fumigating plant materials that might contain *O. nubilalis*.

A phytosanitary treatment must be effective against the most tolerant stage of the pest that could normally be present in the shipped commodity. The radiotolerance of insects increases as they develop (Hallman 2001). Because all immature stages of *O. nubilalis* can be present on host commodities, the most radiotolerant stage likely to be present is the most developed stage, the late pupa.

Results from irradiation of insects for sterile insect technique (SIT) research aimed at pest population suppression may offer guidance to studies on phytosanitary irradiation, and there are several studies on SIT of *O. nubilalis*. Most of these use mating nonirradiated to irradiated insects. Raun et al. (1967) studied the effect of irradiation on larvae, which is not the most tolerant stage for phytosanitary purposes. Walker and Brindley (1963) and Zhang and Lou (1980) studied adults, which are not expected to occur on shipped produce; thus, they are not of concern for phytosanitary treatments. Nabors and Pless (1981) found that 150 Gy applied to late pupae did not diminish male fertility. Roçsea and Bărbulescu (1989) demonstrated that pupal tolerance to radiation increases with pupal age, as measured by percentage adult emergence, and that male *O. nubilalis*, reproductively, are more radiotolerant than females. When nonirradiated males were mated to irradiated females 1.25% of eggs hatched at 250 Gy, whereas none hatched at 300 Gy, indicating that a phytosanitary irradiation treatment may lie within this range.

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Although mating nonirradiated to irradiated insects is a vital part of SIT research it is not a logical part of phytosanitary irradiation research, thus, cannot usually be directly applied to phytosanitary ends. Insects emerging from a phytosanitary treatment system will be expected to have all been treated. Because only irradiated males and females will be present, the dose required for phytosanitary security is reduced compared with radiation doses required for SIT. Another factor that lowers the efficacious dose for phytosanitary irradiation compared with SIT is that only females need be prevented from reproducing, and female reproduction often is more susceptible to radiation than males (Hallman 1998). However, a phytosanitary treatment demands a high level of confidence (>99.9%), whereas an SIT treatment may allow for some fertility as long as the large numbers of reproductively sterile insects released out compete any fertile insects present, whether feral or released.

Levels of radiation required for sterility of Lepidoptera in the F_1 generation may be lower than those required for sterility of the parent generation (Carpenter et al. 2005). This characteristic allows researchers to produce sterility using lower doses of radiation, resulting in less somatic damage and more competitive insects. However, this strategy is not being used in present SIT programs because sterile F_1 larvae may cause economic damage to crops. F_1 sterility also has been explored as an end point for phytosanitary irradiation in homopterans. Follett (2006a,b) used prevention of F_1 females with eggs as the treatment end point after irradiation of parent generation female diaspidid scales.

Major phytosanitary treatments based on heat, cold, and fumigation apparently have failed because live insects have been found by inspectors after treatment (Heather and Hallman 2008). Although the reason for failure may not be definitely known, subsequent research sometimes finds that the treatment is not as efficacious as was originally thought. With these (nonirradiation) phytosanitary treatments, finding no live insects upon inspection (i.e., acute mortality) is the primary means to independently verify treatment efficacy. The measure of efficacy of phytosanitary irradiation, however, is rarely acute mortality, but most commonly prevention of further development or reproduction. There is no easy way to independently verify irradiation efficacy if the treatment is not as efficacious as was originally considered. Consequently, irradiation treatment research and application should be conducted with a relatively higher level of confidence compared with other phytosanitary treatments, and it would be prudent to leave a margin of security around aspects of irradiation treatments in both research and application. For example, it may be advisable to choose a measure of efficacy that falls short of allowing the F_1 generation to develop to the adult stage.

Low oxygen, which is used to prolong shelf life of stored commodities, has been found to reduce efficacy of phytosanitary irradiation (Hallman 2004a, b, 2005). Also, cold (above freezing) has been hypothesized to reduce the efficacy of irradiation (Hallman 2001),

although the only thorough study of the subject found no difference in efficacy between apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), third instars irradiated in apples held at 1 or 24°C (Hallman 2004b).

The objective of this research was to determine absorbed radiation doses required for phytosanitary treatment of *O. nubilalis* hosts under ambient, low oxygen and low temperature conditions.

Materials and Methods

Source of European Corn Borer. *O. nubilalis* pupae in corrugated cardboard pupal rings (Guthrie 1987) were obtained from a colony at Ames, IA, and shipped overnight to a quarantine containment facility in Weslaco, TX, for treatment and observation. The colony was established with ≈ 500 each females and males collected in light traps in Polk and Story counties in Iowa in July of each year (2003–2007). This research was conducted with individuals from generations 2–9 arising from those colonies. Voucher specimens were deposited in the insectary of the ARS Crop Quality and Fruit Insects Research Unit, Building 200 at Weslaco, TX.

The pupal stage of *O. nubilalis* lasts ≈ 9 d at 24°C. Pupae within 3 d of adult emergence were used in all of the experiments. This was accomplished in the case of diet-reared pupae by irradiating cardboard rings containing the pupae after ≈ 10 –15% had emerged as adults (these adults were discarded), allowing additional adults to emerge for 3 d and then discarding the rings.

Radiation Source and Dosimetry. The radiation source was ^{137}Cs (Husman model 521A, Isomedix, Inc., Whippany, NJ) located at the USDA–APHIS Mexican Fruit Fly Rearing Facility at Mission, TX. The unit delivered a gamma ray dose rate of ≈ 40 Gy $\cdot\text{min}^{-1}$. Reference standard dosimetry was done in 1996 with the Fricke system. Routine dosimetry was done with radiochromic film (Gafchromic MD-55, ISP Technologies, Inc., Wayne, NJ) placed inside the center and outside edges of the tubes with *O. nubilalis* pupae, which were the areas with the most extreme dose readings. Dosimeters were read with a spectrophotometer (Milton Roy Spectronic 401, Milton Roy, Ivyland, PA) at 600 nm.

Radiotolerance of Borers Reared on Corn versus Diet. Phytosanitary treatments must be effective against quarantine pests reared on natural host material. However, it is often easier and more economical to work with organisms reared on a laboratory diet. This first experiment tested the radiotolerance of *O. nubilalis* reared on corn ears versus meridic diet (Guthrie 1987). The moths were reared from the first instar to pupation on diet and sweet corn ears obtained from a local grocery store. Pupae within 3 d of emergence were irradiated with 50 Gy and held ($24 \pm 1^\circ\text{C}$, RH $70 \pm 8\%$) in cube-shaped screen cages (0.3 m) for adult emergence; 50 Gy was selected as a discriminating dose because it was known from preliminary research that egg hatch would be greatly reduced, but not eliminated, using this dose level. Emerging adults from each food source were provided sucrose in water (1: 2.5 ratio by weight) gelled with 10 g

of agar per kilogram of diet and water in cotton wicks as food sources and placed together with strips of waxed paper for oviposition. Percentage of eclosion of eggs laid (between 2,000 and 4,000 eggs per experimental unit) was recorded (three replicates of ≈ 55 adults per food source) and subjected to *t*-test.

Effect of Low Oxygen and Cold on Radiotolerance. Pupae reared on diet that were within 3 d of emergence were placed in plastic cylinders (polyvinyl chloride, 37.5 cm inside length, 10 cm i.d.) fitted on one end with a screw cap sealed with vacuum grease. On each end was a brass, barbed-nipple compression hose fitting (2.5 cm in length, 0.4 cm i.d.). Sections of the cardboard rings (0.2 m in length) containing pupae were placed in the tubes and kept under the following conditions: ambient atmosphere and 13°C, ambient atmosphere and 1°C, low oxygen and 13°C, and low oxygen and 1°C. The threshold for development for *O. nubilalis* is $\approx 11^\circ\text{C}$, and the most it will accumulate in 20 h at 13°C is a few day-degrees of the 210 needed to complete the pupal stage, resulting in insects of very near the same developmental stage at both temperatures treated (Mason et al. 1996). Low oxygen was achieved by flushing the cylinders with nitrogen gas at a pressure of ≈ 3 kPa for 2 min at 20, 16, and 2 h before irradiation at 50 Gy. After flushing the hose, fittings were sealed with rubber septa. Two hours after irradiation the cylinders were opened and the pieces of pupal ring were placed in cube-shaped screen cages (0.3 m) for adult emergence. Any adults that emerged before the cylinders were opened were discarded, as were any that emerged after 3 d. Oxygen content in the cylinders was measured 1 h before irradiation by inserting the needle of a meter (CheckMate II, PBI Dansensor, Ringsted, Denmark) through a septum covering one of the fittings.

Percentage eclosion of eggs laid was recorded (four replicates) and tested for normality (Kolmogorov-Smirnov test; Prism 4, GraphPad Software Inc., San Diego, CA) before a two-way (atmosphere and temperature) analysis of variance (ANOVA).

Effect of Irradiation of Late Pupae on Adult Emergence. Prevention of adult emergence was not considered as a measure of efficacy because it was known early in the research that late pupae would largely emerge as adults after irradiation with a few hundred gray. However, it was necessary to know how many might be prevented from emerging so that the number of pupae treated could be estimated. The pupation rings contained pupae of differing ages as pupation dates of larvae varied considerably. To estimate the proportion of late pupae that might not emerge at the highest dose used in this research, one cardboard ring each of pupae was irradiated at 300 Gy in both ambient and low oxygen atmospheres, as described above. One other ring was not irradiated. After 3 d the rings were placed at $<0^\circ\text{C}$ for several days to kill any remaining insects, and the rings were taken apart to determine the number of pupae from which adults did not emerge. The data were converted to percentage of adults from the rings that emerged as adults and analyzed with one-way ANOVA with six replicates. If the irradiation prevented adult emergence the percentage

Table 1. Analysis of variance of effect of temperature and oxygen level on radiotolerance of *O. nubilalis* as measured in percentage of eclosion of F_1 eggs when late pupae were irradiated with 50 Gy

Source of variation	F value	df	Probability
Interaction	0.014	1, 6	0.91
Temperature	0.011	1, 6	0.92
Oxygen level	12.1	1, 6	0.013

Number of eggs was between 2,000 and 4,000 for each experimental unit.

of adults from the irradiated rings would be less than from the control rings.

Determination of Doses to Prevent F_1 Egg Hatch, Larval Development and Pupation. Pupae reared on diet within 3 d of emergence were irradiated as described above with doses beginning at 100 Gy and raised until the objective was met for a large number ($>9,000$ for each objective) pupae. First instars produced from treated adults were placed on diet to determine how far they would develop. Probit analysis was used to estimate doses required for 90 and 99% prevention of egg hatch (PROC PROBIT, SAS 9.1, SAS Institute, Cary, NC).

Effect of Irradiation on Adult Survival. Cohorts of adults (mean = 108.2; range, 20–319) that had emerged from late pupae irradiated at a target dose of 300 Gy in ambient or low oxygen atmospheres or not irradiated were held at $24 \pm 1^\circ\text{C}$, $70 \pm 8\%$ RH, and a photoperiod of 14:10 (L:D) h and fed as described above. The number of dead adults was counted twice per week, and the test was replicated six times.

Results

Dose Distribution. Dosimeters recorded dose distributions from 2% below the target dose in the center of the cylinder to 15% above the target dose on the outside of the treatment cylinder.

Radiotolerance of *O. nubilalis* Reared on Corn versus Diet. *t*-test showed no significant difference in percentage eclosion of eggs laid by emerging adults between corn ($11.6 \pm 2.9\%$) and diet-reared ($11.0 \pm 2.4\%$) *O. nubilalis* late pupae irradiated with 50 Gy ($t = 0.17$, $df = 4$, $P = 0.42$). Therefore, the remainder of the research was conducted with diet-reared *O. nubilalis*.

Effect of Low Oxygen and Cold on Radiotolerance. The oxygen level in the tubes purged with nitrogen was always <0.5 kPa. Eclosion of *O. nubilalis* eggs did not deviate significantly from normality (Kolmogorov-Smirnov test; KS distance = 0.19 and 0.15 for irradiation under low oxygen and ambient conditions, respectively). There was no significant interaction between temperature and oxygen level regarding eclosion when late pupae were irradiated with 50 Gy (Table 1). Temperature (1 versus 13°C) during irradiation did not significantly affect eclosion. Low oxygen resulted in a significantly greater percentage of eclosion of eggs laid by adults irradiated as late pupae. The mean \pm SEM eclosion of F_1 eggs of pupae irradiated with 50 Gy in low oxygen and

Table 2. Probit analysis of F₁ egg hatch when diet-reared *O. nubilalis* are irradiated from 0 to 3 d before adult emergence

Probability density function	Log ₁₀ of dose	Slope ± SE	ED ₉₉ (95% CL)	Probability > χ^2
Gompertz	Yes	7.45 ± 1.13	202 (200–204)	0.96
Gompertz	No	0.014 ± 0.0022	202 (200–205)	0.99
Normal	Yes	14.9 ± 2.57	202 (200–204)	0.90
Normal	No	0.029 ± 0.0051	202 (200–204)	0.95

df = 2.

ambient atmospheres was 31.7 ± 5.8 and $11.7 \pm 2.8\%$, respectively.

Effect of Irradiation of Late Pupae on Adult Emergence. There was no difference in adult emergence rates among irradiated (300 Gy, in ambient or low oxygen atmospheres) and nonirradiated pupae within 3 d of emergence, which means that 300 Gy did not significantly reduce adult emergence ($F = 0.044$; $df = 2, 10$; $P = 0.96$). The means ± SEM for the control, 300 Gy in ambient atmosphere, and 300 Gy in low oxygen were 30.3 ± 5.6 , 32.1 ± 5.5 , and 29.9 ± 4.9 , respectively. Therefore, the number of adults emerged from irradiated pupae reliably represents the number of pupae treated.

Determination of Dose to Prevent F₁ Egg Hatch and Pupation. Percentage F₁ egg hatch when late pupae were irradiated with 100, 200, 250, and 300 Gy was 8.1, 1.17, 0.011, and 0%, respectively. Number of eggs was ≈10,000, 14,000, 9,500, and 30,000, respectively, at 100, 200, 250, and 300 Gy. Egg hatch in the control was 97.2%. The data closely fit the probit model regardless of whether the Gompertz or normal probability density functions with or without log 10 of dose were used (Table 2). In total, 34,760 adults emerged from pupae irradiated 0–3 d before emergence with a target dose of 300 Gy (maximum absorbed dose of 343 Gy), and no F₁ eggs hatched from an estimated total of >3 million eggs laid. Samples of ≈5,000 eggs were closely observed for larval development inside the eggs, and none was found. The sex ratio of adults was 54:46 (female:male).

At 250 Gy, F₁ larvae did not develop past the first instar before dying. In total, 9,468 late pupae were irradiated at a target dose of 250 Gy (dose range maximum absorbed dose = 289 Gy). At 200 Gy, some F₁ *O. nubilalis* developed to the fifth instar but did not pupate and eventually shriveled and died. In total, 14,297 late pupae were irradiated at a target dose of 200 Gy (maximum absorbed dose, 233 Gy).

In total, 13,488 adults emerged from pupae irradiated in <0.5 kPa oxygen 0–3 d before emergence with a target dose of 300 Gy (maximum absorbed dose of 341 Gy) and one F₁ egg hatched from an estimated total of >1 million eggs laid. In 8.0% of nonhatched eggs, some larval development was observed, identifiable by the presence of a head capsule, before the insects died.

Effect of Irradiation on Adult Survival. Figure 1 shows survival of adult *O. nubilalis* when irradiated as pupae within 3 d of adult emergence. Survival of adults in the two treatments (300 Gy at ambient and low

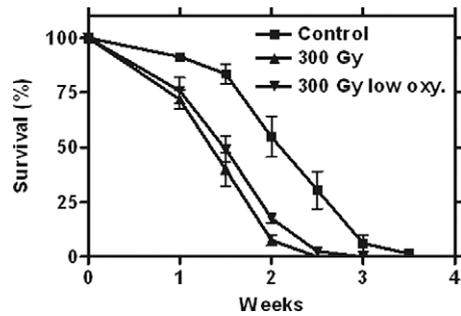


Fig. 1. Survival of adult *O. nubilalis* after late pupae were irradiated with 300 Gy in ambient or low oxygen atmospheres. Controls were not irradiated.

oxygen atmospheres) was significantly lower than the control throughout the experiment. Those irradiated at 300 Gy in ambient and low oxygen atmospheres were all dead by 2.5 and 3 wk, respectively. Controls were all dead by 4 wk.

Discussion

Fundamental to phytosanitary treatment efficacy research is that the experimental conditions do not render the test organism easier to control compared with the feral state. One of these conditions may be diet versus natural hosts. As with *O. nubilalis* in the current study, Hallman (2004a) found no significant difference between a natural diet (whole apple) and a mericid diet for response of irradiated oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae). However, some organisms may be more radiosusceptible when treated in diets than fruits. For example, the dose required to prevent emergence of Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae), adults from irradiated third instars was 60 Gy when in grapefruit, *Citrus paradisi* Macf., and 16 Gy when in diet (Hallman and Worley 1999, Hallman and Martinez 2001). The susceptibility of an organism to a treatment when reared on diet should always be compared with that organism reared on a natural host before a mericid diet is used to develop any phytosanitary treatment.

As has been shown before with other insects (Hallman 2004a,b, 2005), irradiation under low oxygen conditions may reduce efficacy of phytosanitary irradiation against *O. nubilalis*. Hence, irradiation should not be used for commodities stored in low oxygen atmospheres until it can be shown that either the modified atmosphere does not reduce efficacy or a modification of the treatment is developed to compensate for any reduction. For example, an irradiation phytosanitary treatment against plum curculio, *Conotrachelus nenuphar* (Herbst), (Coleoptera: Curculionidae), under low oxygen atmospheres might be made efficacious by doubling the dose found to be effective in ambient atmosphere (Hallman 2005). However, low oxygen storage by itself is toxic to insects and has been studied as a phytosanitary treatment (Heather and Hallman 2008). Any toxic effect of low oxygen storage to quarantine pests of commodities des-

ted to be irradiated may be developed as part of a combination treatment. The research to prove this combination effect, however, could be complicated and result in unwieldy conditions for its application.

Cool storage temperature did not reduce radiosusceptibility in the current study. However, some additional studies may be needed before it can be concluded that cool temperatures will not reduce radiosusceptibility for quarantine pests in general. Cold storage alone is also a phytosanitary treatment (Heather and Hallman 2008).

Depending on the measure of efficacy used, the minimum absorbed dose for phytosanitary irradiation against *O. nubilalis* could vary from 233 Gy (the maximum dose measured when the target was 200 Gy) for prevention of F₁ pupation to 343 Gy (the maximum dose measured when the target was 300 Gy) for prevention of F₁ egg hatch. Possibly the dose could be <233 Gy if greater development was allowed, such as F₁ pupation or adult emergence, as long as the F₁ generation did not successfully reproduce. However, the closer the measure of efficacy approaches successful reproduction of irradiated insects the greater the risk that the treatment may fail. Given that there is no independent verification of phytosanitary irradiation efficacy as there is for all other commercially used phytosanitary treatments (dead insects), failure would probably not be detected and an exotic pest infestation could result. Therefore, irradiation phytosanitary treatments should probably be more conservative than may be possible with other treatments.

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