Ionizing Irradiation Quarantine Treatment Against Oriental Fruit Moth (Lepidoptera: Tortricidae) in Ambient and Hypoxic Atmospheres

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ABSTRACT Oriental fruit moth, *Grapholita molesta* (Busck), is a pest of many rosaceous temperate fruits, including pomes, *Malus* spp., and stone fruits, *Prunus* spp., in much of the world. However, some areas are free of the pest, and shipments of fruit hosts from infested to noninfested areas may be regulated. Current quarantine treatments for oriental fruit moth include methyl bromide fumigation and cold storage for several weeks. Methyl bromide use is being restricted because it is a stratospheric ozone-depleting substance, and alternatives are sought. Cold is not tolerated by many hosts of oriental fruit moth. The objective of this research was to develop irradiation quarantine treatments against the pest under ambient and hypoxic storage conditions because some hosts of oriental fruit moth are stored in hypoxic atmospheres, and hypoxia is known to lessen the effects of irradiation. In ambient atmospheres, no adults emerged from 58,779 fifth instars (the most radiotolerant stage present in fruit) irradiated with a target dose of 200 Gy (195–232 Gy measured). In atmospheres flushed with nitrogen, 5.3% of adults emerged from 44,050 fifth instars irradiated with a target dose of 200 Gy (194–230 Gy measured), but they died at a faster rate than control adults and without laying eggs. A dose of 232 Gy (the maximum recorded when 200 Gy was targeted) is recommended to disinfest any fruit of oriental fruit moth under ambient and hypoxic atmospheres.

KEY WORDS *Grapholita molesta*, phytosanitary treatment, controlled atmosphere, commodity treatment

ORIENTAL FRUIT MOTH, *Grapholita molesta* (Busck), originated in northwestern China and was introduced into the United States from Japan ≈90 yr ago. It has become a serious pest of stone fruits, *Prunus* spp., and to a lesser extent pomes, *Malus*, *Pyrus*, *Cydonia*, and infests other species of the Rosaceae, such as *Rosa* spp. Although oriental fruit moth has become distributed in much of the temperate world, some areas, such as the Scandinavian and low countries of Europe (Belgium, The Netherlands, and Luxembourg) and parts of temperate Brazil, remain free of it. If host fruit from infested areas is to be shipped to a noninfested area where the oriental fruit moth could become established, the fruit must undergo a phytosanitary procedure that will ensure that no viable insects accompany it. Often phytosanitary inspection is sufficient for a pest such as oriental fruit moth that leaves external feeding holes. Inspection leaves a certain risk that an infestation will not be detected, and when that risk is considered unacceptable, a disinfestation treatment may be required. Methyl bromide fumigation is the principal oriental fruit moth disinfestation technique. However, continued use of methyl bromide worldwide is uncertain because the fumigant is considered a significant stratospheric ozone-depleting substance. Although quarantine uses of methyl bromide have been exempted from phasing out, there is no certainty that the fumigant will not be lost as a quarantine treatment or at least become more scarce and expensive in the future. The price of methyl bromide has quadrupled in recent years. Alternative quarantine treatments are needed. Cold storage for 40 d at 0°C is acceptable for apples, but stone fruits will not tolerate it.

Ionizing radiation is a viable quarantine treatment (Hallman 2001). Commodities are exposed to gamma rays from the radioactive isotopes cesium 137 or cobalt 60, electron beams, or X rays converted from an electron beam generator. The mode of action of ionizing irradiation as a quarantine treatment involves breaking chemical bonds. When this happens to DNA, normal development or reproduction of the organism may be prevented. There is no residual activity, and irradiation does not make food harmful to human health (WHO 1994). Currently, several fruits that are hosts of tephritids (Diptera) and sweetpotatoes, *Ipomea batatas* (L.) Lam, at risk of carrying three pests are irradiated in Hawaii and shipped to the rest of the United States. Guavas, *Psidium guajava* L., and sweetpotatoes are irradiated for quarantine control of the Caribbean fruit fly, *Anastrepha suspensa* (Loew), and the sweetpotato weevil, *Cylas formicarius elegantulus*

(Summers), respectively, in Florida for shipment to Texas and California. The United States has accepted irradiation quarantine treatments against 11 fruit fly (Diptera: Tephritidae) species and mango seed weevil, *Cryptorhynchus mangiferae* (F.), and has proposed it for green scale, *Coccus viridus* (Green) (APHIS 2002a,b). Efficacy, host tolerance, consumer acceptance, and economic returns with irradiation have been favorable. The expanded use of irradiation as an effective quarantine treatment with no health or environmental detriments looks positive.

A quarantine treatment must be virtually 100% effective. Irradiation is unique among quarantine treatments in that the objective is not acute mortality but prevention of development or reproduction. Achieving 100% acute mortality requires doses of irradiation not tolerated by fresh commodities. Hosts of oriental fruit moth tolerate ≥400 Gy (Drake and Neven 1997, 1998; Drake et al. 1999).

Hosts of the oriental fruit moth, such as apples, are often stored under hypoxic conditions to prolong shelf life. Hypoxia is known to abate the effects of radiation on organisms because less oxidative radicals are produced (von Sonntag 1987). However well known is this effect, its significance for irradiation quarantine treatments has been scarcely examined. Hallman and Worley (1999) found that Mexican fruit fly, *Anastrepha ludens* (Loew), third instars irradiated in a nitrogen atmosphere were twice as radiotolerant as those irradiated in air.

The objective of this research was to establish irradiation quarantine treatment doses for oriental fruit moth in both ambient and hypoxic atmospheric storage because some hosts of the pests are stored in hypoxic atmospheres.

Materials and Methods

Oriental Fruit Moth. Eggs were obtained from a colony maintained at the USDA–ARS facility in Parlier, CA, and reared on diet (product no. F9649B, Bio-Serv, Frenchtown, NJ) in 210-ml plastic cups. The colony was based on field-collected stock from Fresno County, California. All work was done with fifth instars, the most advanced stage found on shipped fruit, because sufficient evidence demonstrates that, when the same response is measured, radiotolerance increases with development (Hallman 2001).

Irradiation System. Radiation was applied with a ^{137}Cs source (Husman model 521A, Isomedix, Inc., Whippany, NJ) that had a centerline gamma ray dose rate of ≈40 Gy·min^{-1}. Reference standard dosimetry was done in 1996 by using the Fricke system. Routine dosimetry was done with radiochromic film (Gafchromic MD-55, ISP Technologies, Inc., Wayne, NJ) and read with a spectrophotometer at 510 nm (Spectronic 401, Milton Roy, Ivyland, PA). Routine dosimeters were used periodically in the center and the outside center of the load. These locations were shown by reference standard dosimetry to receive the minimum and maximum, respectively, absorbed doses.

Diet Effect on Radiotolerance. Although a quarantine treatment ideally would be devised using feral insects naturally infesting fruit, that approach is rarely taken because of the difficulty in getting sufficient insects of the proper stage in good condition. Often, quarantine treatments are established using a laboratory colony infesting fruit in a cage. Because of the difficulty in rearing large numbers of oriental fruit moth on fruit, it would be easier to rear the insects on diet to the fifth instar and then irradiate them. The experimental technique should not produce insects that are more susceptible to irradiation than insects in a more natural condition, or the resulting treatment could be inadequate. The first experiment compared radiosusceptibility of apple-reared oriental fruit moth versus diet-reared moths. Oriental fruit moth eggs on paper strips were placed on thinning apples ('Red Delicious', mean diameter 3 cm) picked in Washington State in June or diet. After 2 wk at 27°C, the insects had developed to late fifth instars. Both groups in their respective hosts were irradiated with an absorbed dose of 100 Gy (five replicates of ≈500 insects per replicate), and adult emergence was noted. Data were analyzed with a two-tailed, paired *t*-test (Prism 4, GraphPad Software Inc., San Diego, CA).

Oxygen Level and Radiotolerance. A large-scale experiment was conducted to determine the doses required to prevent adult emergence from fifth instars of oriental fruit moth irradiated in ambient atmosphere and under low oxygen conditions. Fifth instars reared on diet were irradiated in ambient atmospheres and in atmospheres of mostly nitrogen. Cylinders (polyvinyl chloride, 37.5 cm inside length, 10 cm i.d.) fitted on one end with a screw cap sealed with vacuum grease and on the other end with two brass, barbed-nipple compression hose fittings (25 mm in length, 4 mm i.d.) were constructed. Approximately 100 larvae each in eight diet cups were placed inside the cylinder, and the atmosphere was purged through the hose fittings with nitrogen at a pressure of ≈3 kPa for 2 min at 20, 16, and 2 h before irradiation. After purging, the hose fittings were sealed with rubber septa and the cylinders held at ≈24°C. Approximately 1.5 h after irradiation, the cylinders were opened to return the larvae to ambient atmosphere. Checks on the effect of hypoxia alone for ≈22 h consisted of the same procedure without irradiation. Another control consisted of larvae in diet that made the trip to the irradiator in cylinders with ambient air that were not irradiated. To test the effect of irradiation alone, larvae in diet cups were irradiated in cylinders with ambient air. Absorbed target doses were 100, 150, 175, and 200 Gy for ambient atmospheres, with >50,000 fifth instars treated at 200 Gy. For hypoxic atmospheres, doses were 100, 200, 300, and 400, with >40,000 fifth instars treated at 400 Gy. The relationship between dose and prevention of adult emergence was analyzed with probit analysis (SAS Institute 1988).

Reproduction of adult survivors of fifth instars treated with 200 Gy while under hypoxic conditions was evaluated. Adults emerging from surviving larvae were placed together in cages with water containing 10% su-

Table 1. Response of oriental fruit moth fifth instars to ionizing irradiation in ambient and hypoxic atmospheres

Dose (Gy)		Oxygen level	*n*	Pupae (%)		Adults (%)		
Target	Range			Normal	Abnormal	Normal	Deformed	Partially emerged
0 (control)	—	Ambient	6,272	94.8	—	92.7	—	—
0 (control)	—	Hypoxic	4,465	90.9	0.58	82.9	3.8	0.22
100	99–114	Ambient	5,048	17.3	—	1.0	—	—
100	98–116	Hypoxic	11,750	80.0	—	68.5	—	—
150	147–170	Ambient	8,489	14.6	—	0.035	—	—
175	171–197	Ambient	18,306	2.3	0	0.006	0	0.016
200	195–232	Ambient	58,779	1.0	0	0	0	0
200	194–230	Hypoxic	44,050	41.2	5.8	5.3	17.3	1.3
300	291–343	Hypoxic	7,800	3.6	1.1	0.14	0	0.10
400	388–457	Hypoxic	13,675	4.3	0.66	0.015	0.029	0.015

Dashes indicate no data taken.

crose and waxed paper strips for oviposition, and their longevity and reproduction were compared with an untreated control for five replicates, comprising a total of 467 surviving adults of irradiated fifth instars.

Results

Host (thinning apples or diet) did not affect adult emergence after irradiation of fifth instars with 100 Gy ($t = 0.50$, df = 4, $P = 0.64$; mean percentage adult emergence ± SEM, 0.80 ± 0.65). Therefore, further research was conducted with larvae reared on diet.

Three adults developed from 8,489 fifth instars irradiated with 150 Gy in ambient atmosphere (Table 1). At 175 Gy, one adult developed from 18,306 fifth instars. Three adults partially emerged from pupal exuvia and were not counted as survivors. At 200 Gy, 58,779 fifth instars were irradiated with no adult emergence. Prevention of the emergence of normal-looking adults fit probit analysis by using the normal probability density function with the $\log_{10}$ of dose (n = 90,622; 6.52 + 0.65 [slope + SE]; ED (effective dose)$_{99}$ = 100.0 Gy; 95% CL, 96.1–103.5 Gy; $\chi^2 = 0.64$).

In hypoxic atmosphere, 44,050 fifth instars were irradiated with 200 Gy, and although 5.3% emerged as normal-looking adults, no eggs were laid and adult longevity was shortened considerably (Fig. 1). Almost 50% of adults of irradiated fifth instars died 1 d after emergence, versus ≈1% of those not irradiated. All adults of irradiated fifth instars were dead by 10 d after emergence, whereas the last of those not irradiated survived until 28 d and laid abundant eggs that hatched.

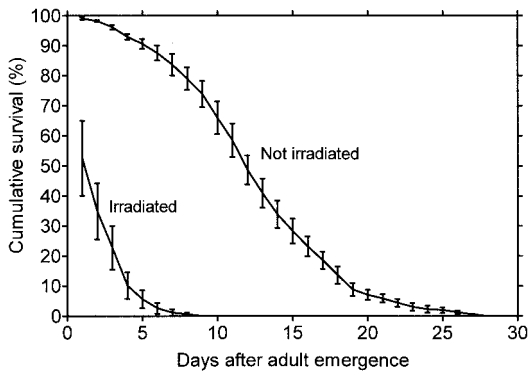

Fig. 1. Cumulative survival of oriental fruit moth adults irradiated as fifth instars with 200 Gy in a hypoxic atmosphere (n = 467) versus adults of fifth instars not irradiated (n = 868).

Under hypoxia, adult emergence was not totally prevented at 400 Gy (Table 1). Prevention of the emergence of normal-looking adults fit probit analysis by using the normal probability density function with the $\log_{10}$ of dose (n = 77,275, 7.00 + 0.051 [slope + SE], ED_{99} = 252.6 Gy; 95% CL, 250.4–254.8 Gy; $\chi^2 = 0.28$).

Discussion

Under ambient atmospheric conditions, a target, absorbed dose of 200 Gy applied to fifth instars of oriental fruit moth prevented adult emergence (Table 1). This dose achieved the same result with the related codling moth, *Cydia pomonella* (L.) (Mansour 2003). Because the maximum dose measured when 200 Gy was sought was 232 Gy, the latter should be the dose recommended for quarantine disinfestation purposes. A dose of 400 Gy did not prevent adult emergence under hypoxic conditions. Although 5.3% of fifth instars irradiated with 200 Gy under hypoxic conditions developed to adults, no eggs were laid, and the insects died earlier than the nonirradiated controls. This would satisfy the requirements of quarantine security. However, some quarantine protocols, such as those for tephritid fruit flies (APHIS 1996), demand that adult emergence be prevented to avoid triggering expensive delimiting trapping when adults, sterile or not, are found.

Because of the effect of a hypoxic atmosphere in increasing adult emergence of oriental fruit moth compared with ambient atmosphere in this case, phytosanitary protocols should prohibit irradiation disinfestation treatments of commodities stored under hypoxic conditions for any pest unless supporting research is successfully conducted.

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