Commodity Treatment and Quarantine Entomology

Potential Increase in Fruit Fly (Diptera: Tephritidae) Interceptions Using Ionizing Irradiation Phytosanitary Treatments

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ABSTRACT Irradiation postharvest phytosanitary treatments are used increasingly and show further promise because of advantages compared with other treatments. Its chief disadvantage is that, unlike all other commercially used treatments, it does not provide acute mortality, although it prevents insects from completing development or reproducing. The objective of this research was to determine to what extent irradiated egg and early instars of tephritids would develop to later instars that could be found by phytosanitary inspectors or consumers. Mexican fruit fly, *Anastrepha ludens* (Loew), eggs and first instars in grapefruit, *Citrus paradisi* Macfayden, were irradiated with 70–250 Gy and held at ≈27°C until third instars completed development. The accepted minimum absorbed phytosanitary dose for this pest is 70 Gy, although higher doses may be applied under commercial conditions. The more developed a fruit fly before it was irradiated, the greater the proportion that survived to the third instar. Also, dose was inversely related to developmental success, e.g., a mean of ≈65 and 35%, respectively, of late first instars reached the third instar when irradiated with 70 and 250 Gy. Of those, 65.1 and 23.4%, respectively, pupariated, although no adults emerged. Irradiation may result in a greater frequency of live (albeit incapable of resulting in an infestation) larvae being found than would be expected compared with other treatments that provide acute mortality. The regulatory community should be aware of this and the fact that it does not increase the risk of irradiation phytosanitary treatments resulting in an infestation of quarantine pests.

KEY WORDS *Anastrepha ludens*, quarantine, commodity treatment, radiation, disinfestation

The use of ionizing irradiation as a phytosanitary treatment is increasing because it possesses advantages over other treatments, such as applicability to packed commodities, broad tolerance by fresh fruit, and efficacy across a broad range of quarantine pests (Hallman 2007). A major disadvantage with phytosanitary irradiation, compared with other treatments, is that irradiation is the only commercially applied treatment that does not result in significant acute mortality, leaving phytosanitary inspectors with no independent verification of treatment efficacy. If inspectors find live quarantine pests for virtually every other treatment, the shipment is rejected or retreated regardless of certification of treatment. It is assumed that the treatment was not properly done or does not work as applied or that the shipment was contaminated with nontreated, infested commodity or reinfested after treatment. Live pests are expected after irradiation, and finding them does not preclude entry of the shipment as long as treatment certification is verified (FAO 2003).

The measure of efficacy of irradiation is prevention of further development or successful reproduction (FAO 2003). Specifically for tephritid fruit flies, the measure of efficacy is prevention of adults capable of flight when all stages that infest fruit (eggs and larvae) are irradiated. A review of the literature found that insects increase in radiotolerance as they mature when the same endpoint is measured (Hallman 2000).

Agricultural quarantine inspectors look for tephritid infestations by slicing fruit and examining it for larvae. They generally find only late instars and only a fraction of those present. Gould (1995) noted that Florida Department of Plant Industry inspectors found a mean of 23% of *Anastrepha suspensa* (Loew) late instars in several fruit species, and this was when they knew that they were being tested and expected to find larvae. Tephritid larval finds in fruit along the U.S.–Mexican border are essentially all third instars (D. Thomas, personal communication).

Fruit examination for quarantine pests can occur at any point in the marketing chain from before or after treatment, when the fruit is in a retail market or confiscated from transporters or consumers while crossing quarantine boundaries. Late in the marketing chain irradiated fruit may lose its identification as irradiated. Some fruit, such as citrus (*Citrus* spp.) and apples (*Malus* spp.), may be held at ambient temperatures that allow for fruit fly development for many days without spoiling. Eggs and early instars could develop to sizes readily noticed by phytosanitary inspectors.

Studies have determined doses to prevent different immature stages of fruit flies from reaching the adult

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stage (Hallman 1999), which is the goal of irradiation phytosanitary treatments against tephritids. However, how far irradiated eggs and early instars develop after irradiation has not been addressed. Although it has been abundantly noted in the literature that insects continue developing to a certain extent after irradiation, the effect of this observation on inspection efficacy has not been quantified (Hallman 1999, 2000; FAO 2003). Because irradiation does not provide acute mortality, it is conceivable that this treatment would allow some eggs and early instars to develop further, thus resulting in a higher probability of inspectors finding late instars in irradiated fruit. This would not increase the risk of rejection of irradiated fruit in properly certified marketing channels, but it might lead to regulatory action being taken when live larvae are found in fruit that has left these channels. Furthermore, the increased chance of finding live larvae may lead consumers to reject fruit.

The objective of this research was to determine the degree to which tephritid fruit flies irradiated as eggs and early instars develop to the point that they could be discovered by inspectors posttreatment.

Materials and Methods

Source of Insects. Lots of ≈80 'Rio Red' grapefruit, *Citrus paradisi* Macfayden, harvested near Weslaco, TX, were placed in each of two screen cages (1.2 by 0.8 by 0.5 m) with ≈20,000 Mexican fruit fly adults for ≈1 h. The flies were from the USDA–APHIS Mexican Fruit Fly Rearing Facility at Mission, TX, and originated with flies collected from mangoes in Morelos, Mexico (Moreno et al. 1991). The strain, continuously cultured since 1953, is used in the Mexican fruit fly sterile release programs in Texas and elsewhere. The flies are reared on a diet developed by Spishakoff and Hernandez-Davila (1968) except that wheat germ has been substituted for the dehydrated carrot powder.

Irradiation Source and Dosimetry. The radiation machine (Husman model 521A, Isomedix, Inc., Whippany, NJ) used ^{137}Cs in a sealed environment and is located at the USDA–APHIS Mexican Fruit Fly Rearing Facility at Mission, TX. It delivered a gamma ray dose rate of ≈40 $\text{Gy} \cdot \text{min}^{-1}$. 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 in areas of the load (center and edges) with the most extreme dose readings. Dosimeters were read with a spectrophotometer (Milton Roy Spectronic 401, Ivyland, PA) at 600 nm.

Insect Irradiation. Grapefruit were removed from the infestation cage, wiped clean of debris and remaining flies, and held in a growth chamber at ≈26.7°C, 75% RH until they were irradiated. Irradiation treatments occurred 0, 3, 7, and 10 d after infestation, at which time flies had achieved approximately early egg, late egg, middle-aged first instar, and late first instar, respectively. Larvae beyond 10 d at 27°C feeding on grapefruit, develop into the second instar and may be found by inspectors, although probably not as readily as third instars. An additional 10 d is required for the majority of Mexican fruit fly in grapefruit to reach the late third instar at 27°C.

Twenty grapefruit infested with each of the four Mexican fruit fly growth stages were irradiated at target doses of 70, 110, 150, 200, and 250 Gy. An additional 20 infested fruit were not irradiated but held as controls to observe Mexican fruit fly development in the absence of irradiation. The minimum absorbed dose accepted by the United States for irradiation of hosts of Mexican fruit fly is 70 Gy (APHIS 2006). Irradiation applied on a commercial scale results in considerably higher doses being applied to much of the load because of attenuation of the dose as the distance from the source increases and by the density of the product irradiated. Therefore, product nearer the source receives higher doses than product farther away. Attenuation is greatest for electron beam sources, followed by isotope sources, and less for X-ray sources. Molecular damage by all three sources is essentially identical and caused by electron shower knocking electrons out of orbit, with resulting changes in molecular structure, such as breaks in DNA. Under commercial conditions the maximum absorbed dose has been up to 3 times the minimum dose when whole pallet-loads were treated with ^{60}Co or as little as 1.4 times when treated in narrower arrangements with X-rays. This is not important if the commodity can tolerate it. Hallman and Martinez (2001) found that citrus fruit grown in southern Texas tolerated at least 500 Gy without significant damage.

Examination of Grapefruit Postirradiation. After irradiation, fruit were placed back in the growth chamber and held in individual plastic containers (2 liter) with screened lids and vermiculite in the bottom until third instars began to emerge from the fruit, ≈3.5 wk after infestation. Fruit were pulled apart into small pieces and examined for larvae. Three trained persons examined each group of fruit before proceeding to the next group. Therefore, it is assumed that any potential differences in capabilities of finding larvae or bias among examiners would occur relatively evenly in all treatments as well as the control. Immatures found were separated into the following categories: dead larvae, live second instars, live third instars, and puparia. The live larvae and puparia were placed in plastic containers (230 ml) with perforated lids and held for further development. Subsequent adult emergence was recorded. The tests were replicated three times.

Data were analyzed by linear regression and probit analysis (Prism 4, GraphPad Software Inc., San Diego, CA, and SAS Institute, Cary, NC).

Results and Discussion

The amount of Mexican fruit flies found ≈3.5 wk after infestation expressed as a percentage of the nonirradiated control, decreased as the dose increased when the same developmental stage was irradiated. At the same dose the amount of Mexican fruit flies found in grapefruit postirradiation increased as development progressed. No early eggs irradiated at ≥70 Gy were later observed as late instars. It is concluded that inspectors will not find evidence of infestation when

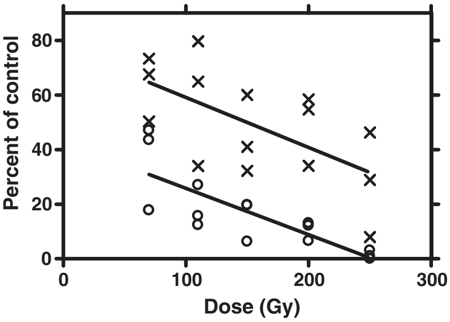

Fig. 1. Irradiated first instar Mexican fruit flies surviving long enough in grapefruit to grow to a size (late instar) found upon inspection. Data for middle-aged first instars are represented by circles and late first instars by Xs. Grapefruit were checked ≈3.5 wk after oviposition. Data expressed as percentage of nonirradiated control.

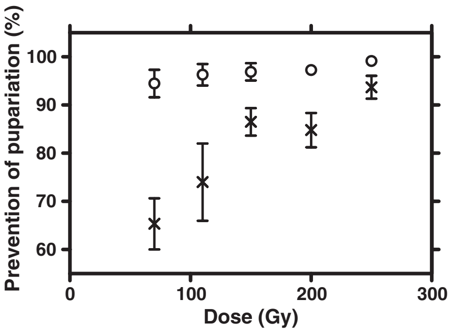

Fig. 2. Irradiated first instar Mexican fruit flies that did not reach pupariation. Percentages are based on total numbers of late instars found in the nonirradiated controls. Data for middle-aged first instars are represented by circles and late first instars by Xs.

early Mexican fruit fly eggs are irradiated. At 70 Gy a mean of 14.2% of 3-d-old eggs developed to latter instars; 24.2% of these were second instars and the rest third instars. A mean of 10.8% of these larvae were dead. At ≥110 Gy, no 3-d-old eggs developed to the second instar. Therefore, the probability of finding late instars of Mexican fruit fly irradiated during the late egg stage is small, because most commercially irradiated fruit receive more than the allowable minimum dose of 70 Gy. The remainder of the results are for first instars.

The number of larvae found when irradiated as middle-aged and late first instars (7 and 10 d after oviposition, respectively) fit a linear regression (Fig. 1; Table 1). A test for equal slopes gave an F value of 0.034 (df = 1, 26), $P < 0.86$; therefore, slopes are not significantly different; the pooled slope is −0.176. The difference between the two lines was highly significant ($F = 51.0$; df = 1, 27; $P < 0.0001$).

It is assumed that the probability of finding second instars present is significantly less (although of unknown quantity) than the probability of finding third instars, due to the small size of the former. Because of the degree of examination to which grapefruit were subjected, I believe that the majority of third instars present were found or at least the relative differences in third instar finds among the treatments and control were similar.

Additional results outside of the main scope of the stated objective (determine the degree to which tephritid fruit flies irradiated as eggs and early instars develop to the point that they could be discovered by inspectors posttreatment) add useful information. Figure 2 presents the percentages of total larvae estimated in each treatment that did not pupariate. Estimates were based on total numbers of larvae found in the nonirradiated controls. Mean failure to pupariate in the controls was 6.7%. Failure to pupariate was inversely related to dose (from a mean of 94.5% at 70 Gy to 99.1% at 250 Gy), and the curves roughly follow the sigmoid shape typical of dose–response data. However, neither set of data for middle-aged or late first instars fit the Gompertz or normal probability density functions for probit analysis, with or without log 10 of dose. No adults emerged from puparia formed by any of the irradiated insects, whereas adult emergence in the controls was 85.4 ± 7.5%.

Figure 3 represents the same data as Fig. 2, but based only on the number of larvae found in each treatment/replicate combination, not the control, i.e., it is based on the number of insects reaching a late enough instar that they were found upon examination. Two contrasting tendencies are shown. Failure to pupariate for late first instars increases slightly at higher doses, as may be expected. However, failure to pupariate for middle-aged

Table 1. Linear regression analysis of the amt (expressed as percentage of nonirradiated control) of late instars found about 3.5 wk after oviposition in grapefruit after irradiation as early or late first instars of Mexican fruit fly

Stage	r^2	$s_{y.x}$	Test of slopes		Runs test P value
			F	P value	
Early first instar	0.64	8.7	23	0.0003	0.972
Late first instar	0.39	16.0	8.3	0.013	0.704

Degrees of freedom for test of slopes were 1 for the numerator and 13 for the denominator.

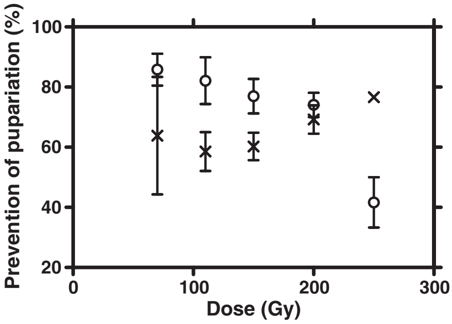

Fig. 3. Irradiated first instar Mexican fruit flies that did not reach pupariation. Percentages based on total numbers of late instars found in each respective treatment (not controls). Data for middle-aged first instars are represented by circles and late first instars by Xs.

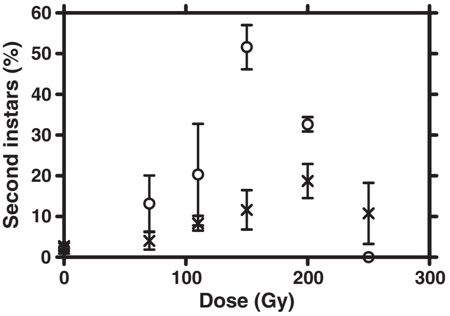

Fig. 4. Percentage of larvae that were second instars (the rest were third instars) when removed from grapefruit ≈3.5 wk after infestation. Larvae were irradiated as first instars. Data for middle-aged first instars are represented by circles and late first instars by Xs.

first instars declines possibly insignificantly until it declines considerably at 250 Gy. This may signify a heterogeneous population in susceptibility to radiation. Perhaps as the number of insects surviving irradiation to grow to latter instars becomes small, those few surviving have an increased probability of developing even further, to the puparial stage. The maximum dose for late first instars may not have been sufficient to show the increased tendency for pupariation among the most radiotolerant individuals, as a mean of ≈30% reached late instars at 250 Gy. Perhaps at a dose that only allowed a few percent of total late first instars present to reach late instars would show a greater probability for these surviving larvae to pupariate. None of the four models fit the data for middle-aged first instars, whereas the Gompertz model using log10 of dose gave the closest fit for late first instars (χ^2 = 2.48, probability > χ^2 = 0.48, df = 3, y-intercept = −3.74 ± 0.65, slope = 1.86 ± 0.30, ED_{50} = 102 [confidence limits = 82–118] Gy).

Figure 4 shows the proportion of larvae found that were second instars as opposed to third instars when larvae were removed from the grapefruit. A reasonable hypothesis might be that increasing radiation doses increase the proportion of insects not developing beyond the second instar. These could remain alive for some time, but not be able to molt to the third instar. There was an increasing tendency in proportion of second instars as dose increased until ≈150 Gy for insects irradiated when early first instars and ≈200 Gy for those irradiated as late first instars. A mean of 2.6 ± 1.8% of nonirradiated controls were second instars when examined ≈3.5 wk after oviposition. Irradiated eggs did not develop far enough to determine tendencies in these categories at the doses used. A mean of 24.2 ± 14.7% of late eggs were second instars ≈3.5 wk after oviposition when irradiated with 70 Gy.

Numbers of dead larvae found when grapefruit were opened varied considerably among replicates from 0 to 19% and showed no apparent trend.

This research demonstrates that the probability of finding live larvae by using ionizing irradiation phytosanitary treatments may be greater than the probability of finding any larvae, dead or alive, with all other commercial treatments where acute mortality of the most tolerant stage is the measure of efficacy. With other treatments further development of early stages is prevented, so they will not be found. This finding does not jeopardize the use of irradiation as a phytosanitary treatment but, instructs regulators and industry that finding more total larvae in irradiated commodities compared with commodities treated by other means does not indicate greater pretreatment infestation levels. Climacteric fruit, such as mangoes, *Mangifera indica* L., and papayas, *Carica papaya* L., may be picked at a riper stage when using irradiation as a phytosanitary treatment (Hallman 2007), and in these cases, fruit could be exposed to infestation longer than that picked for other treatments.

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