Gamma Radiation Doses to Prevent Adult Emergence from Immatures of Mexican and West Indian Fruit Flies (Diptera: Tephritidae)

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ABSTRACT Quarantine treatments against tephritids generally have been concerned only with eggs and larvae because the larva leaves the fruit to pupate in the soil. However, irradiation would usually be applied after fruits are packed, and some packed fruits, such as citrus, could conceivably sit at ambient conditions for a few days before being irradiated, allowing 3rd instars to emerge and pupate within the packaging. Pupae are generally more tolerant of irradiation than are larvae and eggs. This research evaluated tolerance of immatures of Mexican and West Indian fruit flies, *Anastrepha ludens* (Loew) and *A. obliqua* (Macquart), respectively, to ionizing radiation from a ^{137}Cs source. Although tolerance to irradiation generally increased with increasing stage of development, the insect immediately preceding 2 developmental milestones (pupariation and larval to pupal molt) was usually more susceptible than 24 h earlier. We recommend that fruits to be irradiated not remain at ambient temperatures for enough time to enable 3rd instars to develop to the phanerocephalic pupal stage, which is ≈3 d at 25°C for the 2 species studied. The 1st d of the phanerocephalic pupa is ≈40% more tolerant of irradiation than the 3rd instar, whereas the insect 24 h earlier is only ≈14% more tolerant. Mexican fruit fly 3rd instars inside grapefruits, *Citrus paradisi* Macf., were notably more tolerant of irradiation than 3rd instars in ambient air.

KEY WORDS *Anastrepha ludens, Anastrepha obliqua,* ionizing radiation, irradiation, dose-response

THE MEXICAN FRUIT fly, *Anastrepha ludens* (Loew), infests various fruits from the southern tip of Texas through northern Guatemala. The West Indian fruit fly, *A. obliqua* (Macquart), occurs on many Caribbean islands and from Mexico into South America. Hosts grown in these areas are subject to quarantine restrictions by importing countries which often require that the fruits be treated to kill virtually all eggs and larvae which may be present before those fruit are exported. Treatments approved by some importing countries include hot water immersion of mangoes, *Mangifera indica* L. (Sharp 1988); high-temperature forced air of grapefruits, *Citrus paradisi* Macf. (Mangan and Ingle 1994); vapor heat; and methyl bromide fumigation (USDA 1994). Ionizing radiation has received considerable attention as a quarantine treatment (Burditt 1994). Irradiation has distinct advantages as a quarantine treatment, such as application to products that are already packed, low damage incidence to many agricultural commodities, and speed of treatment. A unique problem with the application of irradiation as a quarantine treatment has been that it does not kill insects in a reasonable amount of time at the doses allowed on fresh commodities by the U.S. Food and Drug Administration (≤1 kGy). In any case, doses in excess of 1 kGy would be detrimental to the quality of many fruits (Morris and Jessup 1994). Therefore, the currently accepted criterion for determining efficacy of an irradiation quarantine treatment against tephritids is prevention of adult emergence (Anonymous 1996). The U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA–APHIS), is considering requiring an absorbed dose of 150 Gy to disinfest any fruit host of Mexican and West Indian fruit flies before importation into the United States (Anonymous 1996).

In general, as an insect approaches the adult stage of development, increasingly higher doses of irradiation are needed to prevent adult emergence (Balock et al. 1963). Quarantine treatments against tephritids have targeted only eggs and larvae because the insect pupates outside of the fruit. However, a commodity is usually irradiated after packaging. Conceivably, 3rd instars could emerge from the fruit and pupate inside of the package in the time lag between packaging and irradiation. Some fruits, such as citrus, may remain at ambient temperatures for several days after being packed. It is more difficult to prevent adult emergence by irradiating pupae than larvae.

The objective of this research was to determine minimum absorbed doses necessary to prevent adult emergence from the feeding 3rd instars and postfeeding stages through the 7th d of pupariation of Mexican and West Indian fruit flies.

Materials and Methods

Irradiator. Gamma radiation was applied with a ^{137}Cs self-contained, dry-storage irradiator (Husman model 521A; Isomedix, Whippany, NJ) which was de-

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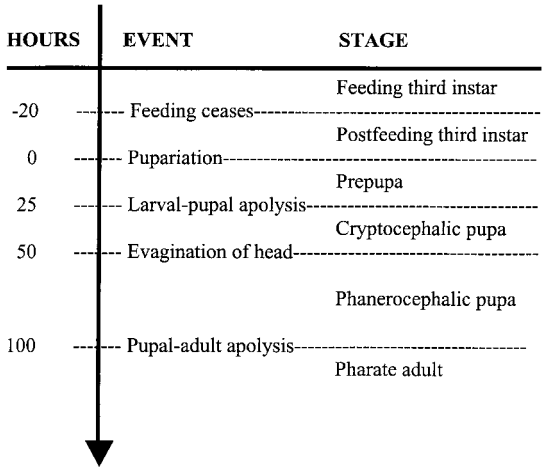

Fig. 1. Mexican and West Indian fruit fly development at 25°C between late 3rd instar and early pharate adult using pupariation as time zero. Hours between each event vary. Terminology follows Fraenkel and Bhaskaran (1973).

livering a calculated centerline absorbed dose rate of ≈0.39 Gy·sec^{-1} during the time of this research (ASTM 1998). Reference standard dosimetry was done in 1985 using the Fricke system. Routine dosimetry during our research was done with radiochromic film (Gafchromic MD-55; ISP Technologies, Wayne, NJ), and absorbance at the 510-nm wavelength was read with a spectrophotometer (Milton Roy Spectronic 401; Ivyland, PA) using the Fricke centerline determination as the standard.

Fruit Flies. Mexican fruit flies were from a colony reared on semiartificial diet (Spishakoff and Hernandez-Davila 1968) at the USDA–APHIS facility at Mission, TX. West Indian fruit flies were from a colony reared on a casein diet at the USDA–ARS Kika de la Garza Subtropical Agricultural Research Center at Weslaco, TX (Moreno et al. 1997). Both were reared at ≈25°C and 75% RH.

Terminology used to describe fruit fly immature stages follows Fraenkel and Bhaskaran (1973) and is illustrated in Fig. 1. Feeding larvae are still actively ingesting food; postfeeding 3rd instars have irreversibly ceased feeding and emptied their crops, but have not yet initiated pupariation. The prepupa has pupariated but has not yet formed the pupa. The cryptocephalic pupa comprises the stage between larval–pupal apolysis (pupation) and evagination of the head. The phanerocephalic pupa is the insect after the head has everted but before pupal–adult apolysis. After pupal–

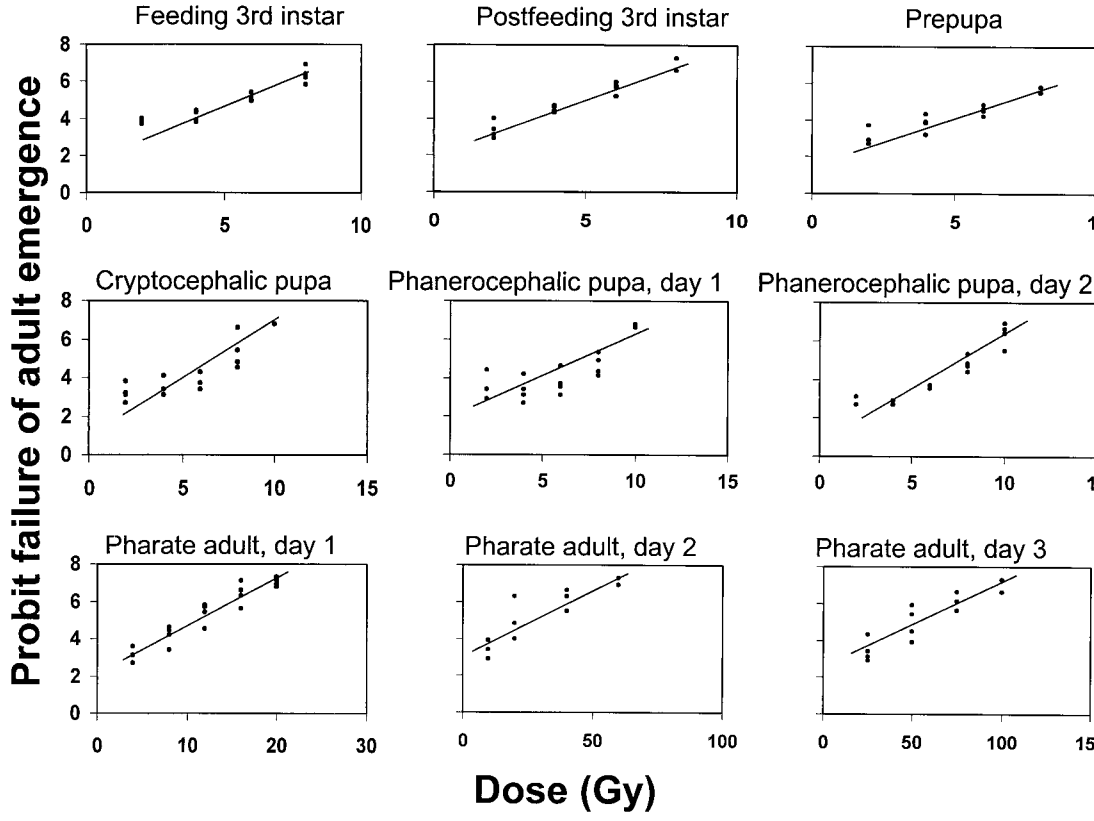

Fig. 2. Probit (5 = 50%) of percentage of Mexican fruit fly stages failing to emerge as normal-looking adults after irradiation. Probit estimates of 0 and 100% not possible and therefore not included. Line is best-fitting model between normal and Gompertz probability density functions (Table 1).

Table 1. Probit analyses of prevention of emergence of normal-appearing adults from irradiated tephritid larvae, pupae, and pharate adults

Species and stage[a] irradiated	n	Probability density function	Slope ± SE	Effective min. absorbed doses (95% CL), Gy			χ^2
				ED_{50}	ED_{99}	$ED_{99.9968}$	
Mexican fruit fly, *Anastrepha ludens*							
Feeding third instar	2,000	Gompertz	0.53 ± 0.041	5.8 (5.3–6.3)	9.4 (8.6–10.5)	14.0 (12.4–16.6)	7.8
		Normal	0.45 ± 0.069	5.4 (4.2–6.7)	10.6 (8.7–15.8)	14.4 (11.3–23.0)	53**
Postfeeding 3rd instar	2,000	Gompertz	0.64 ± 0.04	5.1 (4.7–5.4)	8.0 (7.5–8.7)	11.9 (10.8–13.4)	7.2
		Normal	0.58 ± 0.023	4.8 (4.6–4.9)	8.8 (8.4–9.1)	11.6 (11.1–12.2)	6.1
Prepupa	2,000	Gompertz	0.64 ± 0.026	6.8 (6.6–6.9)	9.8 (9.5–10.0)	13.6 (13.1–14.2)	4.6
		Normal	0.50 ± 0.067	6.4 (5.5–7.5)	11.1 (9.5–14.9)	14.4 (11.9–20.7)	93**
Cryptocephalic pupa	2,000	Gompertz	0.74 ± 0.15	7.6 (6.0–8.9)	10.2 (8.9–15.1)	13.5 (11.1–24.9)	66**
		Normal	0.49 ± 0.16	7.3[b]	12.1[b]	15.5[b]	197**
Phanerocephalic pupa, day 1	2,400	Gompertz	0.61 ± 0.16	7.9 (5.8–9.7)	11.1 (9.4–18.9)	15.1 (12.0–33.1)	174**
		Normal	0.41 ± 0.13	7.6 (3.8–13.6)	13.3 (10.0–56)	17.4 (12.3–89)	298**
Phanerocephalic, day 2	2,400	Gompertz	0.75 ± 0.075	8.5 (8.0–8.9)	11.0 (10.4–12.0)	14.3 (13.0–16.5)	9.5
		Normal	0.56 ± 0.22	8.1[b]	12.3[b]	15.3[b]	77**
Pharate adult, day 1	2,400	Gompertz	0.25 ± 0.038	11.7 (9.0–13.6)	19.4 (17.0–24.4)	29.5 (24.4–41.6)	73**
		Normal	0.25 ± 0.009	11.1 (10.8–11.4)	20.5 (19.9–21.3)	27.4 (26.3–28.6)	6.8
Pharate, day 2	2,000	Gompertz	0.077 ± 0.03	26.9[b]	52[b]	84[b]	173**
		Normal	0.085 ± 0.02	24.9 (12.6–40)	52 (38–139)	72 (50–216)	90**
Pharate, day 3	2,000	Gompertz	0.048 ± 0.009	54 (32–67)	94 (79–138)	145 (114–257)	54**
		Normal	0.049 ± 0.002	52 (50–53)	99 (95–103)	133 (127–141)	2.4
West Indian fruit fly, *Anastrepha obliqua*							
Postfeeding 3rd instar	2,400	Gompertz	0.63 ± 0.043	6.7 (6.4–7.1)	9.7 (9.2–10.4)	13.6 (12.5–15.2)	8.7
Prepupa	2,000	Gompertz	0.68 ± 0.044	6.6 (6.2–6.9)	9.4 (8.9–10.2)	13.1 (12.0–14.7)	7.4
Cryptoecphalic pupa	2,400	Gompertz	0.72 ± 0.06	7.8 (7.4–8.2)	10.4 (9.9–11.2)	13.9 (12.7–15.6)	9.4
Phanerocephalic pupa, day 1	2,400	Gompertz	0.65 ± 0.082	7.7 (7.0–8.4)	10.6 (9.8–12.2)	14.4 (12.7–17.9)	34**
Phanerocephalic, day 2	2,400	Gompertz	0.66 ± 0.15	7.9 (6.1–9.0)	10.7 (9.5–14.7)	14.4 (12.0–24.1)	17**
Pharate adult, day 1	2,400	Gompertz	0.51 ± 0.036	9.2 (8.7–9.6)	12.9 (12.2–13.7)	17.7 (16.4–19.7)	12.3*
Pharate, day 2	2,400	Gompertz	0.20 ± 0.077	17.9[b]	27.1[b]	39.2[b]	222**
Pharate, day 3	2,400	Gompertz	0.057 ± 0.008	48 (38–55)	81 (71–102)	125 (103–175)	72**

*, $P \leq 0.05$; **, $P \leq 0.01$.
[a] Stage terminology follows Fraenkel and Bhaskaran (1973).
[b] 95% CL not calculable because of a poor fit.

adult apolysis, but before emergence from the puparium, the insect is the pharate adult. The pharate adult is the longest stage that occurs within the puparium, lasting almost ¾ of the entire puparial period.

Irradiation. Four replicates each of 100 Mexican and 100 West Indian fruit fly feeding 3rd instars, postfeeding 3rd instars, and puparia (1, 2, 3, 4, 5, 6, and 7 d old) were irradiated in 0.5-liter paper containers with 6 doses between 2 and 200 Gy to estimate the effective dose (ED) needed to prevent adult emergence at the 99.9968% level, which is the statistical goal of most quarantine treatments against tephritids (Shannon 1994). Feeding 3rd instars were irradiated in diet, and all other stages were irradiated with ≈40 ml of moist vermiculite. Doses per insect age group were adjusted with the goal that only the highest dose gave no survivors. A control group of 100 insects of each species and stage was not irradiated. After irradiation, all insects were kept in the paper containers at ≈25°C and 75% RH until the adults emerged and died. Feeding 3rd instars (done only for Mexican fruit fly) were removed from the diet 24 h after irradiation and placed in containers with vermiculite. The 4 replicates were irradiated on different dates. After adult emergence, the insects were classified as successful if emerged adults looked morphologically normal, or as failure if they did not emerge, were partially emerged, or had twisted wings or other notable deformities. Successful emergence in the Mexican fruit fly controls was always >95%, but was often between 80 and 95% for the West Indian fruit fly. Therefore, data for the latter were corrected for mortality in the control using Abbott (1925) before analysis with the PROBIT procedure (SAS Institute 1988) using normal, logistic, and Gompertz (also known as complementary log–log, Weibull, or Gumbel) probability density functions. $ED_{99.9968}$s were estimated using Finney (1971). Because the $ED_{99.9968}$ is such an extreme value, the accuracy of its estimates using mathematical models cannot be assumed even if statistical fits were good. Therefore, trials were performed with large numbers of insects to confirm accuracy. The same methods as before were used, except that up to 1,000 insects were placed in each paper container.

Further studies were done on Mexican fruit flies inside of grapefruits and in low oxygen atmospheres because prevention of adult emergence in the previous experiments was achieved with remarkably low doses.

Grapefruit Infestation and Irradiation. Grapefruits (cultivar Rio Red, mean weight ≈450 g) were placed ≈190 at a time in an aluminum screen cage (228 by 81 by 46 cm) with ≈10,000 Mexican fruit fly adults (about a 50: 50 sex ratio) for 1–2 d. Grapefruits were removed, washed, and placed at ≈25°C for 16–19 d so that larvae could develop to large 3rd instars. At that time the

Table 2. Results of large-scale confirmatory testing of irradiation doses to prevent adult emergences of tephritids at different stages of development

Species/Stage	Min. absorbed dose, Gy	No. treated	No. of normal adults
Mexican fruit fly, *Anastrepha ludens*			
Feeding third instar	14	18,400	3
Feeding third instar	16	108,200	0
Postfeeding third instar	14	40,500	1
Postfeeding third instar	16	108,100	0
Prepupa	12	26,900	1
Prepupa	14	97,200	0
Cryptocephalic pupa	16	28,400	1
Cryptocephalic pupa	18	93,500	0
Phanerocephalic pupa, day 1	16	27,400	2
Phanerocephalic pupa, day 1	20	31,800	1
Phanerocephalic pupa, day 1	22	52,900	1
Phanerocephalic pupa, day 2	16	36,400	1
Phanerocephalic pupa, day 2	25	94,900	0
Pharate adult, day 1	30	5,700	3
Pharate adult, day 1	35	40,000	0
Pharate adult, day 2	100	44,100	0
Pharate adult, day 3	250	31,700	0
West Indian fruit fly, *Anastrepha obliqua*			
Postfeeding third instar	14	8,300	0
Prepupa	14	6,900	0
Cryptocephalic pupa	14	4,300	0
Phanerocephalic pupa, day 1	14	22,600	1
Phanerocephalic pupa, day 2	14	25,500	0
Pharate adult, day 1	20	6,300	0

grapefruits were irradiated 3 at a time inside the aluminum cylinder with 25–60 Gy. About 25 fruits were irradiated at the lower doses and >100 at the higher doses. Strips of radiochromic film (1 by 10 cm), wrapped in wax sheets (Parafilm M; American National Can, Greenwich, CT) to prevent contact of radiochromic film with grapefruit juice, were inserted into slits through the center of some grapefruits to identify any relative differences in absorbed radiation dose within the grapefruits. Some grapefruits were not irradiated (controls). Grapefruits were placed individually in 2-liter plastic containers with ≈50 ml of vermiculite in the bottom. Larvae emerging from the fruits were placed in containers with vermiculite at ≈25°C to observe development. Larval emergence from irradiated grapefruits was not reduced compared with the controls because the relatively low doses used do not kill 3rd instars, but only prevent continued development. Number of larvae ranged from 400 at 25 Gy to several thousand at 50 Gy. Data were analyzed as before.

Low-Oxygen Irradiation. Irradiation in low oxygen often is known to cause less damage to living organisms than irradiation in ambient air (Earle et al. 1979). One hundred Mexican fruit fly postfeeding 3rd instars were placed with ≈30 ml of vermiculite in 125-ml flasks which were then flushed with nitrogen 1 h before irradiation with 6–25 Gy. At 24 h after irradiation, the flasks were opened and the larvae were placed in plastic containers with moist vermiculite for further development. Data were analyzed as before.

Results

Routine dosimetry revealed that the absorbed dose ranged from the set dose to ≈15% greater. Doses presented in this study are minimum absorbed doses; the upper range of the absorbed dose would be ≈1.15 times the minimum.

Percentage nonemergence of normal-looking adults was converted to probits (except for data points of 0 and 100% nonemergence) and is graphed against radiation dose in Figs. 2 (Mexican fruit fly) and 3 (West Indian fruit fly). Results of analyses with normal and Gompertz probability density functions for Mexican fruit fly are presented because the former fit 3 of 9 data sets whereas the latter fit 4 of 9 at the 95% confidence level (Table 1). The Gompertz probability density function was the only model that fit any of the data sets at the 95% confidence level (4 of 8) of the West Indian fruit fly. Results of analyses using lognormal dose did not fit the data in any case, although it has been the analysis most commonly used for quarantine treatment data. Analyses using logit gave illogical results—estimates of $ED_{99.9968}$ which were lower than the observed data for ED_{99}.

Both species showed similar results through the phanerocephalic pupal stage in that 95% CLs largely overlapped. For the pharate adult stages tested, the Mexican fruit fly seemed to be more tolerant than the West Indian fruit fly (Table 1). Estimates of the EDs increased as the fruit flies advanced in development.

Large-scale testing supported those tendencies and furthermore showed that prepupal Mexican fruit flies were slightly more susceptible to irradiation than 3rd instars (Table 2). $ED_{99.9968}$ indicates, on average, there will be 1 survivor for every 31,250 insects treated. From Table 2 it is evident that the $ED_{99.9968}$ for feeding 3rd-instar Mexican fruit flies was >14 Gy but <16 Gy because there were 3 survivors of 18,400 irradiated with 14 Gy and no survivors of 108,200 irradiated with 16 Gy. Likewise, the $ED_{99.9968}$ for postfeeding 3rd instars was near 14 Gy (1 survivor of 40,500 irradiated with 14 Gy). The results of large-scale testing demonstrate that the Gompertz and normal probability

Table 3. Probit analyses of prevention of emergence of normal-appearing adults from irradiated Mexican fruit fly third instars in grapefruits or in a nitrogen atmosphere

Irradiation medium	n	Probability density function	Slope ± SE	Effective min. absorbed doses (95% CL), Gy			χ^2
				ED_{50}	ED_{99}	$ED_{99.9968}$	
Grapefruit	9,706	Gompertz	0.05 ± 0.007	0 (0–0.81)	30.2 (28.0–32.0)	80.1 (70.2–96.9)	0.75
Nitrogen atmosphere	1,800	Gompertz	0.23 ± 0.038	12.5 (10.5–14.1)	20.7 (18.2–26.0)	31.2 (25.9–43.9)	56**

**, $P \leq 0.01$.

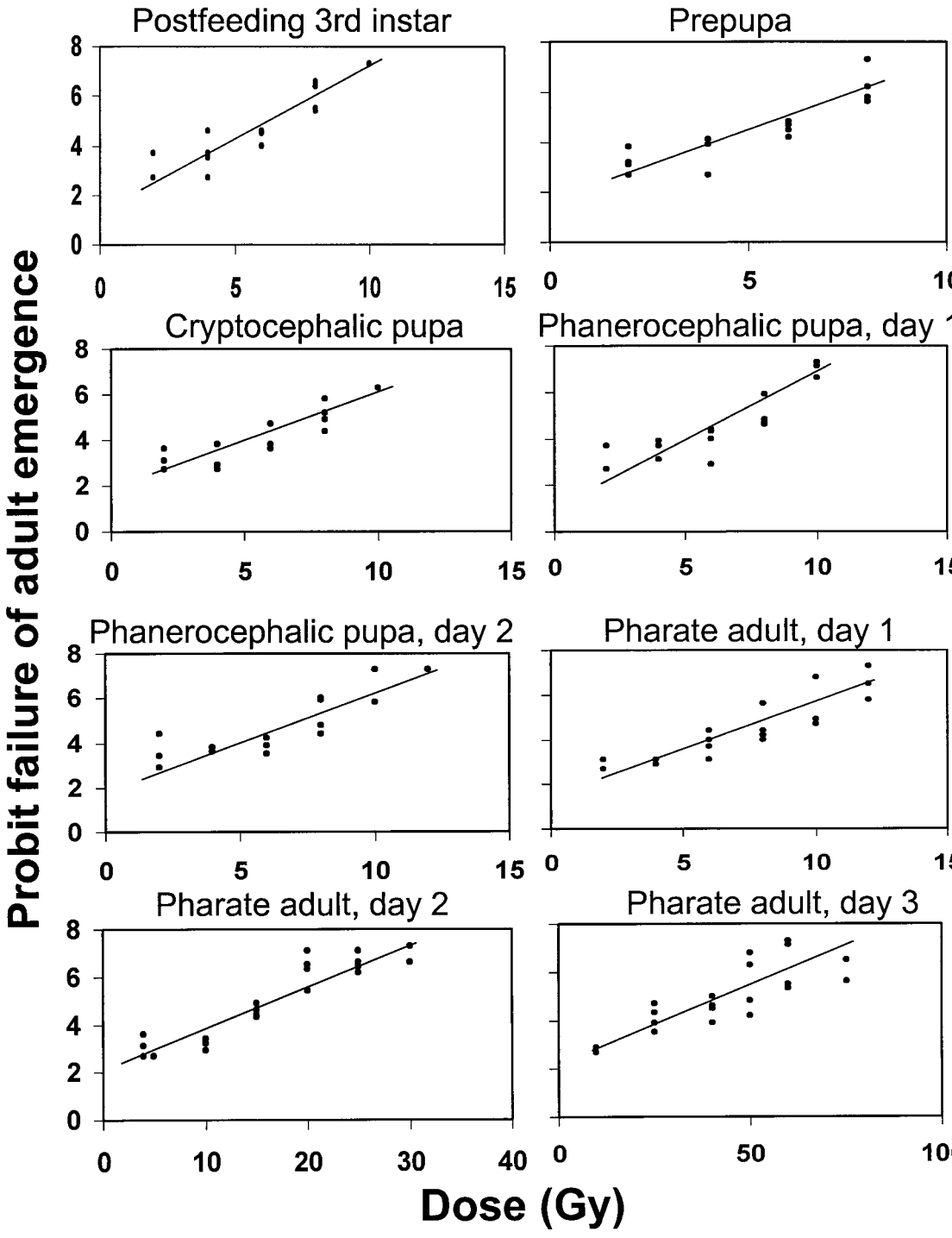

Fig. 3. Probit (5 = 50%) of percentage of West Indian fruit fly stages failing to emerge as normal-looking adults after irradiation (Table 1). Probit estimates of 0 and 100% not possible and therefore not included.

density functions (PDF) gave roughly similar ED estimates, and in those cases with Mexican fruit fly where sufficient insects were tested to make conclusions on the accuracy of $ED_{99.9968}$ estimates (the stages from feeding 3rd instar through 1st-d pharate adult), the estimates were fairly close to the point estimate and well within the 95% CL except for postfeeding 3rd instars where the estimates were low.

Third-instar Mexican fruit flies irradiated in grapefruits (Fig. 4 and Table 3) showed greater tolerance to the process than those irradiated in ambient air (Table 1), which is evident by the large gaps between fiducial limits. Mexican fruit fly 3rd instars irradiated in nitrogen atmospheres (Fig. 4 and Table 3) and left for 24 h before returning to ambient air were more tolerant of irradiation than those irradiated in ambient air (Table 1) but less tolerant than those irradiated in grapefruits. A mean of 98% adult nonemergence of irradiated Mexican fruit fly 3rd instars was reached at 8–10, 20, and 30 Gy, respectively, for larvae irradiated in air, nitrogen, and grapefruits.

Discussion

In general, tolerance of late immature stages of the 2 fruit flies, measured in adult emergence, increased steadily as they matured. Slight reductions in tolerance of fruit flies to irradiation as they matured were related to increases in developmental activity. The prepupa which, based on large scale testing (see Table 2), was slightly more susceptible to irradiation than stages 24h earlier, directly preceded the larval–pupal molt. Another reduction in tolerance to irradiation occurred preceding pupariation which, although it does not involve apolysis, is still a process comprising great changes (Denlinger and Ždárek 1994). Balock et al. (1963) also found a reduction in tolerance to irradiation of prepupae of 3 tephritid species compared with 3rd instars and for the larval stage immediately preceding pupariation in 2 of the 3 species studied.

Oxygen levels inside grapefruits are roughly half of ambient levels (Hallman et al. 1994). Third instars irradiated in a nitrogen atmosphere were less tolerant than those irradiated inside grapefruits. There are obviously other factors in addition to lower oxygen which favor fruit fly survival inside of grapefruits, and they should be explored because they may impact the efficacy of irradiation quarantine treatments. Our results with Mexican fruit fly irradiated in grapefruits are very similar to those found by Bustos et al. (1992) in mangoes; prevention of adult emergence at the level of 98.9% with 30 Gy and 99.89% with 40 Gy in mangoes versus 98.6% with 30 Gy and 99.95% with 40 Gy in grapefruits. Because of the increased susceptibility of insects irradiated in air versus those irradiated in fruits, doses found to prevent adult emergence of insects outside of fruits cannot be used to develop quarantine treatment doses for tephritids inside of fruits, although this has been suggested as a possibility (Anonymous 1996).

The fact that tolerance of fruit flies to irradiation, as measured by adult emergence, increases as the insects develop, suggests that a limit should be placed on the amount of time packed commodities can remain at ambient temperatures before they are irradiated. Compared with feeding 3rd-instar Mexican fruit flies at ≈25°C, this increase is evident by the 2nd d of pupariation (cryptocephalic pupa), doubles by the 5th d (apolysis to pharate adult), and increases by a factor of 6 one day later. We recommend that fruits that will be irradiated as a quarantine treatment against fruit flies do not remain at ambient temperatures after packaging for >2 or 3 d before irradiation, depending on the temperature. Phanerocephalic pupal or later stages should not be present when the fruits are irradiated because of their significantly increased tolerance to irradiation (≥40%) relative to 3rd instars. Based on the data presented by Balock et al. (1963), this recommendation would also suffice for the 3 fly species they studied. We recognize that insects outside of fruits may not receive the benefit of reduced oxygen or other protection that insects inside fruits receive, and hence, may have reduced tolerance to irradiation. But when fruits are stored in modified atmospheres, insects outside of fruits but in the packaging also might benefit from the reduced oxygen and other possible unrecognized factors conferring reduced susceptibility to irradiation.

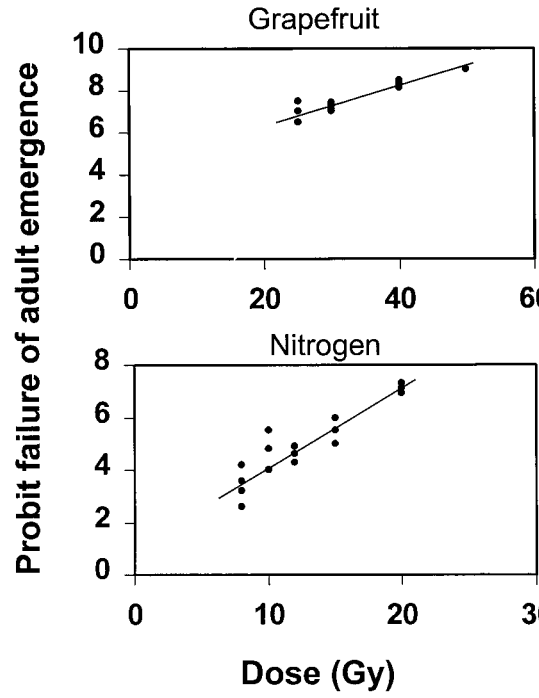

Fig. 4. Probit (5 = 50%) of percentage of Mexican fruit fly 3rd instars failing to emerge as normal-looking adults after irradiation in grapefruits and nitrogen atmosphere.

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