Irradiation to Ensure Quarantine Security for *Cryptophlebia* spp. (Lepidoptera: Tortricidae) in Sapindaceous Fruits from Hawaii

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ABSTRACT Studies were undertaken to determine whether irradiation treatment at 250 Gy, an accepted treatment for disinfestation of fruit flies in spindaceous fruits from Hawaii, would also disinfest fruit of two species of *Cryptophlebia. Cryptophlebia illepida* (Butler) was determined to be more tolerant of irradiation than *Cryptophlebia ombrodelta* (Lower); therefore, *C. illepida* was the focus for detailed tests. Using the criterion of success in developing to the adult stage, the pattern of tolerance to irradiation in *C. illepida* was generally eggs < early instars < late instars < pupae. The most tolerant stage potentially occurring in harvested fruits was late (fourth and fifth) instars. Development to adult was reduced slightly in late instars receiving an irradiation dose of 62.5 Gy, whereas development to adult was dramatically reduced in late instars receiving irradiation doses ≥125 Gy. No *C. illepida* larvae receiving an irradiation dose ≥125 Gy emerged as adults and produced viable eggs, indicating sterility can be achieved at doses well below 250 Gy. In large scale tests, when 11,256 late instars were irradiated with a target dose of 250 Gy, 951 pupated (8.4%) and none eclosed as adults. Within the pupal stage, tolerance increased with age; 7- to 8-d-old pupae (the oldest pupae tested) treated with an irradiation dose of 125 Gy produced viable offspring, whereas those treated with a dose of 250 Gy produced no viable offspring. Irradiation of adults with a target dose of 250 Gy before pairing and mating resulted in no viable eggs. Irradiation of actively ovipositing adult females resulted in no subsequent viable eggs. Therefore, the irradiation quarantine treatment of a minimum absorbed dose of 250 Gy approved for Hawaii's fruits will effectively disinfest fruits of any *Cryptophlebia* in addition to fruit flies.

KEY WORDS *Cryptophlebia*, quarantine treatment, lychee, longan, rambutan

Ionizing radiation with a minimum absorbed dose of 250 Gy is a generic quarantine treatment developed for Hawaii's tephritid fruit fly pests that is approved for eight tropical fruits exported from Hawaii, including lychee (*Litchi chinensis* Sonn.), longan (*Dimocarpus longan* (Lour.) Steud.), and rambutan (*Nephelium lappaceum* L.) (Sapindaceae) (Federal Register 1997). In addition to fruit flies, two species of *Cryptophlebia* (Lepidoptera: Tortricidae) are also pests of quarantine concern on these fruits in Hawaii. *Cryptophlebia illepida* (Butler) is a native Hawaiian species known as the koa seedworm, and *C. ombrodelta* (Lower) is native to Australia and known as the litchi fruit moth. *Cryptophlebia* spp. are the most commonly intercepted insect pests in sapindaceous fruits carried in passenger baggage and arriving in the mail (USDA-APHIS-PPQ 1997).

Cryptophlebia spp. lay their eggs singly on the fruit surface and neonate larvae bore through the skin and feed at the skin/pulp interface. Although many eggs may be laid on the fruit surface, typically only one larva is found feeding in a fruit. *Cryptophlebia* spp. are potentially multivoltine in Hawaii (Jones et al. 1997). Laboratory tests and field surveys in Hawaii indicate that lychee, longan, and rambutan are poor hosts for *Cryptophlebia* (McQuate et al. 2000, P.A.F., unpublished data). Actual damage from larval feeding is often minimal and larval survival to pupation when fruit are on the tree is rare. In a study consisting of >47,000 mature fruits of nine varieties of rambutan harvested from orchards in Hawaii over a 2-yr period, *Cryptophlebia* adult emergence was 0.11% (McQuate et al. 2000). In a concurrent study, *Cryptophlebia* infestation rates for lychee and longan in Hawaii were 1.1 and 0.14%, respectively (G. T. McQuate, USDA, Hilo, HI, personal communication). Successful development in fruit may improve once fruit are harvested (P.A.F., unpublished data).

Current regulations for quarantine treatment of Hawaii-grown lychee and longan with irradiation to kill fruit flies stipulate that fruits also must be found free of *Cryptophlebia* (Federal Register 1997). *Cryptophlebia* and is not yet listed as a regulatory pest on rambutan but is of concern. Thus, the presence of *Cryptophlebia* can prevent export of lychee and longan from Hawaii, and potentially could interrupt rambutan shipments as well. If the accepted irradiation quarantine treatment for pest fruit flies is also effective against *C. illepida* and *C. ombrodelta*, these species could be added to the regulation, preventing potential interruption of shipments caused by their presence. The objectives of the studies with *Cryptophlebia* spp. reported here were to examine the effects of irradiation on egg, larval, and pupal development, and adult reproductive fitness, and thereby determine the efficacy of the accepted irradiation quarantine treatment against these pests.

Materials and Methods

Insect Rearing. A colony of *C. illepida* was started with larvae (≈1,000) collected from macadamia nuts from an orchard in Pahala, HI. Because of difficulties collecting *C. ombrodelta* in the field, *C. ombrodelta* pupae (108) were obtained from a laboratory colony maintained at the Maroochy Experiment Station in Queensland, Australia, to start a colony. For both species, adults were placed in 3.8-liter plastic jars with ventilated lids for mating and provided with a 5% honey water solution through a wick. Eggs were laid on the inside surface of the jar, especially in grooves on the jar lids. Emerging neonates were transferred daily to 28-ml diet cups with laboratory diet (see description below). As larvae approached pupation, diet cups (with larvae) with lids removed were placed in 3.8-liter plastic tubs (Rubbermaid, Wooster, OH) with 30 g sand covering the bottoms. *C. illepida* larvae typically leave the diet trays to pupate in the sand, whereas *C. ombrodelta* typically pupate in the diet. Emerging moths were cooled and transferred to the plastic mating jars. Rearing conditions were 25 ± 2°C) and a photoperiod of 10:14 (L:D) h for the duration of the experiments. For stage-specific tests, larvae were placed into categories (L1, L2/3, and L4/5 corresponding to instars) based on size and known developmental rates (unpublished data).

The laboratory diet for larvae was modified from Sinclair (1974). The diet consisted of boiled white beans ('Great Northern') (234 g), wheat mill (Roger's Foods, British Columbia, Canada) (60 g), yeast flakes (nutritional yeast, Red Star Yeast and Products, Milwaukee, WI) (35 g), ascorbic acid (3.5 g), sodium benzoate (2.2 g), and sorbic acid (1.1 g), mixed together and added to a boiling mixture of distilled water (700 ml) and agar (14 g). The mixture was blended at the lowest setting for 1 min then poured into 28-ml diet cups and cooled uncovered at room temperature. Before use, beans were soaked for 24 h then boiled in distilled water for 2 h and drained thoroughly.

Irradiation Treatment. Irradiation treatment was done at the Hawaii Research Irradiator at the University of Hawaii at Manoa on the island of Oahu. The Hawaii Research Irradiator uses a ^{60}Co source of gamma radiation and the dose rate at the time of the tests was 5.3–5.8 Gy min^{-1}. The internal dimensions of the treatment chamber are 18 by 53 by 58 cm. For irradiation treatment, eggs, larvae, pupae, or adults in 29-ml plastic cups with or without diet were packed in containers, transported to the Hawaii Research Irradiator on Oahu, and treated at the target dose(s). Density of the packed containers was 0.16 g/cm^3. Gafchromic film dosimeters (ISP, Wayne, NJ), read with a spectrophotometer (model 550, Perkin-Elmer, Foster City, CA) at 500-nm absorbance, were used to verify dose accuracy in each replicate. Film dosimeters were calibrated using alanine dosimeter standards

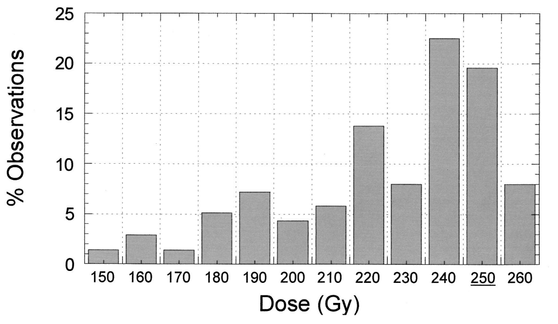

Fig. 1. Dose variation in the full volume of the treatment chamber of the Hawaii Research Irradiator. The target dose was 250 Gy and the maximum/minimum ratio was 1.8. Dose mapping indicated that most of the higher doses (230–260 Gy) were recorded at the center of the chamber. Variation in dose was minimized by positioning samples in the center of the chamber.

supplied and quantified by the National Physical Laboratory, Middlesex, UK. Initially, 900 larvae in individual diet cups were packed into a box equal in volume to the inside of the treatment chamber to maximize the number of individuals treated at the same time. A detailed dose mapping study midway through our studies indicated that filling the chamber resulted in high maximum/minimum values (ratio = 1.8) for irradiation doses (Fig. 1) with highest and most consistent doses in the center of the chamber and lowest doses at the inside periphery. In subsequent tests, all individuals to be treated for a particular age or stage were placed in 3.8-liter plastic containers and positioned in the center of the chamber to minimize variation in irradiation dose (maximum/minimum ratio ≈1.3).

Experimental Design. All irradiation tests used laboratory-reared *Cryptophlebia.* To identify the more tolerant species between *C. illepida* and *C. ombrodelta,* late (fourth and fifth) instars were irradiated with a target dose of 62.5 Gy. This dose was chosen because it was known from preliminary tests to be sublethal. Each of four replicates had 80 individuals of each species and all replicates were treated on the same day. Emerging adults were classified as (1) normal, (2) with deformed wings, or (3) partially emerged from the pupal case. Percentage adult eclosion was the criterion used for selecting the most tolerant species. A single group of 30 late instars of each species was left untreated and held as controls. Based on the results of this test all subsequent tests focused on *C. illepida.*

Eggs (3 d old) and larvae (neonates, second/third instars, and fourth/fifth instars) were irradiated at target doses of 62.5, 125, 250, and 400 Gy. These are the stages that occur in fruit. Although the pupal stage of *Cryptophlebia* is not found in fruits, we tested the effects of irradiation against this stage in the event a larva emerged from a fruit and pupated in a package or shipping container before receiving irradiation treatment. Pupal development to the adult stage is 11 d

Table 1. Effect of low-level irradiation treatment of late instar *C. illepida* and *C. ombrodelta* on adult emergence and fertility

Species	Mean % adult emergence (± SE)				No. adults with eggs	No. eggs	No. neonates
	Normal wings	Deformed wings	Partial emerg.	Total			
				Treated			
C. illepida	5.0 (0.7)	26.9 (3.1)	3.8 (0.9)	35.6 (2.4)a	1	3	0
C. ombrodelta	0.9 (0.3)	4.7 (1.4)	2.5 (0.5)	8.1 (1.9)b	0	0	0
				Control			
C. illepida	76.7	0.0	0.0	76.7	6	246	207
C. ombrodelta	76.7	0.0	3.3	80.0	7	348	305

Means within a column followed by different letters are significantly different using Student *t*-test ($P \leq 0.05$). Irradiation treatment dose range was 65-78 Gy.

under our rearing conditions (25 ± 2°C). Pupae (1–2 d old, 4–5 d old, and 7–8 d old) were treated at 125 and 250 Gy only. For each larval or pupal age/stage, four to seven replicates were irradiated on different dates, and in each replicate a control group of 25–50 insects was not irradiated.

Irradiation doses were selected based on the accepted irradiation treatment for exporting Hawaii's fruits (250 Gy) (Federal Register 1997) and the generic dose established by California for insects other than fruit flies (400 Gy). After treatment, development of each individual in a test was followed until death. For each age–dose combination, individuals that developed to the adult stage were mated *inter se* when possible (i.e., when there was synchronous emergence of male and female moths) to examine irradiation effects on mating success, fecundity, and fertility. Insects received irradiation treatment while on artificial substrates because preliminary artificial infestation tests indicated that lychee, longan, and rambutan were poor hosts for *Cryptophlebia* (i.e., high control mortality in artificially infested fruit). Eggs were treated naked in plastic cups, and neonates emerging from treated eggs were transferred to diet cups for development. Larvae and pupae were treated in plastic cups with laboratory diet. The efficacy of irradiation treatments against immature *C. illepida* was evaluated based on the number of treated individuals pupating, adult eclosion, and adult female fecundity and fertility.

Premating and postmating studies were conducted to examine the effects of irradiation of adults on fecundity, fertility, and egg development. In the first study, individuals were isolated in individual plastic cups as pupae, and after eclosion, unmated moths (≤2 d old) were irradiated with a target dose of 125 or 250 Gy or left untreated. Irradiated and un-irradiated males and females in all combinations were then mated as pairs in individual cups for 24 h. Males were removed after 24 h and eggs were counted daily until adult female death. Actively ovipositing females were transferred to new cups every 2 d. Egg development was scored every 2 d using a five-stage maturity rating system: (0) eggs clear (no observable development, or infertile), (1) pink spots appearing on surface, (2) pink coloration throughout, (3) developing larvae visible within, (4) sclerotized head capsule evident. This test was replicated twice. In a second unreplicated study, a cohort of mated females that had begun ovipositing was irradiated at 250 Gy or left un-irradiated as controls. Moth longevity, the number of eggs laid, and number of eggs eclosing were measured.

All larval dose–response tests and the first five replicates of the large-scale confirmatory test used the full volume of the irradiation chamber during treatment. The last six replicates of the large scale confirmatory test, and all pupal and adult mating tests involved placing insects in a 3.8-liter plastic Rubbermaid container and placing the container in the center of the chamber.

Data Analysis. Adult emergence data for the comparative study between *C. illepida* and *C. ombrodelta* were arcsine transformed and subjected to analysis of variance (ANOVA), and means separation was done using a *t*-test at the 0.05% level of probability (SAS Institute 1997). Simple linear regressions were done on untransformed data from studies of life stage tolerance to irradiation. For each life stage, data used in the linear regression model included control mortality (O Gy), any dose causing mortality between 0 and 100%, and the lowest dose causing 100% mortality. Linear regression was used instead of probit or logit models because of the limited number of irradiation doses examined and the high mortality rates at the higher doses (i.e., for five out of seven life stages, only three points could be used in the analysis) (Robertson et al. 1994). Data on eggs/female and percent egg eclosion from the adult reciprocal matings study were subjected to ANOVA and means separation were done for each of the two irradiation doses separately using a *t*-test test at the 0.05% level of probability.

Results

Cryptophlebia illepida fourth–fifth instars were more tolerant of irradiation than *C. ombrodelta* at the target dose of 62.5 Gy (Table 1). Adult emergence including all classifications averaged 35.6% (range, 28.8–40.0%) for irradiated *C. illepida* and 8.2% (range, 3.8–11.3%) for irradiated *C. ombrodelta* (Table 1), which was a highly significant difference ($t = 8.0$; df = 1, 4; $P < 0.0002$). Of the emerging *C. illepida*, 85% had deformed wings or exhibited only partial emergence from the pupal case. One surviving adult female *C.*

Table 2. Effects of irradiation on maturation of 3-d-old *C. illepida* eggs

Dose (Gy)	Reps	*n*	No. eggs hatched	No. pupae	No. adults	No. reproductive females	Total eggs	Total neonates
0	6	421	257	141	112	23	1,245	1,052
62.5	6	647	488	190	71	0	—	—
125	6	1,047	597	24	0	—	—	—
250	5	1,082	162	0	—	—	—	—
400	6	2,144	94	0	—	—	—	—

For treatments 0, 62.5, and 125 Gy, approximately 70% of neonates hatching from eggs were placed on diet and reared. Numbers under column headings for pupae, adults, reproductive females, total eggs and total neonates are extrapolations from the subset of individuals observed for these treatments.

Table 4. Effects of irradiation on maturation of *C. illepida* pupae

Age	Dose (Gy)	Reps	*n*	No. adults	No. reproductive females	Total eggs	Total neonates
1–2 d	0	6	160	118	18	730	633
	125	6	525	147	0	—	—
	250	6	805	4	0	—	—
4–5 d	0	6	150	136	26	841	677
	125	6	435	64	0	—	—
	250	6	595	16	0	—	—
7–8 d	0	7	215	187	25	1,238	1,065
	125	7	495	335	36	623	3
	250	7	620	325	1	6	0

All pupae were reared at 25 ± 2°C.

illepida laid three infertile eggs, whereas no *C. ombrodelta* laid eggs. Actual irradiation doses ranged from 65 to 78 Gy. Based on these results, detailed life stage-specific irradiation studies focused on *C. illepida*, the more tolerant species.

When 3-d-old eggs were treated with a target dose of 62.5 Gy, 29.4% pupated and 11% eclosed as adults and laid no eggs (Table 2). None of the 4,273 eggs treated at 125, 250 or 400 Gy eclosed as adults. When neonates (L1) were treated with a target dose of 62.5 Gy, 14.4% reached the adult stage and one female laid 20 infertile eggs (Table 3). When neonates were treated with 125 Gy, 0.2% eclosed as adults. No individuals eclosed as adults in the 250 and 400 Gy treatment. When second and third instars (L2/3) were treated with the target dose of 62.5 Gy, 16.2% eclosed as adults and laid no eggs. None of the 1,371 second and third instars treated at 125, 250, or 400 Gy eclosed as adults. When fourth and fifth instars were treated with the target dose of 62.5 Gy, 35.1% eclosed as adults, seven females laid 117 eggs and one egg was fertile (Table 3). In the 125 Gy treatment, 1.4% reached the adult stage but failed to reproduce. One adult with deformed wings emerged from 654 treated individuals in the 250 Gy treatment.

A large-scale confirmatory study was conducted with late instars because this appeared to be most tolerant stage of the life stages found in fruit. The confirmatory test focused on a target dose of 250 Gy, which is the minimum absorbed dose currently required for export of Hawaii's fruits that are hosts for pest fruit flies (namely *Bactrocera dorsalis*). Of 11,256 late instars irradiated with a target dose of 250 Gy, 951 pupated (8.4%) and none eclosed as adults (Table 3). Actual absorbed doses measured by dosimetry ranged from 150 to 289 Gy (maximum/minimum = 1.9) for the first five replicates, and 210 to 273 Gy (maximum/minimum = 1.3) for the last six replicates.

In general, tolerance of pupae to irradiation treatment increased with increasing age (Table 4). No eggs were produced by surviving females when 1- to 2-d-old and 4- to 5-d-old pupae were treated with target doses of 125 and 250 Gy. In 7- to 8-d-old pupae, survival to adult after treatment with target doses of 125 and 250 Gy was 67.7% and 52.4%, respectively. In the 7- to 8-d-old pupae group treated at 125 Gy, 36 females laid 623 eggs and three were fertile, whereas in the 250 Gy treatment only one female laid six eggs that were infertile (Table 4).

Linear regression on the data from Tables 2–4 was used to predict the irradiation dose needed to prevent adult emergence in *Cryptophlebia.* The 7-to 8-d-old pupa was predicted to require the highest irradiation dose to prevent adult emergence (estimated 617 Gy), whereas the egg and early larval stages were predicted to require the lowest doses to prevent adult emergence (Table 5). Based on this regression analysis the accepted treatment of 250 Gy would not prevent adult emergence of late instars and 7- to 8-d-old pupae (but see later discussion concerning efficacy against late instars).

Irradiation treatment of adult moths affected successful reproduction (Table 6). In the premating irradiation experiment, when both adults were irradiated (IF × IM) at 125 Gy, 0.7% of eggs laid developed to stage 3 (larva visible), and no eggs reached stage 4 (head capsule visible) or hatched. When both parents were treated at 250 Gy, 0.9% of eggs laid developed to stage 2 (pink color) and no eggs developed farther or

Table 3. Effects of irradiation on maturation of *C. illepida* larvae

Instar	Dose (Gy)	Reps	*n*	No. pupae	No. adults	No. reproductive females	Total eggs	Total neonates
L1	0	5	255	173	143	24	954	762
	62.5	5	459	212	66	1	20	0
	125	5	807	36	2	0	—	—
	250	5	873	0	—	—	—	—
	400	5	1,035	0	—	—	—	—
L2/3	0	4	204	132	106	12	408	328
	62.5	4	291	159	47	0	—	—
	125	4	399	191	0	—	—	—
	250	4	489	33	0	—	—	—
	400	6	483	0	—	—	—	—
L4/5	0	4	146	119	81	18	812	686
	62.5	4	222	168	78	7	117	1
	125	4	414	295	6	0	—	—
	250	4	654	318	1	0	—	—
	400	4	822	290	0	—	—	—
L4/5 confirmatory test								
	0	5	696	—	597	169	6,858	4,441
	250	5	11,256	951	0	—	—	—

Table 5. Linear regressions on prevention of adult emergence when various life stages of *C. illepida* were irradiated at target doses of 0, 62.5, 125, 250, and 400 Gy

Stage	Obs.	Y-intercept ± SE	Slope ± SE	R^2	Predicted dose for 100% mortality, Gy
Egg	3	74.2 ± 1.7	0.21 ± 0.02	0.99	123
L1	4	60.5 ± 15.0	0.20 ± 0.10	0.64	197
L2/3	3	51.3 ± 7.3	0.42 ± 0.09	0.95	116
L 4/5	5	60.1 ± 12.3	0.13 ± 0.06	0.63	307
Pupa, 1–2 d old	3	29.3 ± 6.8	0.29 ± 0.04	0.98	244
Pupa, 4–5 d old	3	20.0 ± 23.9	0.35 ± 0.15	0.85	229
Pupa, 7–8 d old	3	13.7 ± 1.5	0.14 ± 0.01	0.99	617

hatched. When one parent was irradiated and the other left untreated (IF × UM or UF × IM), egg development and hatch was more common in the 125 Gy treatment compared with the 250 Gy treatment, and more common when the male was irradiated compared with when the female was irradiated (Table 6). This pattern has been observed in other irradiation studies with lepidopteran pests (e.g., Johnson and Vail 1987). When the male was irradiated and the female untreated, six eggs out of 411 (1.5%) hatched in the 125 Gy treatment, five of which developed to pupation and three emerged as normal appearing adults. When the female was irradiated and the male was untreated, one egg out of 331 (0.3%) hatched in the 125 Gy treatment and died as a neonate. The mean number of eggs laid per female was not significantly different among mating treatments at 125 Gy ($F = 1.1$, df = 2, 1; $P < 0.44$) or at 250 Gy ($F = 0.13$; df = 2, 1; $P < 0.87$). The mean number of eggs for each treatment at 250 Gy was about half of that at 125 Gy. At 125 Gy, eggs laid by females in the UF × IM treatment at 125 Gy had a significantly higher mean percent eclosion ($F = 15.8$; df = 2, 1; $P < 0.01$) than in the IF × IM and IF × UM treatments. No eggs eclosed from the pairings treated with 250 Gy. The untreated control groups ($n = 2$, total = 49 females) laid 27.4 (1.4) (mean ± SE) eggs and 39.4 (8.8)% eclosed. Mean longevity of females was 7.7 ± 0.43, 8.2 ± 0.35, 8.1 ± 0.4, and 7.5 ± 0.35 in the 125 Gy test, and 7.7 ± 0.38, 8.0 ± 0.37, 7.9 ± 0.32, and 7.6 ± 0.38 d in the 250 Gy test for the IF × IM, UF × IM, IF × UM, and UF × UM (control) treatments, respectively.

In the postmating irradiation experiment, irradiation of ovipositing females caused immediate sterilization. No fertile eggs were produced by 134 previously mated and ovipositing females that were irradiated with a dose of 250 Gy, whereas 14 untreated control females laid 527 viable eggs. Mean longevity after irradiation was 5.4 ± 0.8 d (mean ± SEM) for irradiated females compared with 4.6 ± 1.0 d for control females.

Discussion

Using a single diagnostic dose, *C. illepida* was determined to be more tolerant of irradiation than *C. ombrodelta.* Therefore, the efficacy of irradiation treatments against *C. illepida* is probably applicable for *C. ombrodelta* as well. Using the criterion of success in development to the adult stage, the pattern of tolerance to irradiation in *C. illepida* was generally eggs < early instars < late instars < pupae. For the stages likely to be found in harvested fruits (egg and larvae), fourth and fifth instars (L4/5) were determined to be the most tolerant stage of *C. illepida.* Development to the adult was reduced slightly in fourth and fifth instars receiving an irradiation dose of 62.5 Gy, whereas development to the adult was dramatically reduced in fourth and fifth instars receiving irradiation doses ≥125 Gy. No *C. illepida* larvae receiving an irradiation dose ≥125 Gy emerged as adults and produced viable eggs, indicating sterility can be achieved at doses well below 250 Gy. Large-scale tests irradiating fourth- and fifth-instar *C. illepida* at a target dose of 250 Gy resulted in no adult emergence. *C. illepida* typically leaves the fruit to pupate, therefore pupae would not normally be encountered in export

Table 6. Fitness of *C. illepida* when both parents, one parent, or neither parent receive(s) irradiation treatment

Dose, Gy	Pairing	No. pairs	No. females with eggs[a]	Total no. of eggs	Mean (± SE) no. eggs/female	Egg development maturity rating[b]						Mean (± SE) % eggs eclosed
						0	1	2	3	4	Eclosed	
125	IF × IM	36	28	695	24.7 (1.0) a	578	65	46	6	0	0	0.0a
	UF × IM	45	31	663	21.1 (3.1) a	501	19	22	35	73	13	0.42 (0.07) b
	IF × UM	42	30	637	21.2 (0.8) a	427	66	105	22	16	1	0.03 (0.03) a
250	IF × IM	41	24	384	14.0 (7.9) a	348	33	3	0	0	0	0.0a
	UF × IM	42	35	368	10.6 (0.2) a	342	6	7	3	10	0	0.0a
	IF × UM	38	32	414	12.6 (1.8) a	373	24	9	6	2	0	0.0a

I, irradiated; U, unirradiated; F, female; M, male. Means (± SE) within a column for a dose followed by different letters are significant using Student *t*-test ($P < 0.05$).

[a] Some pairings resulted in no eggs.

[b] Number of eggs developing to a given maturity rating: (0) eggs clear (no development, or infertile); (1) pink spots appearing on surface; (2) pink coloration throughout; (3) developing larvae visible within; (4) sclerotized head capsule evident.

shipments. An unlikely delay in irradiation treatment after harvesting and packaging of fruit might result in the presence of young pupae. Within the pupal stage, tolerance increased with age; 7- to 8-d-old pupae treated with an irradiation dose of 125 Gy produced viable offspring, whereas those treated with a dose of 250 Gy produced no viable offspring. Likewise, adult *Cryptophlebia* would not be expected in export shipments. Irradiation of unmated adult pairs before mating, or previously mated adult females, with an irradiation dose of 250 Gy resulted in no viable eggs after treatment. Therefore, the irradiation quarantine treatment of a minimum absorbed dose of 250 Gy approved for Hawaii's fruits will effectively disinfest fruit of any *Cryptophlebia* in addition to fruit flies.

Dose variation around the target dose can lead to erroneous conclusions about the dose required to ensure quarantine security. Detailed dose mapping of the full volume of the treatment chamber at the Hawaii Research Irradiator indicated that the maximum/minimum value is on the order of 1.8. Equally important to the maximum/minimum statistic is the distribution of doses (Fig. 1). By mapping with a large number of dosimeters (216) we could estimate the distribution of doses received by a population of insects evenly distributed in the chamber. The dose distribution in the chamber of the Hawaii Research Irradiator indicates that a few individuals might receive a dose that is almost one-half the target dose (Fig. 1). Dose variation probably accounted for the one L4/5 surviving to adult out of 654 treated individuals at 250 Gy in our stage specific tests using the full volume of the chamber (Table 3). In the large-scale confirmatory test at 250 Gy, where we treated insects in the center of the chamber only, to minimize dose variation, no L4/5s survived to adult out of 11,256 treated individuals.

Previous studies have suggested that insects irradiated in air are less tolerant than those irradiated in fruit because of lower oxygen levels inside fruits or because of other factors (Earle et al. 1979, Hallman and Worley 1999). Therefore, irradiation tests should involve treating insects in fruit. In our experiments, *C. illepida* was irradiated in diet because survivorship on its commercial fruit hosts is poor. Confirmatory studies with artificially infested fruit are impractical because of high control mortality (unpublished data).

The effects of gamma irradiation has been tested for a limited number of lepidopteran pests (Hallman 1998), including two tortricids, the codling moth, *Cydia pomonella* (L.) (Tortricidae) (Hathaway 1966, Burditt and Hungate 1989, Toba and Burditt 1992) and the leafroller *Clepsis spectrana* (Hallman 1998). Different treatment dose intervals and methods employed in each study make it difficult to make direct comparisons between species in tolerance to irradiation. Hallman (1998) estimated the dose required to achieve 100% sterility in adult males and females as 100 and 150 Gy for *Clepsis spectrana*, and >500 and 200 Gy for codling moth, respectively. In our study, adult males and females of *C. illepida* were sterilized at 250 Gy but not at 125 Gy. Sterilization of late pupae of *C. illepida* in our study required 250 Gy, also, supporting Hallman's (1998) suggested required dose range for tortricids and pyralids of 200–300 Gy. However, in our tests no *C. illepida* larvae receiving an irradiation dose ≥125 Gy produced viable eggs. Therefore, because *Cryptophlebia* larvae are the target, quarantine security for Hawaii's sapindaceous fruits could be achieved with an irradiation dose of 125 Gy.

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References Cited

Burditt, A. K., and F. P. Hungate. 1989. Gamma irradiation as a quarantine treatment for apples infested by codling moth (Lepidoptera: Tortricidae). J. Econ. Entomol. 82: 1386–1390.

Earle, N. W., L. A. Simmons, and S. S. Nilakhe. 1979. Laboratory studies of sterility and competitiveness of boll weevils irradiated in an atmosphere of nitrogen, carbon dioxide, or air. J. Econ. Entomol. 72: 687–691.

Federal Register. 1997. Papaya, carambola, and litchi from Hawaii. Rules and Regulations. Fed. Reg. 62 (132): 36967–36976, July 10, 1997.

Hallman, G. J. 1998. Ionizing radiation quarantine treatments. Ann. Soc. Entomol. Bras. 27: 313–323.

Hallman, G. J., and J. W. Worley. 1999. Gamma radiation doses to prevent adult emergence from immatures of Mexican and West Indian fruit flies (Diptera: Tephritidae). J. Econ. Entomol. 92: 967–973.

Hathaway, D. O. 1966. Laboratory and field cage studies of the effects of gamma radiation on codling moths. J. Econ. Entomol. 59: 35–37.

Johnson, J. A., and P. V. Vail. 1987. Adult emergence and sterility of indianmeal moths (Lepidoptera: Pyralidae) irradiatied as pupae in dried fruits and nuts. J. Econ. Entomol. 80: 497–501.

Jones, V. P., C.H.M. Tome, and L. C. Caprio. 1997. Life table for the koa seedworm (Lepidoptera: Tortricidae) based of degree-day demography. J. Econ. Entomol. 90: 1291–1298.

McQuate, G. T., P. A. Follett, and J. M. Yoshimoto. 2000. Field infestation of rambutan fruits by internal-feeding pests in Hawaii. J. Econ. Entomol. 93: 846–851.

Robertson, J. L, H. K. Preisler, E. R. Frampton, and J. W. Armstrong. 1994. Statistical analyses to estimate efficacy of disinfestations treatments, pp. 47–65. *In* J. L. Sharp and

G. J. Hallman [eds.], Quarantine treatments for pests of food plants. Westview, San Francisco, CA.

SAS Institute. 1997. JMP user's guide. SAS Institute, Cary, NC.

Sinclair, E. R. 1974. A life system study of *Cryptophlebia ombrodelta* (Lower) (Lepidoptera: Tortricidae) in Southwest Queensland. Ph.D. dissertation, University of Queensland, Australia.

Toba, H. H., and A. K. Burditt. 1992. Gamma irradiation of codling moth (Lepidoptera: Tortricidae) eggs as a quarantine treatment. J. Econ. Entomol. 85: 464–467.

USDA-APHIS-PPQ. 1997. Pest risk assessments: Hawaiian fruits. USDA, Riverdale, MD.

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