Cold Storage Enhances the Efficacy and Margin of Security in Postharvest Irradiation Treatments Against Fruit Flies (Diptera: Tephritidae)

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ABSTRACT Cold storage is used to preserve fruit quality after harvest during transportation in marketing channels. Low temperature can be a stressor for insects that reduces survivorship, and cold storage may contribute to the efficacy of postharvest quarantine treatments such as irradiation against quarantine insect pests. The combined effect of irradiation and cold storage was examined in a radiation-tolerant fruit fly, *Bactrocera cucurbitae* Coquillet (melon fly), and a radiation-intolerant fruit fly, *Ceratitis capitata* (Wiedemann) (Mediterranean fruit fly) (Diptera: Tephritidae). Third instars on diet or in papaya were treated with a sublethal radiation dose of 30 Gy and stored at 4 or 11°C for 3–13 d and held for adult emergence. For both fruit fly species, survival of third instars to the adult stage generally decreased with increasing cold storage duration at 4 or 11°C in diet or papaya. Survivorship differences were highly significant for the effects of substrate (diet > papaya), temperature (11 > 4°C), and irradiation (0 > 30 Gy). Few Mediterranean fruit flies survived in any cold storage treatment after receiving a radiation dose of 30 Gy. No melon fly larvae survived to the adult stage after irradiation and 11 d cold storage at 4 or 11°C in papayas. Cold storage enhances the efficacy and widens the margin of security in postharvest irradiation treatments. Potentially irradiation and cold storage can be used in combination to reduce the irradiation exposure requirements of quarantine treatments.

KEY WORDS irradiation, quarantine, postharvest, phytosanitary treatment, systems approach

Postharvest cooling and cold storage are the most important methods to slow quality loss in perishable commodities. Optimal cold storage conditions depend on the product (Kader 2002, Thompson et al. 2002). For exported, imported and domestic shipments of products, postharvest treatments such as fumigation, heat, or irradiation may be required to control quarantine pests, and these treatments are integrated into a product handling system that accommodates commodity-specific storage temperature conditions. Low temperature can be a stressor for insects (Denlinger and Lee 1998), and cold storage of products may cause mortality in quarantine insect pests. In fact, cold storage was one of the first methods used to control quarantine pests in traded commodities (Armstrong 1994, Gould 1994), with early research focusing on Mediterranean fruit fly control in peaches and apples (Back and Pemberton 1916, Mason and McBride 1934). Currently, temperatures of 0.6–2.2°C are used as a quarantine treatment for only a limited number of fresh commodities (citrus, grapes, and kiwis) because of the relatively long treatment time (12–22 d) (Willink et al. 2006), the strict requirements for maintaining cold temperatures for the duration of the treatment, and the fact that the temperature required to control insects can result in loss of quality (Gould 1994). However, use of slightly warmer storage temperatures (3–15°C) is critical to maintaining quality during the marketing of produce. Cold storage at these temperatures may enhance insect mortality and provide a margin of security for required postharvest quarantine treatments.

Tephritid fruit flies are among the most significant quarantine pests of perishable fruits and soft vegetables. Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) and melon fly, *Bactrocera cucurbitae* (Coquillet), are important fruit fly quarantine pests, and their introduction and establishment in countries where they do not exist would result in crop loss, increased control costs, and trade restrictions on the movement of fresh produce. Irradiation is a postharvest quarantine treatment option for horticultural commodities to prevent movement of viable tephritid fruit flies. In 2006, U.S. Department of Agriculture–Animal and Plant Health Inspection Service (USDA–APHIS) approved generic radiation doses of 150 Gy for any tephritid fruit fly and 400 Gy for all other insects except the pupa and adult stages of Lepidoptera (that may require higher doses) (USDA–APHIS 2006, Follett 2009, Follett et al. 2011). At the same

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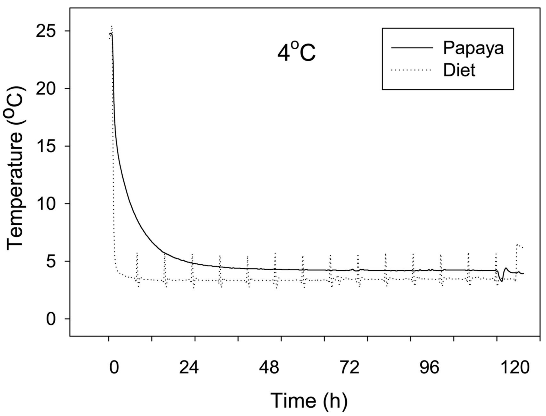

Fig. 1. Cold storage temperature profile at the surface of the diet and inside the papaya for the 4°C treatment.

time, lower doses were approved for 10 specific quarantine pests for which there was sufficient information. A radiation dose of 100 Gy was shown to be effective against Mediterranean fruit fly (Follett and Armstrong 2004, Torres–Rivera and Hallman 2007), and this dose has been adopted by USDA–APHIS and the International Plant Protection Convention (IPPC) (USDA–APHIS 2009, IPPC 2009). Melon fly is significantly more radiation-tolerant than Mediterranean fruit fly (Follett and Armstrong 2004), and the generic 150 Gy dose is required for this species.

Irradiation is increasingly used as a phytosanitary treatment (Follett 2009). Irradiation is an approved treatment to control quarantine pests in 17 fruits and 7 vegetables for export from Hawaii to the U.S. mainland (Follett and Weinert 2012). In recent years, India, Mexico, Pakistan, South Africa, Thailand, and Vietnam have obtained approvals to export fruits to the United States using generic radiation treatments, and Australia is exporting fruit to New Zealand and Malaysia using generic radiation treatments (Follett and Neven 2006). Fruit in transit are stored at low temperatures specific to the commodity, and the duration of cold storage depends on the distance to the export market and mode of transportation. For example, irradiated mangosteens are shipped from Thailand to the United States at 13–15°C, with a transit time of 4 wk; irradiated lychee are shipped from Australia to New Zealand at 5°C, with a transit time of 3–4 d; irradiated guavas are trucked from Mexico to the United States at 8°C and the trip requires 3–4 d; and irradiated longans are flown from Hawaii to the United States mainland and are maintained at 8–10°C for 3–4 d until they reach retail markets (Table 1).

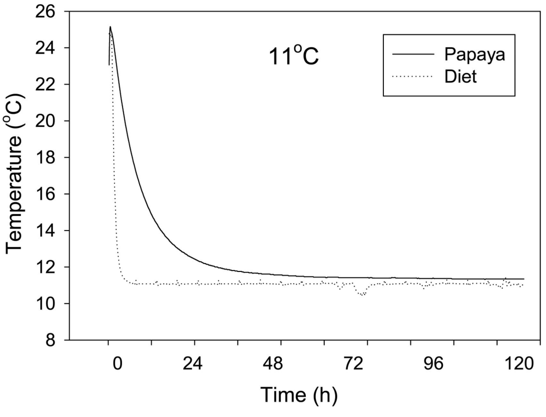

Fig. 2. Cold storage temperature profile at the surface of the diet and inside the papaya for the 11°C treatment.

The conceptual recognition that cold storage following phytosanitary treatment contributes to overall treatment efficacy is an extension of the systems-concept (Vail et al. 1993, Jang 1996), which integrates biological and physical factors with operational procedures to cumulatively address elements of quarantine security. However, documenting the extent that cold storage supplements efficacy relative to singular treatments has not been well-documented, particularly with respect to irradiation treatments. In the past, USDA–APHIS has considered cold storage temperature as a factor in measuring the likelihood of surviving shipment when preparing pest risk assessments for imported commodities. The effect of cold storage after radiation treatment was studied in Mediterranean fruit fly and melon fly to determine its ability to enhance the efficacy of the quarantine treatment and provide an added margin of security compared with no cold storage.

Materials and Methods

Mediterranean fruit flies and melon flies were obtained from laboratory colonies maintained at the USDA–ARS (Agricultural Research Service) Pacific Basin Agricultural Research Center in Hilo, HI, and reared on a standard diet for each species (Vargas 1989). Laboratory and wild strains of these fruit flies were shown to be equally susceptible to ionizing radiation (Follett and Armstrong 2004). Third instars were used in all tests because this is the most radiotolerant life stage found in fruit (Follett and Armstrong 2004, Follett et al. 2011).

For each species, eggs were placed on diet in plastic food service trays (TriPak, Toronto, Canada) with screened lids for 4 d at 24.5°C to obtain third instars. For diet experiments, cohorts of 100 early third instars were haphazardly selected from the food service trays and transferred to 60-ml plastic food service cups (Fabri-Kal Greenware, Kalamazoo, MI) with ≈50 ml of standard diet. The larvae in diet cups were placed inside 500-ml clear food service containers (Del-Pak, Rolling Meadows, IL) with 50 g of sand at the bottom for pupation, and covered with a screened lid. A 2-mm layer of dry diet was spread on top of the prepared diet to prevent mold. For papaya experiments, cohorts of 100 early third instars were haphazardly selected from the food service trays and introduced to the cavity at the center of a one half to three fourths ripe papaya through a hole made with a 12-mm cork borer, then sealed inside with a fruit plug and hot glue. Larvae

Table 1. Examples of fresh fruit and vegetable exports using phytosanitary irradiation and recommended cold storage temperatures during transit

Commodity	Cold storage temp (°C)	Origin and destination	Time in transit (d)	Mode of transportation
Dragon fruit	10–12	Vietnam to United States	11–14	Surface
Guava	8	Mexico to United States	1–4	Surface
Lemons	14	Mexico to United States	1–4	Surface
Longan	2–4	Thailand to United States	28	Surface
	8–10	Hawaii to the United States mainland	3–4	Air
Lychee	5	Australia to New Zealand	3–4	Surface
Mango	16–18	Australia to New Zealand	3–4	Surface
	10	Mexico to United States	1–4	Surface
Mangosteen	13–15	Thailand to United States	28	Surface
Papaya, banana	10	Hawaii to the United States mainland	3–4	Air
Oranges, mandarins	1–3	Hawaii to the United States mainland	8–10	Surface
	5–10	Mexico to United States	1–4	Surface
Sweet potato	8	Hawaii to the United States mainland	8–10	Surface

were transferred to papaya or diet 24 h before irradiation to allow larvae to feed and distribute themselves in the fruit.

Larvae on diet in plastic containers or in papayas were treated at a nearby commercial X-ray irradiation facility (CW Hawaii Pride, Keaau, HI) by using an electron linear accelerator (5MeV, model TB-5/15, Titan Corp., San Diego, CA). Detailed dose mapping was conducted before initiating the experiments, and ROW dosimeters (Opti-chromic detectors, FWT-70–40M, Far West Technology, Goleta, CA) were placed in representative diet trays or fruit with each replicate to measure dose variation. Dosimeters were read with an FWT-200 reader (Far West Technology) at 600-nm absorbance. A sublethal radiation dose of 30 Gy (measured doses 30–35 Gy) was used to allow comparisons between treatments. This was the lowest dose that could be applied using the commercial X-ray radiation equipment. To minimize the dose uniformity ratio (the ratio of the maximum/minimum dose), wooden racks holding a single row of papayas or diet trays were placed perpendicular to the X-ray beam and elevated by placement on a cardboard box positioned in the center of the carrier. With X-ray radiation, product moves in front of the beam on a conveyor belt, so individual fruit or diet trays pass in front of the beam sequentially and each can be considered a replicate.

Irradiated larvae on diet or in papayas were held in a walk-in cold chamber (Controlled Environmental Systems, Cypress, TX) at 4 or 11°C for 0, 3, 5, 7, 9, 12, or 15 d (Figs. 1 and 2). For Mediterranean fruit fly, cold storage intervals were shortened because of high mortality in the irradiation plus cold treatment. Temperature and humidity in the cold chamber were recorded with a HOBO Pro V2 datalogger (Onset, Pocasset, MA). HOBO dataloggers were placed in the food service container with diet, or in a cardboard box with papayas. At the end of each cold storage interval, larvae were transferred to a controlled environment room and held at 23°C and a photoperiod of 12:12 (L:D) h for pupation and adult emergence. Pupae were counted approximately 10 d after removal from the cold chamber, and adult flies were counted approximately 1 mo later after all adults had emerged and died. Controls were subjected to the same cold treatments (0, 3, 5, 7, 9, 12, and 15 d at 4 and 11°C) but did not undergo irradiation. The diet and papaya experiments were replicated a minimum of four times for each fruit fly species at each temperature in a completely randomized design. Prevention of adult emergence is the criterion for efficacy for phytosanitary irradiation treatment of tephritid fruit flies (Follett and Armstrong 2004). Adult emergence data were arcsine transformed and subjected to three-way analysis of variance (ANOVA) using the standard least squares model, with substrate, temperature, and radiation dose as main effects (Chew 1994, SAS Institute 2010). Data were also subjected to linear regression to estimate the cold storage time required for 99.99% mortality ($LT_{99.99}$). A significant negative slope of the regression line indicates a decrease in survivorship to adult with increasing duration of cold storage, and all cold intervals are assumed to be significant in their effects (Chew 1976).

Results

For both fruit fly species, survival of third instars to the adult stage generally decreased with increasing cold storage duration at 4 or 11°C in diet or papaya (Tables 2–7). For Mediterranean fruit fly, survivorship differences were highly significant ($P < 0.001$) for the main effects of substrate (diet > papaya), temperature (11°C > 4°C), and irradiation (0 > 30 Gy), and for the two-way interaction effects of substrate by irradiation and temperature by irradiation. The two-way interaction of substrate by temperature was not significant for Mediterranean fruit fly ($P = 0.23$). For melon fly, survivorship differences were highly significant ($P < 0.001$) for the main effects of substrate (diet > papaya), temperature (11°C > 4°C), and irradiation (0 > 30 Gy), and for the two-way interaction effect of temperature by irradiation. The two-way interactions of substrate by irradiation and substrate by temperature were not significant for melon fly ($P = 0.14$, $P = 0.07$, respectively).

For Mediterranean fruit fly not exposed to irradiation, survivorship in diet was 0.8% after 5 d at 4°C, compared with 93.3% in control insects not exposed to cold storage (Table 2). Survivorship in papaya was

Table 2. Effect of irradiation (30 Gy) plus cold storage at 4°C on development of third instar Mediterranean fruit fly to the adult stage

Substrate	Days in cold storage	4°C			Irradiation + 4°C		
		No. larvae treated	% pupation (mean ± SE)	% adult emergence (mean ± SE)	No. larvae treated	% pupation (mean ± SE)	% adult emergence (mean ± SE)
Diet	0	400	97.8 (1.3)	93.3 (1.8)	400	97.0 (3.0)	0.3 (0.3)
	1	400	76.0 (15.7)	72.8 (16.6)	400	94.5 (3.0)	0.5 (0.3)
	2	400	88.3 (6.20)	85.0 (7.9)	400	66.0 (2.7)	0.0
	3	400	38.3 (3.1)	28.3 (5.8)	400	60.0 (0.7)	0.0
	5	400	1.8 (0.9)	0.8 (0.3)	400	71.8 (0.9)	0.0
Papaya	0	400	65.8 (5.2)	43.8 (5.9)	400	71.5 (6.9)	0.3 (0.3)
	3	400	61.3 (7.1)	41.3 (6.3)	400	45.0 (12.0)	0.0
	5	400	26.3 (7.7)	9.0 (4.4)	400	17.0 (8.8)	0.0
	7	400	0.3 (0.3)	0.0	400	0.5 (0.5)	0.0
	9	400	1.3 (0.8)	0.0	400	1.0 (0.6)	0.3 (0.3)
	11	400	0.3 (0.3)	0.0	400	0.0	0.0
	13	400	0.3 (0.3)	0.0	400	0.0	0.0

9.0% after 5 d at 4°C, and no larvae in papaya survived to the adult stage after 7 d at 4°C. The effect of cold storage on survivorship was significantly less pronounced at 11°C compared with 4°C, as shown by the significantly shallower slopes of the regression lines ($P < 0.001$) for diet and papaya (Table 6). Survivorship in papaya not irradiated was ≈40–60% after 3–9 d cold storage at 11°C, but decreased to 16.5% after 13 d cold storage (Table 3). The predicted time to 99.99% mortality ($LT_{99.99}$) for Mediterranean fruit fly in papaya was 11.5 d at 4°C and 30.4 d at 11°C (Table 6). The $LT_{99.99}$ in diet was 5.1 d at 4°C and 41 d at 11°C. Mediterranean fruit fly was relatively sensitive to irradiation and few larvae survived to the adult stage after radiation treatment at 30 Gy, with or without cold storage (Tables 2 and 3), which prevented any examination of the interplay between irradiation and cold storage. Slopes for all 30 Gy treatments were not significantly different from 0 (Table 6).

For melon fly, survival of third instars to the adult stage generally decreased with increasing cold storage duration at 4°C or 11°C in diet or papaya (Tables 4, 5, and 7). Survivorship in diet not exposed to irradiation was 23.6% after 5 d at 4°C, compared with 64.6% in control insects not exposed to cold storage. (This relatively high rate of control mortality in diet [≈30%] is not unusual for melon fly and has been observed in other studies [Vargas et al. 2000, Follett and Armstrong 2004, Gayle et al. 2013].) Survivorship in papaya was 1.0% after 9 d at 4°C, and no larvae in papaya survived to the adult stage after 11 d at 4°C, whereas survivorship in control insects not exposed to cold storage was 38.3% (Table 4). (Likewise, other studies with melon fly have also shown increased control mortality in papaya of 20–30% compared with mortality on diet [Follett and Armstrong 2004, Gayle et al. 2013].) The effect of cold storage on survivorship was significantly less pronounced at 11°C compared with 4°C for melon flies in diet ($P < 0.001$) and papaya ($P < 0.001$). Survivorship in papaya not exposed to irradiation was ≈16.0% after 9 d cold storage at 11°C, and decreased to 8.8% after 13 d cold storage, compared with 57.3% in control insects not exposed to cold storage (Table 5). The predicted time to 99.99% mortality ($LT_{99.99}$) for melon fly in papaya was 12.1 d at 4°C and 15.4 d at 11°C (Table 7). The $LT_{99.99}$ in diet was 11.8 d at 4°C and 21.3 d at 11°C. Melon fly was more radiation tolerant than Mediterranean fruit fly. Survivorship in diet was 6.0% after radiation treatment at 30 Gy and cold storage at 4°C for 5 d, but no larvae survived after radiation treatment and 7 d at 4°C (Table 4). Survivorship in papaya was lower than in diet,

Table 3. Effect of irradiation (30 Gy) plus cold storage at 11°C on development of third instar Mediterranean fruit fly to the adult stage

Substrate	Days in cold storage	11°C			Irradiation + 11°C		
		No. larvae treated	% pupation (mean ± SE)	% adult emergence (mean ± SE)	No. larvae treated	% pupation (mean ± SE)	% adult emergence (mean ± SE)
Diet	0	900	97.2 (1.0)	85.3 (2.2)	500	95.8 (1.7)	0.0
	3	900	98.1 (0.60)	89.2 (1.7)	500	92.4 (0.9)	0.0
	5	900	93.3 (1.8)	78.4 (2.4)	500	85.8 (5.8)	0.0
	7	1,000	95.0 (2.5)	78.8 (2.7)	500	92.4 (2.8)	0.0
	9	900	90.3 (2.0)	64.9 (4.2)	500	79.6 (3.7)	0.2 (0.2)
Papaya	0	400	64.8 (21.7)	41.5 (15.3)	400	44.0 (20.4)	0.3 (0.3)
	3	400	69.8 (18.6)	62.8 (17.4)	400	48.8 (20.1)	0.3 (0.3)
	5	400	60.3 (14.3)	38.5 (13.3)	400	62.0 (7.9)	0.5 (0.5)
	7	400	68.0 (11.9)	60.5 (11.6)	400	85.3 (8.4)	0.0
	9	400	59.0 (7.8)	51.5 (8.1)	400	51.5 (11.2)	0.0
	11	400	62.8 (9.9)	33.3 (7.3)	400	65.3 (4.3)	0.0
	13	400	51.8 (8.0)	16.5 (3.7)	400	63.0 (4.3)	0.0

Table 4. Effect of irradiation (30 Gy) plus cold storage at 4°C on development of third instar melon fly to the adult stage

Substrate	Days in cold storage	4°C			Irradiation + 4°C		
		No. larvae treated	% pupation (mean ± SE)	% adult emergence (mean ± SE)	No. larvae treated	% pupation (mean ± SE)	% adult emergence (mean ± SE)
Diet	0	900	87.2 (3.0)	64.6 (5.4)	1000	86.1 (4.1)	18.2 (5.1)
	3	900	72.1 (2.4)	39.3 (2.9)	1100	64.5 (6.60)	15.3 (3.8)
	5	500	61.8 (5.1)	23.6 (4.9)	500	33.4 (6.1)	6.0 (1.1)
	6	400	35.3 (8.0)	13.0 (4.5)	600	15.8 (1.4)	0.2 (0.2)
	7	500	13.6 (3.1)	2.6 (1.2)	500	1.0 (0.6)	0.0
	9	900	0.3 (0.2)	0.1 (0.1)	1,100	0.1 (0.1)	0.0
	11	900	0.0	0.0	1,100	0.0	0.0
	13	900	0.0	0.0	1,100	0.0	0.0
Papaya	0	400	53.3 (13.2)	38.3 (10.3)	400	50.5 (14.2)	17.3 (7.9)
	3	400	60.8 (16.4)	42.8 (14.2)	400	0.5 (0.5)	0.0
	5	400	42.0 (22.6)	28.0 (15.5)	400	0.5 (0.5)	0.3 (0.3)
	7	400	1.8 (1.8)	0.8 (0.8)	400	0.3 (0.3)	0.0
	9	400	2.5 (2.5)	1.0 (1.0)	400	0.5 (0.3)	0.3 (0.3)
	11	400	0.0	0.0	400	0.0	0.0
	13	400	0.0	0.0	400	0.0	0.0

with only one survivor out of 400 treated larvae after irradiation at 30 Gy and 5 or 9 d cold storage at 4°C. Survivorship was 5–10% after irradiation and cold storage in diet at 11°C for 0–11 d, but decreased to <1% at 13 d (Table 5). No larvae survived to adult after radiation treatment at 30 Gy and cold storage in papaya at 11°C for 11 d. Y-intercepts for the regression response (mortality × cold storage duration) were lower for irradiated (30 Gy) compared with nonirradiated (0 Gy) melon flies at 4 and 11°C on diet and papaya (Table 7) because of the additive mortality stemming from cold storage. The shallower slopes of the response for irradiated compared with nonirradiated melon flies is counterintuitive, but reflects the consistently low survival to the adult stage in irradiated larvae. Y-intercepts for the regression response were consistently higher in unirradiated (0 Gy) treatments at 11°C compared with 4°C, indicating that lower temperatures cause higher mortality (Table 7). This trend was obscured in irradiated melon flies because of lower overall survivorship. The predicted time to 99.99% mortality ($LT_{99.99}$) for irradiated melon fly in papaya was 10.5 d at 4°C and 12.4 d at 11°C (Table 7).

Discussion

Although cold storage is used mainly to preserve fruit quality after harvest, it can be a stressor for insect pests that reduces survivorship, particularly after irradiation treatment. The insecticidal effect of cold storage depends on temperature and duration. Melon fly was more tolerant of irradiation than Mediterranean fruit fly. Whereas survival of melon fly third instars irradiated at 30 Gy was 5–18% without cold storage, survival of irradiated Mediterranean fruit fly was <1%. The low radiation dose of 30 Gy was selected to allow survivors so that the effect of cold duration with and without irradiation could be tested. Unfortunately, this radiation dose was still too high for Mediterranean fruit fly, and therefore the interplay between irradiation and cold storage could not be analyzed with this species.

Table 5. Effect of irradiation (30 Gy) plus cold storage at 11°C on development of third instar melon fly to the adult stage

Substrate	Days in cold storage	11°C			Irradiation + 11°C		
		No. larvae treated	% pupation (mean ± SE)	% adult emergence (mean ± SE)	No. larvae treated	% pupation (mean ± SE)	% adult emergence (mean ± SE)
Diet	0	400	97.8 (1.0)	72.0 (8.8)	500	98.0 (2.0)	4.8 (1.5)
	3	400	95.0 (2.7)	74.0 (3.8)	500	90.8 (2.1)	10.0 (1.5)
	5	400	96.3 (2.1)	79.3 (3.4)	500	84.4 (2.2)	6.8 (2.6)
	7	400	93.8 (2.5)	66.8 (4.6)	400	68.3 (1.9)	8.8 (1.8)
	9	400	89.5 (2.4)	60.5 (7.2)	400	84.0 (5.9)	7.5 (0.5)
	11	400	89.3 (6.0)	35.8 (5.4)	400	69.5 (5.1)	6.0 (1.5)
	13	400	90.5 (3.2)	14.0 (1.9)	400	75.3 (4.6)	0.8 (0.5)
Papaya	0	300	82.3 (4.3)	57.3 (6.4)	400	83.0 (10.6)	13.3 (6.2)
	3	400	77.5 (7.2)	54.0 (3.8)	400	78.8 (1.9)	7.8 (5.5)
	5	400	82.8 (6.7)	52.5 (11.2)	400	81.5 (5.3)	7.5 (1.7)
	7	400	75.3 (7.4)	34.3 (13.0)	400	60.3 (9.5)	3.0 (1.9)
	9	400	51.8 (6.4)	16.0 (5.0)	400	67.5 (5.5)	0.8 (0.5)
	11	400	49.8 (8.6)	7.8 (1.4)	400	49.8 (7.5)	0.0
	13	400	67.8 (1.8)	8.8 (1.3)	400	60.3 (4.3)	0.0

Table 6. Regression analysis of the effect of irradiation and cold storage on percent survival of Mediterranean fruit fly third instars to the adult stage

Substrate	Temperature	Radiation dose (Gy)	Y-intercept (±SE)[a]	Slope (±SE)	R^2	Predicted $LT_{99.99}$ (d)[b]
Diet	4°C	0	96.8 (7.4)	−19.2 (2.6)	0.76	5.1 (4.3–6.3)
		30	0.3 (0.1)	−0.08 (0.05)	0.14	ns
	11°C	0	90.0 (2.5)	−2.2 (0.4)	0.36	40.8 (30.4–65.3)
		30	−0.04 (0.07)	0.02 (0.01)	0.08	ns
Papaya	4°C	0	36.7 (4.4)	−3.2 (0.5)	0.61	11.5 (9.7–14.2)
		30	0.13 (0.1)	−0.008 (0.01)	0.02	ns
	11°C	0	57.2 (8.5)	−1.9 (1.0)	0.13	30.4 (18.0–)
		30	0.33 (0.2)	−0.03 (0.02)	0.07	ns

[a] Linear regression of percent mortality (calculated from data in Tables 2 and 3) against numbers of days of cold storage.
[b] ns, slope was not significantly different from 0; otherwise slope was significantly negative ($P < 0.05$).

For both fruit fly species, survival of third instars to the adult stage generally decreased with increasing cold storage duration at 4 or 11°C in diet or papaya. Survivorship differences were highly significant for the effects of substrate (diet > papaya), temperature (11°C > 4°C), and irradiation (0 > 30 Gy). No melon fly larvae survived to the adult stage after irradiation in papaya and 11 d cold storage at 4 or 11°C, whereas survivorship averaged 13–17% when treated by irradiation alone. This demonstrates that cold storage can enhance the efficacy and widen the margin of security in postharvest irradiation treatments. Cold storage is ubiquitous during transportation in market channels, and therefore, it could be included as part of any systems approach for quarantine security, as it provides redundancy in reducing the risk of accidental introductions.

Potentially cold storage and irradiation could be used in combination to reduce the duration of a quarantine cold treatment or the dose level of a quarantine radiation treatment compared with when these treatments are used alone. The desired endpoint for research into a combination treatment would need to be determined. The desired response for cold treatments against fruit fly larvae is mortality or failure of larvae to pupate, whereas the desired response in irradiation treatment is typically prevention of adult emergence or adult sterility. An irradiation–cold combination treatment can be thought of as the use of cold to modify response to irradiation, or as the use of irradiation to modify response to cold. We chose to measure prevention of adult emergence because cold storage at sublethal temperatures is a common practice after irradiation treatment. Furthermore, the ultimate goal of a quarantine treatment is to prevent reproduction, which is most easily achieved in tephritid fruit flies by stopping development to the adult stage (Follett and Neven 2006).

An irradiation plus cold storage combination treatment would allow for wider use of cold as a quarantine treatment by reducing the duration of cold storage and potentially allowing the use of temperatures that are less damaging to the commodity. Presently quarantine cold treatments are used mainly for tephritid fruit fly control in citrus. The USDA–APHIS approved cold treatments for Mediterranean fruit fly in various fruits during overseas transit require 1.1–2.2°C for 14–18 d (USDA–APHIS 2002, 2004). South Africa exports citrus to Japan using a cold treatment of −0.6°C (±0.6°C) for 12 d to control any Mediterranean fruit fly. A cold treatment of <0°C for 14 d was approved separately for mandarins, and recent data suggest a cold treatment of <1.4°C for 16 d will provide quarantine security against Mediterranean fruit fly in oranges (Grout et al. 2011). Australia ships citrus to Japan at <1.0°C for 16 d for control of Mediterranean fruit fly (De Lima et al. 2007). Cold storage of carambola at 1.1 + 0.6°C for 12 d controlled melon fly, *B. cucurbitae*, and oriental fruit fly, *Bactrocera dorsalis* (Hendel), but allowed a small number of Mediterranean fruit fly survivors (Armstrong et al. 1995).

Irradiation treatments of 100 and 150 Gy are approved for Mediterranean fruit fly and melon fly, respectively, on all fresh commodities. For combination treatments, Von Windeguth and Gould (1990) showed that radiation treatment at 50 Gy followed by cold

Table 7. Regression analysis of the effect of irradiation and cold storage on percent survival of melon fly third instars to the adult stage

Substrate	Temperature	Radiation dose (Gy)	Y-intercept (±SE)[a]	Slope (±SE)	R^2	Predicted $LT_{99.99}$ (d)[b]
Diet	4°C	0	50.7 (3.2)	−4.3 (0.4)	0.72	11.8 (10.8–13.0)
		30	15.2 (1.9)	−1.3 (0.3)	0.36	11.7 (9.9–14.5)
	11°C	0	87.3 (4.9)	−4.0 (0.6)	0.67	21.3 (18.0–27.1)
		30	8.4 (1.3)	−0.3 (0.2)	0.11	ns
Papaya	4°C	0	39.5 (6.4)	−3.2 (0.7)	0.43	12.1 (9.7–17.3)
		30	8.21 (2.5)	−0.8 (0.3)	0.22	10.5 (6.9–22.7)
	11°C	0	62.8 (5.5)	−4.1 (0.6)	0.64	15.4 (13.1–19.4)
		30	11.2 (2.1)	−0.9 (0.2)	0.35	12.4 (9.4–19.6)

[a] Linear regression of percent mortality (calculated from data in Tables 4 and 5) against numbers of days of cold storage.
[b] ns, slope was not significantly different from 0; otherwise slope was significantly negative ($P < 0.05$).

storage at 1.1°C for 5 d provided control of Caribbean fruit fly, *Anastrepha suspensa* Loew, in grapefruit. Palou et al. (2007) demonstrated that 30 Gy X-ray radiation and subsequent exposure to 2 d at 1.0°C controlled Mediterranean fruit fly in clementine mandarins. In our study, melon fly and Mediterranean fruit fly third instars treated by a radiation dose of 30–35 Gy followed by 11 d at 4°C in papaya failed to pupate. An irradiation plus cold combination treatment might save treatment costs at an irradiation facility and could minimize any quality problems in radiation-sensitive produce. Reducing the duration of cold treatment, or increasing the required temperature, with the addition of irradiation would shorten the required treatment time, reduce costs, and improve quality by getting produce to markets sooner. Additional research is needed to demonstrate the efficacy of irradiation plus cold combination treatments by treatment of large numbers of insects in specific commodities under commercial conditions while assessing commodity quality (Follett and Neven 2006).

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