Large-Scale Confirmatory Tests of a Phytosanitary Irradiation Treatment Against *Sternochetus frigidus* (Coleoptera: Curculionidae) in Philippine Mango

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ABSTRACT The mango pulp weevil, *Sternochetus frigidus* (F.), is an important quarantine pest preventing the export of mangoes from the Philippines to the United States and other countries. Previously, a radiation dose of 100 Gy was proposed for phytosanitary treatment of *S. frigidus* based on dose–response studies with larvae, pupae, and adult weevils. To validate an irradiation treatment, large-scale confirmatory tests were conducted with adults (the most radiation-tolerant stage) in mangoes at 100 and 150 Gy. After treatment, adults were removed from fruit, sexed, and mated in pairs to observe any reproduction. At 100 Gy, adults laid a small number of eggs but none of the eggs hatched. At 150 Gy (measured doses 96.7–164.1 Gy), 4,559 treated weevils laid no eggs, indicating that this dose caused complete sterility. Irradiation treatment with a minimum absorbed dose of 165 Gy will therefore provide quarantine security for *S. frigidus* in exported Philippine mangoes.

KEY WORDS *Sternochetus frigidus*, mango pulp weevil, postharvest phytosanitary treatment, quarantine pest, disinfestation

Mango is a major Philippine fruit in terms of production and export. The mango pulp weevil, *Sternochetus frigidus* (F.) (Coleoptera: Curculionidae), is an important economic and quarantine pest of Philippine mango. *S. frigidus* is native to northeast India (Assam and Tripura provinces), Bangladesh, Burma, Thailand, Malaysia, Singapore, and Indonesia, including Irian Jaya (Australian Quarantine and Inspection Service [AQIS] 1990). It was first reported in the Philippines in 1987 in the southern-most city of Bataraza in Palawan province (Basio et al. 1994). In 2011, the area planted to mango in Palawan was 1,872 ha with production of 7,042 mt. The most popular cultivar is 'Carabao' (or Super), which is planted on 1,400 ha with production of 4,782 mt (Bureau of Agricultural Statistics [BAS] 2011). The presence of *S. frigidus* in Palawan has prevented the export of mangoes from the Philippines to the United States and other countries because of quarantine restrictions and the absence of an effective postharvest treatment. A small amount of mangoes is exported from Guimaras Island, which is certified free of the mango seed weevil, *Sternochetus mangiferae* (F.), and the mango pulp weevil by the United States Department of Agriculture–Animal and Plant Health Inspection Service (USDA–APHIS) and the AQIS.

In *S. frigidus*, mating and oviposition occur when developing Carabao mango fruits reach an average of 5.5 cm length (De Jesus et al. 2003). The female lays eggs singly on the fruit peel and covers it with secretions, which hold the eggs in place. Five larval instars develop inside the fruit. The fifth-instar pupa is voracious and prepares a hole that later becomes a pupal chamber or cell. The larvae of this weevil are the most destructive, feeding and developing on the pulp. Total development from egg to adult stage is 32 d. The adult remains in its pupal cell inside the fruit until the latter is fully rotten (De Jesus and Gabo 2000). In backyards in Brooke's Point, southern Palawan, infestation of trees was as high as 43% (De Jesus and Cortez 1998). An integrated pest management program developed involved the use of control strategies, namely, cultural methods (open-center pruning and sanitation), pest monitoring, and chemical control (Medina et al. 2005).

The difficulty in identifying *S. frigidus*-infested fruit by visual inspection makes postharvest treatment necessary for disinfestation. Postharvest disinfestation of mango against mango weevils has not been successful by using various treatments such as heat, cold, and fumigation because the weevil completes its life cycle inside a dry chamber or pupal cell, often times in the middle section of the fruit close to the seed (De Jesus and Gabo 2000), and treatments that control the weevil cause damage to the fruit in the process. Irradiation is a viable alternative for the disinfestation of mango

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against *S. frigidus*, as it is already an approved treatment against its close relative *S. mangiferae* in Hawaiian mangoes (Follett 2001, USDA–APHIS 2002). Irradiation does not negatively affect mango quality, does not leave toxic residues, and does not make food harmful to human health (World Health Organization [WHO] 1994).

The goal of irradiation for *S. frigidus* is adult sterility or prevention of reproduction because pupation and adult eclosion occur inside the host fruit (Follett and Neven 2006). Previous irradiation research on weevils attacking mango has focused on *S. mangiferae,* which does not occur in the Philippines. Small-scale research suggested that *S. mangiferae* might be sterilized at 100 Gy (Seo et al. 1974, Follett 2001). However, because of the low confidence in research supporting the 100-Gy dose, an irradiation dose of 300 Gy was eventually approved by USDA–APHIS for quarantine control of this pest in imported mangoes (USDA–APHIS 2002). The dose of 300 Gy has been used to export Hawaii mango to the U.S. mainland, but on a limited scale. The development of a quarantine treatment protocol against mango weevils has been difficult because the insect has only one generation per year, there is no artificial diet to facilitate rearing, and all life stages occur inside the fruit. Therefore, the use of Probit 9 (99.9968% mortality)-level testing as with tephritid fruit flies is not practical for mango weevils (Follett 2001).

USDA–APHIS has approved generic doses of 150 Gy for tephritid fruit flies and 400 Gy for all insects except pupa and adult Lepidoptera (Follett 2009). The 400-Gy generic dose is used to treat mangoes from India and Pakistan, several tropical fruits from Thailand, guava and mangoes from Mexico, and dragon fruit from Vietnam for export to the United States (Hallman 2012). Lowering the dose level below 400 Gy for treatment of *S. frigidus* will be beneficial to Philippine super mango because it will minimize any problems with fruit quality resulting from treatment and increase the capacity of the treatment facility owing to shorter treatment time.

Obra et al. (2013) studied radiation tolerance in *S. frigidus* for the first time. Based on dose–response tests with different life stages, the adult was determined to be the most tolerant life stage and 100 Gy (measured doses 93.4–114.4 Gy) appeared to be sufficient to prevent reproduction in adult weevils. The current study was conducted to demonstrate through large-scale confirmatory tests that the radiation dose predicted to control *S. frigidus* is sufficient to provide quarantine security.

Materials and Methods

Source of Insects. *S. frigidus* used in the irradiation studies were obtained from Brooke's Point, Palawan. Because of the absence of an artificial diet for *S. frigidus,* mass rearing of the insect was done under field condition in Palawan by using developing fruits on mango trees (Obra et al. 2013). The Philippine Carabao mango (internationally known as Philippine super mango) was the variety used in all tests. Mango trees were chemically induced to flower by using potassium nitrate. At 65 d after flower induction, immature green fruits ≈6 cm in length were individually enclosed in nylon bags. A pair of male and gravid female weevils collected from infested ripe fruit and reared on fresh mango fruit in plastic containers in the laboratory was released into each bag for oviposition. Dissection of a sample of fruit was done weekly to determine weevil development to the adult stage (De Jesus and Gabo 2000). When weevils had developed to the adult stage, infested mangoes were harvested, packed in carton boxes, placed inside crates (five boxes/crate), and shipped to Manila by airfreight. The following day, fruit were transported to the Philippines Nuclear Research Institute (PNRI) for irradiation. Shipments were made under a special quarantine permit issued by the Bureau of Plant Industry, Department of Agriculture, and guarded by a Plant Quarantine Officer. Voucher specimens of adult *S. frigidus* were deposited in the University of the Philippines Museum of Natural History.

Irradiation. Irradiation was carried out at the PNRI Multipurpose Irradiation Facility, which was upgraded from a pilot scale to a semicommercial facility in 2008. Studies with *S. frigidus* were performed during the upgrade and used both the pilot-scale and semicommercial-scale configurations. In 2009, additional Co-60 isotope sources were loaded. The pilot-scale facility had eight individually operated source racks and four turntables for product handling. The semicommercial facility has a single source rack with four modules and products are placed in tote boxes. The initial trial of the confirmatory test at 100 Gy was carried out by using the pilot-scale facility; the activity of the Co-60 source was 33.87 kCi and the average dose rate was 324 Gy/min.

The three trials for the final confirmatory test at 150 Gy were performed by using the semicommercial facility. For trials 1 and 2, mangoes were irradiated by using the batch mode. In this set-up, mango boxes (46 by 32 by 14 cm) were placed in tote boxes and 14 tote boxes were loaded per batch and the tote boxes rotated automatically around the source during irradiation. For trial 3, the stationary mode of irradiation was used because of increased activity of the Co-60 source after reloading. In this setting, the boxes were rotated manually at half the irradiation time. For trials 1 and 2, only one mango box was placed per tote to improve the maximum/minimum dose ratio or dose uniformity ratio (DUR). In total, 50 boxes were irradiated in four batches. For trial 3, only three boxes were used per batch and positioned farther from the source to maintain a low DUR. In total, 50 boxes were irradiated in 17 batches. The activity of the Co-60 source as of January 2009 was 180 kCi. The average dose rate obtained from the first two trials was 311.7 Gy/min and for the last trial 83.6 Gy/min.

Fifty boxes each containing 20 mangoes (1,000 mangoes total) infested with adult weevils were irradiated to validate the target dose of 100 Gy. One hundred irradiated mangoes were cut-open at the time of irradiation to serve as check samples (to confirm the

Table 1. Large-scale confirmatory tests irradiating adult *S. frigidus* in mangoes at target doses of 100 and 150 Gy

Target dose (Gy)	Measured dose (Gy)				No reps	No. tested	No. eggs laid	No. eggs hatching	Mean no. eggs laid/female	Percentage hatchability
	Avg. min.	Avg. max.	Overall min.	Overall max.						
0 (control)	—	—	—	—	1	160	30,877	29,087	483	94.2
100 Gy	91.9	105.6	89.5	108.3	1	1,480	95[a]	0	—	—
0 (control)	—	—	—	—	3	440	87,431	84,451	510	95.9
150 Gy	114.4	137.7	96.7	164.1	3	4,549	0	—	—	—

[a] All collected from one adult female.

presence of adult weevils), whereas 100 unirradiated mangoes served as the control (to estimate the number of treated weevils). After irradiation of 800 infested mangoes, it was found that one adult female laid eggs that did not hatch. The criterion for efficacy was complete sterility (no oviposition), and therefore the target dose of 100 Gy was increased to 150 Gy in an attempt to prevent any oviposition.

Three trials each consisting of 55 boxes (1,100 mangoes) that were irradiated at a target dose of 150 Gy were carried out. One hundred mangoes served as check samples and another 100 unirradiated mangoes served as the control. In the final confirmatory tests using a target dose of 150 Gy, irradiated weevils from the minimum dose area of treated boxes were held separately from weevils in the area of boxes receiving the maximum dose to identify any possible reproduction that might have been because of dose variation. Pairing of adults for mating and data collection were done separately on the two groups.

Postirradiation. After conducting irradiation treatments, all test fruits, both treated (irradiated) and control (unirradiated or untreated) mangoes, still in boxes and placed inside crates, were held in the laboratory. Two days after irradiation or as soon as the mangoes became partly ripened, they were dissected to remove adult weevils. Surviving adult weevils were held in a perforated plastic container (maximum of 20 adults per container) and provided fresh green mango cubes for food. Approximately 10 d after fruit dissection, weevils were sexed following the procedure of De Jesus et al. (2002) and then paired to determine reproductive potential. Pairing of adults, feeding of paired adults, egg collection, and egg hatch determination followed the procedure of Obra et al. (2013). Ten males and 10 females were placed together in perforated plastic containers for mating. Fresh mango fruit cubes were provided daily, together with the tissue paper lining, for oviposition throughout the period of oviposition. Weevils laid eggs on the tissue paper. Eggs were collected by spraying the tissue paper with water and by using a pair of fine-pointed forceps, eggs were removed individually together with a small portion of the tissue paper to prevent egg damage. Eggs were transferred onto a black cloth square moistened by a wet absorbent cloth underneath in a petri dish (to prevent egg desiccation) and covered for hatch. Assessment of adult mortality in the perforated plastic containers was made daily.

The number of eggs laid per irradiated female and percentage egg hatchability were calculated. Similar procedures were performed with control weevils. The efficacy of treatments was determined based on adult sterility (i.e., no oviposition or egg laying). *S. frigidus* were held at a room temperature of 27.05 ± 0.93°C and 81.23 ± 7.14% relative humidity (RH) and a photoperiod of 12:12 (L:D) h.

Dosimetry. Fricke dosimeters were placed in boxes of mangoes to measure radiation dose during irradiation, and procedures followed guidelines according to ASTM Standards (American Society For Testing and Materials [ASTM] 2004) and traceable to the National Physical Laboratory of the United Kingdom. Dose mapping was performed on the mango boxes to determine the positions of the minimum and the maximum dose, the minimum dose rate, and the DUR. During irradiation of test samples, dosimeters were placed in the minimum and the maximum dose positions of the product load to monitor the dose received by the mangoes.

Statistical Analysis. For large-scale confirmatory tests, the level of confidence associated with treating a number of insects with zero survivors or offspring is given by the equation,

$$C = 1 - (1 - p_u)^n$$

where p_u is the acceptable level of survivorship (as a proportion) or reproduction and n is the number of test insects (Couey and Chew 1986). Confidence levels were calculated for the number of treated *S. frigidus* adults assuming the required efficacy ($[1 - p_u] \times 100$) is 99.99% (Follett and Neven 2006).

Results

The first shipment of mangoes infested with *S. frigidus* adults from Palawan to PNRI for confirmatory test was made on September 2008. This was used to validate the 100-Gy dose obtained in the dose–response test (Obra et al. 2013). Approximately 48% of the fruits were infested with *S. frigidus*, with approximately one adult weevil per infested fruit and a sex ratio of 0.51: 0.49 (male: female). For the final confirmatory tests using the target dose of 150 Gy, infested mangoes were shipped to PNRI during January and May 2009. Approximately 49.7% of these fruits were infested with, *S. frigidus*, with approximately two adult weevils per infested fruit and a sex ratio of 0.51:0.49 (male: female).

Table 1 shows the result of the confirmatory tests with adult *S. frigidus* at 100 and 150 Gy. Although the dose–response tests suggested that a dose of 100 Gy would be sufficient to prevent reproduction in adult *S. frigidus* (Obra et al. 2013), confirmatory tests using 100 Gy resulted in one adult that laid 95 eggs that did not hatch. We did not separate the adults obtained from mangoes in the minimum dose area from adults in the maximum dose area in this trial, but we speculated that this adult might have possibly received the lowest minimum absorbed dose of 89.5 Gy. For the control, the mean reproduction per female was 483 eggs with 94.2% hatch rate, indicating that conditions at the laboratory were suitable for mating and oviposition by *S. frigidus.* The a priori criterion for efficacy was prevention of oviposition. Therefore, the confirmatory dose was increased to a higher dose (150 Gy) for the remaining three trials. The total number of insects treated with a target dose of 150 Gy was 4,549 adults, whereas 440 adults served as the untreated control. No eggs were laid by any irradiated females in the three replications or trials, indicating complete sterility. The highest dose measured during confirmatory testing at the target dose of 150 Gy was 164.1 Gy. For the control, the mean number of eggs laid per female was 510 eggs with percentage hatchability of 95.9%, indicating that experimental conditions were optimal for mating and reproduction.

In large-scale validation tests, a radiation dose of 150 Gy applied to adult weevils resulted in no reproduction in 4,549 treated individuals (Table 1). Assuming a required efficacy of 99.99%, $C = 1 - (1 - 0.0001)^{4,549}$, and our confidence level is 36.6% that the true sterility of *S. frigidus* is <0.0001.

Discussion

The initial large-scale confirmatory tests used a target dose of 100 Gy based on results from dose–response tests with *S. frigidus* (Obra et al. 2013). The stringent criterion for efficacy was no oviposition. The confirmatory test at 100 Gy resulted in one adult female laying infertile eggs, indicating sterility. In some cases, the predicted dose from dose–response tests may be exceeded by the actual dose needed to achieve quarantine security at a given level of precision. In the case of the melon fly, *Bactrocera cucurbitae* (Coquillett), dose–response tests suggested 120 Gy applied to late third instars would be sufficient to prevent adult emergence, but a small number of flies emerged and the quarantine dose was increased to 150 Gy (Follett and Armstrong 2004). Although *S. frigidus* did not lay fertile eggs after treatment with a radiation dose of 100 Gy, increasing the dose to 150 Gy for validation testing added a margin of safety. Increasing the dose to 150 Gy resulted in no oviposition by irradiated adult females.

In our study, the DUR that was calculated during treatment from all batches and replications was 1.21 and this is comparable with the typical DUR for a Gamma Cell 220, which has a small radiation chamber volume. In large-scale commercial irradiators, the DUR could be as high as 3.0. The DUR needs to be minimized to reduce variation in experimental tests (Follett et al. 2007). The highest or overall maximum absorbed dose measured from all of the three trials irradiated at 150 Gy was 164.1 Gy. The maximum dose measured during confirmatory testing becomes the minimum absorbed dose for quarantine treatment in commercial applications (nominally 165 Gy) (Heather 2004, Follett and Neven 2006).

It is recommended that Philippine mangoes for export should receive an irradiation treatment of 165 Gy because this dose was found sufficient to prevent reproduction based on confirmatory tests. Although the number of insects tested was relatively low by quarantine treatment standards (Follett and Neven 2006), the 165 Gy dose includes a significant margin of safety and should provide quarantine security for *S. frigidus.* The International Plant Protection Convention has recently established a dose of 165 Gy for another curculionid species, *Cylas formicarius elegantulus* (Summers) (Hallman 2012), and USDA–APHIS has approved a dose of 150 Gy for this species (Follett 2006). Lowering the dose from 400 to 165 Gy for *S. frigidus* will minimize fruit quality problems, lower treatment costs, and increase the capacity of the treatment facility because of shorter treatment time (Follett 2009).

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