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Research Article

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Irradiation for Quarantine Control of Coffee Berry Borer, *Hypothenemus hampei* (Coleoptera: Curculionidae: Scolytinae) in Coffee and a Proposed Generic Dose for Snout Beetles (Coleoptera: Curculionoidea)

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Abstract

Coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae), is the most serious insect pest of coffee worldwide. Green coffee used in blending and roasting is traded between countries and may be subjected to fumigation for disinfestation of CBB. For example, green coffee shipped to Hawaii from the U.S. mainland must be treated with methyl bromide. Irradiation is an alternative disinfestation treatment option. Dose-response tests were conducted with adult beetles to identify a sterilizing dose, followed by large-scale confirmatory tests with adults infesting coffee berries at 100 Gy (measured doses 84–102 Gy). In total, 6,598 adult CBBs naturally infesting dried coffee berries were irradiated at 100 Gy and produced no viable offspring, whereas 1,033 unirradiated controls produced 327 eggs, 411 larvae, and 58 pupae at 3 wk post treatment. This is the first study to develop a postharvest irradiation treatment for a scolytine bark beetle and supports other studies suggesting 150 Gy is sufficient to prevent reproduction in snout beetles in the superfamily Curculionoidea.

Key words: phytosanitary irradiation, quarantine pest, x-ray, coffee berry borer, Curculionoidea

The coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae), is the most important pest of coffee worldwide, with damage exceeding US$500 million annually (Jaramillo et al. 2006). CBB was first discovered in Hawaii in 2010 on the Kona side of the island of Hawaii where there are about 800 small farms on 1,050–1,660 ha (Gaertner et al. 2017), and it has since moved to the island of Oahu and recently to the island of Maui. CBB is the greatest threat to Hawaii's coffee industry. Current levels of coffee infestation by CBB average 15–20%, which causes yield loss, reduced quality and price, and increased costs (Aristizabal et al. 2017). If left unchecked, CBB can infest >90% of coffee berries at harvest. CBB has not yet established on the island of Kauai, a major coffee production area with about 2,000 ha coffee.

All CBB life stages develop inside the coffee berry (Aristizabal et al. 2017). Adult females bore into hardened developing green berries and excavate tunnels and galleries in which they lay their eggs during a 3-wk period (Baker et al. 1992). Inside the berry, CBB develops through egg, larva, prepupa, pupa, and adult stages during 1–2 mo (Baker 1999). Berry development requires 200–250 d from flowering to harvest, and therefore, individual berries can support multiple generations and large numbers of CBB (Baker et al. 1992). CBB will continue to breed in coffee berries as they ripen, senesce, and dry to the "raisin" stage on the coffee tree.

Green coffee used in blending and roasting is traded between countries and may be subjected to fumigation or other treatment for disinfestation of CBB and the fungal disease coffee leaf rust. Green coffee shipped to Hawaii from the U.S. mainland is typically treated by methyl bromide in California and must carry a certificate of treatment upon arrival. Within Hawaii, CBB is still not established on the islands of Kauai, Lanai and Molokai, and green coffee berries moved from infested islands (Hawaii, Oahu, Maui) to these islands require treatment by fumigation (methyl bromide or sulfuryl fluoride), heat (157.2°C for 5 min) or freezing (–15°C for 48 h) (Hollingsworth et al. 2013). Roasters wishing to move coffee between islands are registered with and certified by the Hawaii Department of Agriculture (HDOA) (L. Sakaino, personal communication). Irradiation is a potential alternative disinfestation treatment to provide for safe movement of coffee berries between infested and uninfested islands in Hawaii. Studies were conducted to identify an irradiation dose that will sterilize CBB in coffee and verify a treatment protocol that provides quarantine security.

Methods

Insect Rearing

Adult CBBs were collected from over-ripe coffee in the field and reared in the laboratory on an artificial diet modified from

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Brun et al. (1993). Diet modifications included first dissolving methyl-p-hydroxybenzoate, sodium propionate, and ascorbic acid in 25-ml 95% ethanol before mixing, baking the completed diet at 55°C for 8–10 h followed by autoclaving, dipping adult beetles collected from the field in 95% ethanol for 5 s (to minimize *Beauveria bassiana* infection), and rinsing in distilled water before placement on diet in 50-ml plastic cups (Follett et al. 2016, Sim et al. 2016). The CBB colony was infused with new adults from the field approximately every 2 mo during the study to ensure a sufficient number of test insects.

Dose-Response Tests

For irradiation, colony adults were extracted from the diet and transferred to 29.6-ml polystyrene portion cups (Solo Cup Company, Highland Park, IL) containing 10-ml fresh diet. CBB has a female-biased sex-ratio of 10:1 (Baker 1999), so most of the beetles used in the tests were assumed to be reproductive females. Depending on available numbers, 20–30 beetles were transferred to each cup and held for 24 h to allow beetles to bore into the diet. Cups with plastic tops containing beetles were transported to a nearby commercial x-ray irradiation facility (CW Hawaii Pride, Keaau, HI), and treated with x-rays using an electron linear accelerator (5 MeV, model TB-5/15, Titan Corp., San Diego, CA). This facility was designed to apply low-dose irradiation for phytosanitation of fresh produce. Adult beetles were exposed to 50, 75, 100, or 125 Gy of x-ray irradiation or left untreated as a control. Radiation treatments were replicated five times. To control dose uniformity (the ratio of the maximum/minimum dose), a wooden rack holding a single row of the cups with beetles was placed perpendicular to the x-ray beam and elevated by placement on two 1.9-liter plastic containers (polypropylene El-610–64, Highland Plastics, Inc., Mira Loma, CA) and positioned in the center of the carrier. ROW dosimeters (Optichromic detectors, FWT-70-83M, Far West Technology, Goleta, CA) were placed in empty 29.6-ml cups in four locations (one at both ends and two in the middle) on each wooden rack to estimate dose delivery and variation during treatment. Dosimeters were read with an FWT-200 reader (Far West Technology) at 600-nm absorbance. Dosimeters were calibrated and certified by the National Institute of Standards and Technology (NIST) (Gaithersburg, MD) according to international standards. Measured doses were 47–59 Gy for the 50-Gy treatment, 73–88 Gy in the 75-Gy treatment, 87–118 Gy in the 100-Gy treatment, and 120–146 Gy in the 125-Gy treatment. After irradiation, cups with insects were placed in controlled temperature cabinets at 24°C and 24 h darkness. At 3 mo post treatment, cups with insects were opened, and the diet removed and carefully inspected under a stereomicroscope to count the number of eggs, larvae, pupae, and adults present.

Large-Scale Confirmatory Tests

CBB-infested coffee “raisins” (dried berries) were collected from the University of Hawaii Kainaliu Experimental Station (Kainaliu, HI) in January 2016 and January–February 2017 for four large-scale irradiation tests to determine if 100 Gy would prevent CBB reproduction. Thirty replicates of 100 raisins were set up in 118.3-ml Whirl–Pak bags (Zefon International, Ocala, FA). Approximately, 12–16 h after placing infested raisins in whirl paks, samples were transported to the Hawaii Pride commercial x-ray irradiation facility (Keaau, HI) and treated using x-ray irradiation at a radiation dose of 100 Gy as described above. With x-ray radiation, product moves in front of the beam on a conveyor belt, so individual bags of cherries pass in front of the beam sequentially, and each bag can be considered a replicate. Measured doses during the large-scale tests were 84–102 Gy.

Immediately after irradiation, the raisins were brought back to the laboratory and placed in modified Berlese funnels with a 40-W bulb for 20–26 h. After 20 h, CBB that had left the coffee raisins were counted and placed on CBB diet in 118.3-ml glass jars (Wheaton Science Products, Millville, NJ). The glass jars containing diet were placed in an environmental chamber at 25°C for 21 d (3 wk) to allow egg laying and life stage development up to the pupal stage. CBB adults were followed for a 3 wk, which is the normal reproductive period and sufficient time for fertile eggs to hatch and potentially (for eggs laid soon after treatment) develop to the pupal stage. After 21 d, diet containers were removed from the environmental chamber and carefully dissected, and all life stages (eggs, larvae, pupae, alive adults, and dead adults) were counted. If eggs were found on diet from treated CBB, they were placed on a moist filter paper in a Petri dish for an additional 10 d. These eggs were inspected daily to observe any hatch.

Statistical Analysis

For the dose-response studies, data on numbers of different life stages at 3 mo post treatment were used to calculate the average reproduction per adult. The average reproduction per adult was calculated as the numbers of (eggs + larvae + pupae + live adults)/adults tested in each replicate and averaged for each radiation dose including the 0 Gy control. Average reproduction values were subjected to linear regression to estimate a predicted dose for no reproduction or zero survivors. For large-scale confirmatory tests, the level of confidence associated with treating a number of insects with zero survivors is given by the equation,

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

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

Results

In the dose-response tests, adults irradiated at 75 Gy produced a small number of eggs that did not hatch, and adults irradiated at 100 and 125 Gy were completely sterilized (zero survivors at 3 mo) (Table 1). The regression line describing average reproduction per adult was 5.78–0.06 (dose) ($R^2 = 0.60$, $n = 28$), and the predicted dose (±95% CL) for zero reproduction was 96.2 (82.1–116.7) Gy. After review of the dose-response results, 100 Gy was chosen for subsequent large-scale confirmatory tests. In large-scale tests, a total of 6,598 adult CBBs naturally infesting coffee berries were irradiated at 100 Gy with no survivors (no offspring) at 3 wk post treatment (Table 2). The small number of eggs that were laid by irradiated adults at 100 Gy did not hatch. In unirradiated controls, a total of 1,033 adults produced 327 eggs, 411 larvae, and 58 pupae at 3 wk post treatment. Assuming a required efficacy of 99.99%, $C = 1 - (1 - 0.0001)^{6598}$ and the confidence level was 48.3% that the true survival of CBBs irradiated at 100 Gy was less than 0.0001.

Discussion

This study showed that irradiation of CBB adults infesting coffee berries at 100 Gy prevented further reproduction. Thus, irradiation could be used as a postharvest disinfestation treatment to prevent the movement of CBB during interisland shipment of green coffee.

Table 1. Dose-response experiment with CBB adults

Total at 3-mo post treatment								
Dose (Gy)	Reps	No. tested	No. eggs	No. larvae	No. pupae	No. live adults	No. dead adults	Avg. reprod.*
0	5	108	118	117	34	546	105	7.9 (1.3)
50	6	116	49	1	1	12	104	0.5 (0.2)
75	6	138	9	0	0	2	136	0.1 (0.07)
100	6	142	0	0	0	0	142	0
125	5	119	0	0	0	0	119	0

Adults were irradiated then placed on fresh diet to measure reproduction. All life stages were counted after 3 mo.

The regression line describing average reproduction per adult = 5.78–0.06 (dose) (R^2 = 0.60, n = 28).

The predicted dose (±95% CL) for $y = 0$ (no reproduction) = 96.2 (82.1–116.7) Gy.

*Average reproduction per adult = (eggs + larvae + pupae + live adults)/adults tested.

Table 2. Large-scale testing of CBB adults

Total at 3-wk post treatment					
Dose (Gy)	Replicate	No. tested	No. eggs	No. larvae	No. pupae
0	1	238	141	204	21
100		2,390	32*	0	0
0	2	213	23	19	21
100		1,186	0	0	0
0	3	162	18	25	8
100		1,088	4*	0	0
0	4	420	145	163	8
100		1,934	3*	0	0

Adults were irradiated at 100 Gy in infested coffee beans then removed and placed on diet to measure any reproduction and development after 3 wk. Tests were conducted on four separate dates which served as replicates.

Total number of adults sterilized at the target dose of 100 Gy = 6,598.

*Eggs did not hatch.

This study also contributes important new information about a previously unstudied group of beetles to aid in the establishment of a generic dose for the family Curculionidae or more broadly for the superfamily Curculionoidea.

In 2006, the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (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 (which may require higher doses) (USDA APHIS 2006). The generic radiation treatments apply to all fresh horticultural commodities. USDA APHIS has expressed interest in developing additional generic doses for broad groups of quarantine pests to lower the dose from 400 Gy. USDA APHIS recently published a generic dose of 290 Gy for quarantine treatment of eggs and larvae of tortricid moths (Lepidoptera: Tortricidae) (USDA APHIS 2017, Nadel et al. 2018). Generic doses should be developed for the highest taxonomic level possible as supported by available data to maximize their utility and prevent gaps in coverage due to changing phylogenetic relationships.

Snout beetles (Coleoptera: Curculionoidea) including the large family Curculionidae are an important group of internal quarantine pests that may have sufficient information to propose a generic radiation dose (Table 3) (Barkai-Golan and Follett 2017). CBB is placed in the subfamily Scolytinae (bark beetles) within the family Curculionidae. Table 3 contains species that attack fresh fruits and vegetables and stored products. A generic dose of 150 Gy was first proposed for the family Curculionidae in 2009 (Follett 2009). Although the phylogeny and evolution of weevils (superfamily Curculionoidea) have been extensively studied, especially the Curculionidae (>50,000 spp.), many relationships remain uncertain (Shin et al. 2017). We propose here to adopt a broader generic dose for the superfamily Curculionoidea, which includes the Curculionidae and other families, to avoid gaps in the future that may be created due to changing phylogenetic relationships and taxonomy. For example, at one time, *Cylas formicarius elegantulus* (sweetpotato weevil) was placed in the subfamily Cyladinae within the family Curculionidae (Borror et al. 1981), then later, it was placed in the family Apionidae (Borror et al. 1989), and currently, it resides in the family Brentidae (Arnett 2000). The taxonomic position of the bark beetles, to which CBB belongs, has changed over time as well. In the past, bark beetles were placed in the family Scolytidae separate from the Curculionidae (Borror et al. 1989). Crowson (1955) first proposed transfer to the subfamily Scolytinae within the Curculionidae, and this was firmly supported by Kuschel (1995) leading to its current taxonomic placement. Shifts in taxonomic position pose a challenge to regulatory rules that approve treatments, such as generic irradiation treatments, based on broad taxonomic groups (e.g., Tephritidae). Approving a generic dose for the superfamily Curculionoidea rather than the family Curculionidae would provide broader application and help avoid drops in coverage for quarantine pests in this group due to future shifts in taxonomic position.

The information presented in this paper on radiation tolerance in CBB is the first detailed study of irradiation for a scolytine bark beetle. While scolytine beetles are not typically pests of fresh horticultural commodities per se, in some cases, they may be found in traded fresh products and treated as quarantine pests, such as CBB in green coffee and tropical nut borer, *Hypothenemus obscurus* (Coleoptera: Curculionidae: Scolytinae), in macadamia nuts (Delate et al. 1994). Scolytine bark beetles are also significant quarantine pests in wood-packaging material and timber (Schortemeyer et al. 2011), and irradiation may be a useful treatment option to prevent movement of wood pests in these products. If the present study with *H. hampei* (CBB) is representative of other scolytine bark beetles, our data suggest this group can be sterilized at doses of approximately 100 Gy or less. These new data are consistent with the radiation tolerance information supporting a generic dose of 150 Gy for Curculionoidea.

The apparent outlier in the list of APHIS-approved irradiation treatments is the mango seed weevil, *Sternochetus mangiferae* (Coleoptera: Curculionidae). In 2006, a 300-Gy irradiation treatment was approved for mango seed weevil, a monophagous pest of mangos (Follett 2001, USDA APHIS 2003). This approval was a milestone for phytosanitary

Table 3. Radiation doses reported to sterilize adult curculionid weevils

Species	Common name	Dose (Gy)	No. tested	Milieu	Reference
Anthonomus grandis	Boll weevil	100	120	N_2	Earle et al. 1979
		100	~25	Bolls	Davich and Lindquist 1962
Blosyrus asellus	Rough sweetpotato weevil	50	53	Air	Follett et al. 2016
Conotrachelus nenuphar	Plum curculio	92	25,000	Apple	Hallman 2003
Cylas formicarius elegantulus	Sweetpotato weevil	150	60,000	Sweet potato	Follett 2006
Diaprepes abbreviatus	Diaprepes root weevil	50	220	Air	Gould and Hallman 2004
Euscepes postfasciatus	West Indian sweetpotato weevil	150	62,323	Sweet potato	Follett 2006
Hypera postica	Alfalfa weevil	80	30	Air	Burgess and Bennett 1966
Hypothenemus hampei	Coffee berry borer	100	6,598	Coffee	Present study
Pissodes strobi	White pine weevil	50	160	Air	Jaynes and Goodwin 1957
Phlyctinus callosus	Banded fruit weevil	80	200	Air	Duvenhage and Johnson 2014
Sternochetus mangiferae	Mango seed weevil	100	76	Mango	Follett 2001
		100	60	Air	Seo et al. 1974
Sternochetus frigidus	Mango pulp weevil	100	515	Mango	Obra et al., 2013
		165	4,549	Mango	Obra et al. 2014
Sitophilus oryzae	Rice weevil	120	32,025	Rice	Follett et al. 2013
Sitophilus granarius	Granary weevil	100	80	Wheat	Aldryhim and Adam 1999
Sitophilus zeamais	Maize weevil	70	280	Rice	Hu et al. 2003

Modified from Barkai-Golan and Follett 2017.

irradiation as it was the first approval for a nonfruit fly insect (a weevil), the first approval for an adult insect in fruit, and the first approval based on a less-then-probit 9 approach to quarantine security by USDA APHIS (Follett and Neven 2006, Follett and Griffin 2013). Although 300 Gy became the approved treatment, doses as low as 100 Gy had been shown to sterilize this weevil (Seo et al. 1974, Follett 2001), and initially, 100 Gy was recommended as an effective treatment for the export of mangos from Hawaii to the U.S. mainland (USDA APHIS 2002). The higher 300 Gy dose was adopted later due to concerns about the limited number of insects tested at the lower doses and the need, therefore, for a margin of safety. The closely related mango pulp weevil, *Sternochetus frigidus*, was sterilized at a radiation dose of 100 Gy (Obra et al. 2013), and large-scale confirmatory tests showed complete adult sterility of this weevil at a target dose of 150 Gy (maximum dose of 165 Gy) (Obra et al. 2014). Results with *S. frigidus* support eventually lowering the regulatory dose for *S. mangiferae*.

Before recommending a generic dose, radiation tolerance information should be available for a representative sample of species within the taxonomic group or for the important quarantine pests within the group that may be the target of irradiation disinfestation treatment (Follett 2009, 2014). Radiation tolerance has been studied in approximately 15 species of Curculionidae of economic and quarantine importance (Table 3). This list includes a significant level of taxonomic diversity within the Curculionoidea, including two families (Curculionidae, Brentidae) and eight subfamilies (Hallman 2017), and many of the Curculionoidea of phytosanitary concern. The list also reflects significant host diversity, including pests of fruits, vegetables, wood and wood products, and stored products. Large-scale testing, which is often required for approval of a treatment for a specific pest, has been conducted with adult insects in six species: *Conotrachelus nenuphar* (25,000 individuals tested), *Cylas formicarius* (60,000), *Euscepes postfasciatus* (63,232), *H. hampei* (6,598 [present study]), *Sitophilus oryzae* (32,025), and *S. frigidus* (4,538). USDA APHIS (2006) has approved 150 Gy as a specific treatment for *C. formicarius* (sweetpotato weevil) and *E. postfasciatus* (West Indian sweetpotato weevil), and 92 Gy for *C. nenuphar* (plum curculio). A 150-Gy treatment might also have been approved for *S. frigidus*, except that measured doses during large-scale testing exceeded the target dose, thereby leading to approval of 165 Gy instead for this species (Obra et al. 2013). The radiotolerance data available for Curculionoidea is comparable to the data used for establishing a generic dose for the lepidopteran family Tortricidae (280 Gy), which included a total of 12 species and large-scale testing of 6 species (Nadel et al. 2018). Establishing a generic treatment for Curculionoidea below 400 Gy would reduce treatment time for certain commodities, thereby minimizing any negative effects that irradiation treatment may have on commodity quality, reducing treatment costs, and increasing capacity for irradiation facilities (Follett 2009). A generic dose will also prevent interruption of shipments in the event of an incursion of a new snout beetle of quarantine importance.

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