Gamma Radiation as a Quarantine Treatment for Fuller Rose Beetle Eggs (Coleoptera: Curculionidae) on Citrus Fruit

JUDY A. JOHNSON, EDWIN L. SODERSTROM, DAVID G. BRANDL, LAURIE G. HOUCK, AND PAMELA L. WOFFORD

Horticultural Crops Research Laboratory,
Commodity Protection and Quarantine Insect Research Unit,
USDA-ARS, Fresno, California 93727

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ABSTRACT Since 1985 when eggs of the Fuller rose beetle, *Pantomorus cervinus* (Boheman), were found by Japanese fruit inspectors under the calyxes of California citrus, researchers have sought to develop alternatives to methyl bromide fumigation as a suitable quarantine treatment for this pest. Three different ages of Fuller rose beetle eggs laid on waxed paper were exposed to gamma radiation doses of 10, 50, 100, and 150 Gy. The oldest age class (10–13 d old) was the least susceptible. Egg hatch of the two younger age classes (1–3 and 6–8 d old) was prevented by 50 Gy, whereas 150 Gy was needed to prevent hatch of older eggs. To confirm the efficacy of the method, lemons infested with 10- to 13-d-old Fuller rose beetle eggs were placed in the center of standard cardboard lemon cartons and irradiated at doses averaging 174.1 Gy. Egg hatch from egg clusters infesting untreated lemons averaged ($\bar{x}$ ± SEM) 42.5% ± 4.66 per lemon. None of the estimated 6,500 eggs infesting irradiated lemons hatched. Damage of irradiated fruit varied but did not exceed a 6.1% increase compared with damage found in controls. These data show that irradiation of lemons could be an effective quarantine treatment against Fuller rose beetle eggs.

KEY WORDS Insecta, *Pantomorus cervinus*, gamma radiation, *Citrus*

FULLER ROSE BEETLE, *Pantomorus cervinus* (Boheman), a flightless, parthenogenic weevil with a broad distribution and host range, is believed to be present in most citrus-producing areas of the world, and was reported in California as early as 1879 (Woodruff & Bullock 1979). Until recently, California citrus growers considered this insect to be a problem only on newly planted trees (Griffiths et al. 1986). In 1985, eggs of the Fuller rose beetle were found under the calyxes of California citrus fruit exported to Japan, where the beetle is a quarantined pest (Haney et al. 1988).

Currently, Japanese authorities inspect all incoming shipments of fresh California citrus and require fumigation with methyl bromide of shipments found to be infested with the beetle. Such treatments cause considerable fruit damage, resulting in 5–15% loss in oranges and up to 65% in lemons (Griffiths et al. 1986). A deadline of June 1990 has been set by the Japanese for complete or near compete control of the beetle, with no beetle eggs in packinghouses or at shipping points (Anonymous 1988). If these conditions cannot be met, the Japanese may impose more severe quarantine measures, such as exclusion or mandatory fumigation of California citrus. Exclusion would be disastrous to California's citrus industry because Japan is the largest single export market for California citrus. In 1985, for example, Japan received 290 million kg of fresh lemons, grapefruit, and oranges worth $187 million (Haney et al. 1988). Mandatory fumigation would also be prohibitive because of the cost of treatment and resulting fruit loss.

Most current research on this problem deals with control of beetle populations in the orchard before harvest, including skirt pruning, trunk barriers, trunk and foliar pesticide application, and the development of a degree-day model (Morse et al. 1988). Although these solutions seem promising, they can be expensive and labor-intensive and may not provide the level of control stipulated by the Japanese. An alternative approach is development of treatments to replace methyl bromide fumigation after harvest. Unfortunately, neither cold storage nor alternative chemical treatments appear promising, and much more research is needed on high-temperature treatments (Haney et al. 1988; E.L.S., unpublished data).

Another possible alternative quarantine treatment is exposure to ionizing radiation. Because insect eggs are generally the stage most sensitive to radiation, relatively low doses can provide acceptable control of this stage by preventing either egg hatch or adult eclosion (Tilton & Brower 1983). Consequently, for Fuller rose beetle control on citrus, we sought to identify doses that would not adversely affect the quality of treated fruit. The objectives of our study were to determine the relative susceptibility of three different ages of Fuller rose beetle eggs, to estimate the dose necessary to provide suitable control, and to confirm this esti-

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mate with a larger scale test with infested lemons. We also examined the effect of radiation on product quality.

Materials and Methods

Collection and Maintenance of Adults. Fuller rose beetle adults were collected with beating sheets from citrus groves near Orange Cove, Fresno County, Calif., and stored until needed in 4-liter jars at a density of approximately 200 beetles per jar. The jars were covered with cheesecloth fastened with rubber bands and held in an environmental chamber kept at 12 ± 0.5°C, 60 ± 5% RH, and a photoperiod of 10:14 (L:D). Stem cuttings with citrus leaves (usually lemon) were provided as food. The stem ends were embedded in floral vials containing Floralife (Floralife, Chicago) in water to improve the longevity of the leaves. Spent cuttings were replaced weekly with fresh ones.

Dose–Mortality Response. To obtain eggs for preliminary dose determination, adults were removed from storage and placed in an environmental chamber kept at standard conditions of 27 ± 0.5°C, 60 ± 5% RH, and a photoperiod of 16:8 (L:D). Beetle density was reduced to approximately 30–40 beetles per jar and fresh cuttings were provided three times each week. Ovipositional sites were provided by using a heat sealer to make parallel heat seams (12–15 mm apart) across a stack of four waxed paper sheets (20 by 30 cm). A paper cutter was used to cut between the heat seams, resulting in strips about 12–15 mm wide and 20 cm long. The strips were folded along the heat seal, creating a narrow book with eight waxed paper pages. Beetles readily deposited eggs between the pages.

Soaking the waxed paper strips in water for at least 2 h before oviposition dramatically improved egg viability. After soaking, 6–8 strips were placed in each jar and adults were allowed to oviposit for 2–3 d. The pages of ovipositional strips were peeled apart and carefully cut into squares carrying individual egg clusters. Clusters were held in plastic Petri dishes at 27 ± 0.5°C, 85 ± 5% RH, and a photoperiod of 16:8 until needed. At this temperature, eggs began to hatch in 14–16 d.

Eggs were irradiated at doses of 10, 50, 100, and 150 Gy when they were 1 to 3, 6 to 8, or 10 to 13 d old. Doses were applied in a J. L. Shepherd Mark I S/N 1030 137 cesium irradiator at the Laboratory for Environmental Health Research (LEHR), University of California, Davis, Calif. A revolving turntable within the chamber (20 by 15 by 25 cm) and 14 cm from the source provided uniform treatment. The three highest doses were applied with an unattenuated dose rate of 11.03 Gy/min. The dose rate was reduced to 5.01 Gy/min by lead attenuators for the lowest dose.

One day before irradiation, egg clusters were placed in plastic snap-cap vials (23 mm diameter by 60 mm high). A 2-cm diameter screen-covered hole in the side of each vial provided ventilation. Depending upon availability, 5–19 egg clusters were placed in each vial. The average number of eggs per vial was about 500 (range, 255–846). Three vials of egg clusters for each age class and dose were used.

The nine vials for each dose (three per age class) were securely fixed for shipment to Davis by pushing the vials into circular Styrofoam boards (150 mm diameter by 25 mm thick). The vials were evenly spaced along the perimeter of each board, which fit the irradiator turntable. One Styrofoam board was used for each dose; another served as an untreated control. The boards were carefully packed in an ice chest along with a moist sponge to increase humidity and a small chart recorder to monitor temperatures. The ice chest was shipped by air freight to LEHR the afternoon before treatment, and returned to Fresno, Calif., the morning after. Egg clusters were immediately removed from test vials and held in plastic Petri dishes at 27 ± 0.5°C, 85 ± 5% RH, and a photoperiod of 16:8.

Because no method was available to rear the root-feeding larvae, prevention of egg hatch was chosen as the control criterion. Counts of hatched and unhatched eggs in each cluster were made and percentage of egg mortality was determined 4 and 7 wk after treatment. Egg mortality was corrected for control mortality with Abbott's (1925) formula.

Treatment of Infested Fruit. To obtain fruit infested with Fuller rose beetle eggs, lemons with fresh, intact calyxes were selected just after waxing from the processing line of a Tulare County packinghouse. After careful examination to eliminate any eggs already present, 65–70 lemons were placed in a single layer in plastic trays (10 by 30 by 60 cm). The lemons were oriented calyx up and covered with loose leaves and 3–4 citrus shoots that had been kept fresh in floral vials as described above. After 100 beetles per tray had been added, the trays were closed with screened lids and held under standard conditions. The adults were allowed to oviposit for 3 d. Fruit was then examined under a dissecting microscope for the presence of eggs. Infested lemons were returned to standard environmental conditions until needed.

Fruit infested with 10- to 13-d-old eggs were irradiated at the Department of Energy Pacific Northwest Laboratory, near Richland, Wash. Infested lemons were irradiated in groups of 10 in the center of commercial cardboard lemon cartons (28 by 28 by 43 cm). Between 98 and 131 uninfested lemons were packed around the infested lemons, which were wrapped in cheesecloth bags secured by rubber bands. Two days before irradiation, the bags of infested lemons were packed into an ice chest with the lid propped slightly open to prevent accumulation of carbon dioxide and driven to Richland, Wash. Temperatures in the chest were monitored with a datalogger (Model 516C, Omnidata International, Logan, Utah); they remained between 20 and 25°C. Because uninfest-

ed lemons were also to be used in fruit quality studies, they were transported separately from the infested fruit and were kept refrigerated at 18 ± 1.0°C during the drive to Richland, Wash.

Fruit was irradiated in a Gammabeam-650 irradiator (Atomic Energy of Canada, Ontario). The unit had been reloaded in 1986 with approximately 50,000 Ci (185×10^{13} Bq) of cobalt-60 in 12 pneumatically controlled source tubes (Burditt & Hungate 1989). Just before treatment, a single bag of infested lemons was placed at the center of a carton of uninfested lemons. For each replicate, five lemon cartons were spaced evenly along a circular platform rotating around the source tubes at 3 rpm. The centers of the cartons were placed 100 cm from the perimeter of the source tubes. Halfway through the 49-min treatment, the cartons were turned 180° so that a uniform dose was applied. The treatment was replicated three times.

The applied dose was monitored with TLD-700 LiF dosimeter chips (Harshaw/Filtrol, Cleveland). Uniformity of dose throughout the cartons was determined in the first treatment by placing one set of dosimeters in each of five locations: along the edges, on the ends, and in the center of a single carton. Each set of dosimeters included four LiF chips. In the remaining treatments, a single set of dosimeters was placed in the center of each of two cartons. Immediately after treatment, the dosimeters and bags of infested lemons were removed from the cartons. Each infested lemon was placed in a 0.48-liter jar closed with a filter paper lid. The jars were packed into cartons and transported, along with the cartons of filler lemons, back to Fresno, Calif., in the refrigerated truck. During the return trip, temperatures in the jars were monitored with a datalogger; they remained between 15 and 20°C. Jars were held under standard environmental conditions and examined every week for neonate larvae. After 11 wk, eggs from half the untreated lemons were removed from underneath the calyxes and percentage egg hatch was estimated.

Fruit Quality. Uninfested lemons were used to determine the effect of the treatment on fruit quality. After normal washing, waxing, and degreening procedures, marketable, yellow fruit was collected from two packinghouses located in the coastal and San Joaquin Valley growing areas of California. Because packinghouses routinely receive coastal lemons of different maturity and color, four classes of fruit were selected from the coastal packinghouse based on fruit color at time of harvest. These classes were: yellow, harvested 8 April; silver (between yellow and green), harvested 23 March; light green, harvested 6 March; and dark green, harvested 25 January. Because lemons from the San Joaquin Valley mature uniformly and are only picked when nearly ripe, fruit collected from the Valley packinghouse was picked yellow on 2 May.

Fruit for quality studies was transported to and from the irradiation facilities at Richland, Wash., as described above. A single carton of lemons from each maturity class or location was used in each of the three irradiation treatments and as untreated controls. At Fresno, fruit was stored at 10 ± 1°C and inspected for rind injury and decayed fruit at weekly intervals for 4 wk. Damage levels for each irradiated box were corrected for control damage with Abbott's (1925) formula. Data for each color and location were subjected to a one-way analysis of variance (SAS Institute 1987).

Results and Discussion

Dose–Mortality Response. Results from irradiation tests with naked eggs are summarized in Table 1. Eggs from the two youngest age classes (1–3 and 6–8 d old) were similar in their sensitivity to the radiation doses used. For these younger eggs, the lowest treatment dose (10 Gy) caused severe reduction in egg hatch. No eggs hatched after treatment with ≥50 Gy. In contrast, the oldest eggs (10–13 d old) were far less radiosensitive. The lowest treatment dose had little or no effect on hatch of the oldest eggs, while 50 Gy produced variable results. A dose of 100 Gy drastically reduced hatch of the older eggs, and no eggs hatched with a dose of 150 Gy.

These results agree with findings from earlier studies done on the eggs of numerous beetle species. Generally, insect eggs have been found to be the stage most sensitive to radiation (Tilton & Brower 1983), presumably due to the high degree of mitotic activity and lack of differentiation that is usually correlated with radiosensitivity (Bergonie & Tribondeau 1959). The 150-Gy dose indicated by our studies as preventing hatch of Fuller rose beetle eggs falls well within the range of doses determined for other beetle eggs (Brower & Tilton 1973, Brown et al. 1972, Dawes et al. 1987). Although a lower treatment level could probably be recommended if prevention of adult eclosion rather than egg hatch were used as the criterion for effectiveness, the difficulty in rearing the Fuller rose beetle makes adult eclosion an impractical criterion for control.

Studies with beetles and other insects also have indicated a marked change in radiosensitivity during embryonic development (Balock et al. 1963; Brower 1972, 1974; Larsen 1963). Younger, less-developed embryos are typically more sensitive, especially during cleavage and blastulation (Tilton & Brower 1983). As development proceeds, resistance usually levels off (and may even drop slightly) before beginning a steady increase (Balock et al. 1963; Brower 1972, 1974; Larsen 1963). The onset of increased resistance has been correlated with dorsal closure in *Blaberus craniifer* Burmeister (Larsen 1963) and blastokinesis in *Bombyx mori* (L.) (Murakami & Miki 1972). Preliminary observations on the embryology of Fuller rose beetle eggs (E.L.S., unpublished data) indicates that at 27°C, dorsal closure may occur 9–10 d after oviposition. This may account for the great increase

Table 1. Percentage of adjusted mortality ($\bar{x}$ ± SEM) of Fuller rose beetle eggs exposed to gamma radiation

Dose, Gy	Age of eggs, days		
	1–3	6–8	10–13
10	90.3 ± 1.26	97.7 ± 0.14	5.3 ± 5.14
50	100	100	56.1 ± 16.91
100	100	100	99.9 ± 0.05
150	100	100	100

Values are means of three replicates, adjusted by Abbott's (1925) formula.

Table 2. Effect of gamma radiation on lemon fruit quality ($\bar{x}$ ± SEM)

Location	Color	% Total damage	% Rind damage	% Decay
Coast	Yellow	4.2 ± 0.28	0.5 ± 0.16	3.6 ± 0.16
	Silver	4.4 ± 1.59	0.5 ± 0.16	3.8 ± 0.16
	Light green	0.5 ± 0.16	0	0.5 ± 0.16
	Dark green	3.7 ± 1.23	2.1 ± 0.81	1.6 ± 0.53
Valley	Yellow	6.1 ± 1.51	0.3 ± 0.19	5.8 ± 1.70

Values are means of three replicates, adjusted by Abbott's (1925) formula. Lemons were treated with between 166 and 202 Gy of gamma radiation.

in resistance observed for Fuller rose beetle eggs irradiated when 10 to 13 d old.

Treatment of Infested Fruit. The results from our studies with naked eggs led us to choose 10 to 13 d old eggs as the target stage and 150 Gy as the dose for confirmatory tests with infested lemons. Larval emergence from eggs infesting untreated lemons was variable, ranging from 1 to 92; an average ($\bar{x}$ ± SEM) of 22.5 ± 3.05 larvae emerged per lemon. Examination of the egg clusters from half of the untreated lemons 11 wk after treatment showed that total eggs per fruit averaged (±SEM) 43.5 ± 6.45 and that egg hatch ranged from 17.7 to 100%, averaging (±SEM) 42.5% ± 4.66. Based on the average number of eggs obtained from untreated fruit, approximately 6,500 Fuller rose beetle eggs were irradiated. None of these irradiated eggs hatched, confirming radiation as an effective disinfestation technique.

Dosimeter readings taken at the centers of the treatment cartons showed an average ($\bar{x}$ ± SEM) of 174.1 Gy ± 8.01. One dosimeter gave a reading of 204.7 Gy, which seems abnormally high compared with the results of the other dosimeter exposed during the same treatment (166.5 Gy). The average (±SEM) for the center dosimeters was 166.5 ± 3.15 when the high value was omitted. Although we have no explanation for this abnormally high reading, the actual applied dose appeared to be closer to the average value obtained with the high reading omitted.

Readings from dosimeters located throughout a single lemon carton showed that the edge exposure (201.9 Gy) was about 15% greater than exposure at the center, and that exposure along the length of the carton was reasonably uniform, with not more than 5% variation.

Fruit Quality. Damage to irradiated fruit was variable, making statistical analysis difficult. After correction with Abbott's (1925) formula, the average percentage of damaged, irradiated fruit (including rind damage and decay) ranged from 0.5 to 6.1% (Table 2). No significant differences ($P < 0.05$) in corrected damage values were detected between color and location classes. Although the experimental design did not allow direct statistical comparison of controls and treatments, radiation at the applied dose appeared to accelerate existing decay slightly. However, the increase in decay and rind damage was negligible in coastal fruit picked when light green, and even the highest increase in damage (6.1%) may be acceptable.

Earlier studies dealing with the effect of radiation on fruit quality show a wide range of response within the genus *Citrus*. Doses as high as 2.0 kGy have been suggested for increasing shelf life of oranges (Akamine & Moy 1983). Although research by Moy & Nagai (1985) indicates that doses of 750–1,000 Gy had no detrimental effect on the quality of either oranges or lemons, Maxie et al. (1969) showed radiation injury to lemons at doses as low as 500 Gy. Obviously, additional work is needed before conclusions about the effect of radiation on lemon quality can be made.

Should gamma radiation be used as a quarantine treatment for Fuller rose beetle eggs on citrus, higher applied doses than those used in our study may be necessary to obtain a minimum of 150 Gy throughout the product. Commercial irradiators can be designed to give a maximum/minimum dose ratio of ≤2.0 (O'Sullivan & Gunby 1985). Although the results of our quality studies must not be considered as conclusive, it is possible that, given a minimum dose of 150 Gy, the resulting applied dose of 300 Gy could accelerate existing decay. Careful selection of fruit or working to reduce normal decay levels may be possible means to minimize radiation damage. Combining radiation with cold storage during shipment could improve the effectiveness of the treatment (Ohta et al. 1989).

The importance of the Japanese market to California citrus exports warrants the timely development of suitable quarantine treatments for Fuller rose beetle eggs as alternatives to methyl bromide fumigation. Our results show that a radiation dose of 150 Gy prevents hatch of all ages of beetle eggs and confirm that the method is effective as a treatment for infested lemons. Although radiation may accelerate decay of treated lemons, more research is needed to determine if the increase is significant. Even so, the maximum 6% increase in loss we observed in our study is a great improvement compared with the 65% loss recorded in lemons fumigated with methyl bromide (Griffiths et al. 1986).

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