# Irradiation as a Phytosanitary Treatment for *Aspidiotus destructor* (Homoptera: Diaspididae)

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**ABSTRACT** Coconut scale, *Aspidiotus destructor* Signoret (Homoptera: Diaspididae), is a quarantine pest of banana (*Musa* spp.) and many tropical crops. Irradiation was examined as a potential phytosanitary treatment to control coconut scale. Dose–response tests were conducted with second-stage nymphs, adult females without eggs, and adult females with eggs at a series of irradiation doses between 60 and 200 Gy to determine the most tolerant stage. The adult female with eggs was the most tolerant stage. In large-scale validation tests and dose–response tests, a total of 32,716 adult female scales with eggs irradiated with doses between 100 and 150 Gy produced no $F_1$ adults with eggs. Irradiation treatment with a minimum absorbed dose of 150 Gy should provide quarantine security for coconut scale on exported commodities.

**KEY WORDS** phytosanitary treatment, quarantine pest, x-ray, coconut scale

Coconut scale, *Aspidiotus destructor* Signoret (Homoptera: Diaspididae), is distributed throughout tropical and subtropical regions of the world, particularly on islands, and was first discovered in Hawaii in 1968. Coconut scale is an economic pest on many tropical crops, including banana (*Musa* spp.) (Wright and Diez 2005). Green bananas are exported from Hawaii to the U.S. mainland by using a nonhost status protocol for tephritid fruit flies, but other pests [e.g., coconut scale; green scale, *Coccus viridis* (Green) (Homoptera: Coccidae); *Opogona sacchari* (Bojer) (Lepidoptera: Tineidae); mealybugs; thrips; and ants] are occasionally found on bunches, and their appearance can cause delays at ports-of-entry or rejection (Armstrong 2001). Coconut scale is quarantined by California, and a single live insect can cause regulatory action (Miller and Chang 1998).

Irradiation is a quarantine treatment option for bananas to prevent disruption of shipments due to the presence of insect pests other than fruit flies. The U.S. Department of Agriculture (USDA)–Animal and Plant Health Inspection Service recently published a rule approving a generic irradiation quarantine treatment of 400 Gy for control of all insect pests except lepidopteran pupae and adults (Federal Register 2006). Commercial treatment to achieve a minimum dose of 400 Gy can result in some fruit receiving doses up to 700 Gy. Bananas are relatively tolerant of irradiation, but doses >600 Gy may cause scald damage (M. Wall, personal communication). Therefore, determining effective irradiation doses for the main regulatory pests so that the 400-Gy dose can be lowered may improve banana quality. Hawaii's fruit flies are controlled at an irradiation dose of 150 Gy (Follett and Armstrong 2004), and studies with green scale suggest that it is controlled with an irradiation dose of 250 Gy (Hara et al. 2002). Irradiation studies with *O. sacchari* are in progress (Hollingsworth and P.A.F., unpublished data). Until now, there was no information on the radiotolerance of coconut scale and little information on diaspidid scales in general (Angerilli and Fitzgibbon 1990, Sanchez 1991, IDIDAS 2005). Irradiation studies were conducted to determine the most tolerant life stage and an effective irradiation dose to control coconut scale. Unlike other disinfestation techniques, irradiation does not need to kill the pest immediately to provide quarantine security; therefore, live (but nonviable or sterile) insects may occur with the exported commodity (Follett and Griffin 2006). The objective of an irradiation quarantine treatment is to stop the insect's ability to reproduce and thereby prevent its introduction and establishment into new areas.

## Materials and Methods

A colony of *A. destructor* was started from newly hatched crawler-stage scales collected from a banana plantation in Hilo, HI, in 2004. Crawlers were transferred using a long-haired sheep's wool brush. The colony was maintained in the laboratory on Japanese pumpkin, *Curcurbita moschata* (Duchesne) variety *chirimen*, in ventilated 2- or 3-liter round plastic tubs (Sweetheart, Fort Howard Corp., Green Bay, WI or Berry Plastics Corp., Evansville, IN). Rearing conditions were 21°C (range 19.8–23.3°C), 40–85% RH, and a photoperiod of 14:10 (L:D) h. *A. destructor* has four life stages: egg, crawler (first-stage nymph), second-stage nymph, and adult. The crawler stage and adult male are mobile, and the other stages are sessile. Male and female scales can be distinguished at the late second-stage nymph. Adult females are parthenogenic; therefore, irradiation tests focused on females. The adult female scale is translucent, and eggs are visible underneath the scale cover. For purposes of the tests, the adult stage was divided into adults without eggs, adults with eggs, and adults with hatching eggs.

Approximately 100 crawlers were introduced to each pumpkin to establish cohorts of even age. Pumpkins carrying scales were irradiated when most individuals had matured to the desired stage. Dose–response tests were conducted with second-stage nymphs, adult females without eggs, and adult females with unhatched and newly hatched eggs (crawlers) at a series of irradiation doses between 60 and 200 Gy to determine the most tolerant stage. After irradiation treatment, immature female scales on pumpkins were examined weekly to determine whether they had molted to the next stage, and adult females were examined to determine whether they had laid eggs or whether eggs had hatched and crawlers settled. Untreated controls for each stage were held under identical conditions and examined similarly. The average number of eggs per female was 99.8 ($n = 23$, SEM = 2.1, range 88–122). Due to difficulties accurately counting eggs underneath adult female scales, this number was used to indirectly estimate the number of eggs irradiated when reproductive adult females were tested. Often, second generation scales on untreated control pumpkins numbered in the thousands. When large numbers of scales were present, the surface of the pumpkin was divided with marking pens into equal sections, and counts were made on two or three randomly selected sections and then multiplied by the appropriate number to estimate the total number treated. Tests were usually terminated after 60 d, at which time all irradiated scales had died or pumpkins had rotted, and final counts were made of the numbers of all live and dead females at each stage of development. Validation testing was done with large numbers of adult females with eggs at 100 and 150 Gy to determine the efficacy of these doses as a potential quarantine treatment.

All stages of coconut scale can occur on exported commodities. Gravid adults are often present. Therefore, the required response for an effective irradiation treatment was to prevent generation turnover by preventing development to the adult stage or reproduction by the adult in the subsequent ($F_1$) generation.

Irradiation treatment was conducted at a nearby commercial x-ray facility (Hawaii Pride LLC, Keaau, HI) by using an electron linear accelerator (5 MeV, model TB-5/15, L-3 Communications Titan Corp., San Diego, CA) at ambient temperature. Dosimeters (Opti-chromic detectors, FWT-70-83M, Far West Technology, Goleta, CA) were placed on the sides and upper surface of pumpkins at each dose in each replicate to measure dose variation. The dosimeters were read with a FWT-200 reader (Far West Technology) at 600-nm absorbance to verify the minimum absorbed dose and dose variation in each replicate. To minimize the dose uniformity ratio (the ratio of the maximum/minimum dose), infested pumpkins were placed upright in plastic tubs in a single row perpendicular to the x-ray beam. Dose mapping demonstrated that doses were sometimes lower near the sides and floor of the metal carrier, so the tubs with pumpkins were elevated by placement on a cardboard box and positioned in the exact center of the carrier. Each carrier passed in front of the beam in a forward then reverse orientation. The dose uniformity ratio during this current research was consistently <1.2. After irradiation treatment, pumpkins were held in ventilated plastic tubs for scale development. The maximum dose received during large-scale validation testing becomes the minimum dose for a quarantine treatment. During validation testing, all measured irradiation doses during the 100- and 150-Gy treatments were below 100 and 150 Gy, respectively.

Data on mortality were subjected to analysis of variance (ANOVA) after testing for equal variances and normality. Mortality data were arcsine transformed to improve normality. Welch's ANOVA was used to evaluate data when a Levene's test suggested variances were unequal (SAS Institute 2002). Means separations were done using a Tukey's test. Scale count data in the $F_1$ generation were divided by the numbers of individuals in the parent generation, transformed using log $(x + 1)$, and subjected to ANOVA. Only pumpkins that remained intact for the duration of the test were included in data analysis. Because of deteriorating host material, treatments in the dose–response tests often had unequal numbers of replicates.

**Table 1. Effect of irradiation on maturation of female second-stage *A. destructor* nymphs**

| Dose (Gy) | No. pumpkins | No. second-stage nymphs irradiated | Adult females[a] | | Adult females with eggs | | No. $F_1$ generation second-stage nymphs |
|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | |
| Control | 5 | 429 | 256 | 81.6a | 222 | 70.9a | 8,907a |
| 60 | 9 | 1,683 | 532 | 49.4ab | 132 | 11.9b | 0b |
| 100 | 8 | 1,843 | 803 | 43.6ab | 149 | 8.7b | 0b |
| 150 | 7 | 1,763 | 899 | 36.4ab | 176 | 9.0b | 0b |
| 200 | 5 | 3,659 | 103 | 10.3b | 13 | 1.2b | 0b |

Means within a column followed by different letters were significantly different by a Tukey's test ($P < 0.05$).
[a] Second-stage nymphs that have matured to the adult stage.

**Table 2. Effect of irradiation of *A. destructor* adult females without eggs on reproduction and progeny development**

| Dose (Gy) | No. pumpkins | No. adult scales irradiated | Adult females with eggs | | No. $F_1$ generation adults with eggs | $F_2$ generation nymphs |
|---|---|---|---|---|---|---|
| | | | No. | % | | |
| Control | 4 | 388 | 324 | 90.1a | 776a | 62,080 |
| 60 | 4 | 691 | 377 | 31.8a | 0b | 0 |
| 100 | 2 | 1,069 | 951 | 89.6a | 0b | 0 |
| 150 | 8 | 823 | 445 | 27.4a | 0b | 0 |
| 200 | 3 | 1,511 | 180 | 16.6a | 0b | 0 |

Means within a column followed by different letters were significantly different by a Tukey's test ($P < 0.05$).

## Results and Discussion

Irradiation of second-stage nymphs resulted in reduced survivorship to the adult stage without eggs ($F = 2.7$, df = 4, $P = 0.05$) and the adult stage with eggs ($F = 9.4$, df = 4, $P < 0.0001$) (Table 1). At an irradiation dose of 150 Gy, 36.4% of treated second-stage nymphs matured to the adult stage and 9.0% laid eggs, whereas 81.6% of untreated scales matured to the adult stage and 70.9% laid eggs. None of the second-stage nymphs treated with 60, 100, 150, or 200 Gy succeeded in producing second-stage nymphs in the $F_1$ generation.

Irradiation of adult females with no eggs resulted in a reduced number of adult females with eggs compared with controls ($F = 3.2$, df = 4, $P = 0.04$) (Table 2). Means comparisons using a Tukey's test were not significant. Lack of significance was due to the wide variation in results from different replicates. This variation could have resulted because of differences in adult female maturity, i.e., gravid adult females are more likely to lay eggs after irradiation than newly formed adult females. Nevertheless, none of the progeny of adult female scales irradiated at any dose became $F_1$ adults with eggs (Table 2).

Irradiation of adult females with eggs and hatching crawler-stage nymphs resulted in a reduction of $F_1$ adult females compared with the untreated control treatment ($F = 64.4$, df = 4, $P < 0.001$) (Table 3). For example, in the 140-Gy treatment, 984 adult female scales with an estimated 97,416 eggs produced 18,392 second-stage nymphs but only 31 $F_1$ adult females, whereas in the 1,677 adult female scales in the untreated control group with an estimated 166,023 eggs produced 81,655 second-stage nymphs and 19,528 $F_1$ adult females. No $F_1$ adult females with eggs were produced in the 100-, 120-, 140-, and 200-Gy irradiation treatments, whereas 19,528 $F_1$ adult females were produced by the untreated controls (Table 3). One $F_1$ adult female with eggs was produced in the 80-Gy treatment, but none of its progeny reached the second-stage nymph in the $F_2$ generation. In the first 60-Gy treatment shown in Table 3, all adult females had eggs, but none were hatching. In this case, no $F_1$ adults were produced. The second 60-Gy treatment included adult females with eggs and adult females with hatching eggs (newly emerged crawlers still under the female scale). In this case, 757 irradiated scales produced 860 $F_1$ adult females, illustrating that egg maturity at the time of irradiation can strongly influence the resulting numbers of scales surviving in the next generation. Indeed, an inability to precisely determine egg maturity resulted in contradictory results. When adult females with eggs and hatching crawlers were irradiated at doses ranging from 80 to 140 Gy, the number of $F_1$ generation adults did not decrease with increasing dose as would be expected (Table 3). This result reflects natural variation in the developmental stage of eggs and crawlers at the time of treatment, and this is not an unusual level of variation for scale insects over such a narrow range of irradiation doses.

For large-scale validation tests, 100 and 150 Gy were selected as potential quarantine treatment doses. Irradiation of 18,822 adult female scales without and with eggs, and an additional 10,167 adult females with

**Table 3. Effect of irradiation of *A. destructor* adult females with eggs and hatching crawlers on progeny survival and maturation**

| Dose[a] (Gy) | No. pumpkins | No. adult scales irradiated | Estimated no. eggs irradiated[b] | No. $F_1$ generation second-stage nymphs | No. $F_1$ generation adults[c] | No. $F_1$ generation adults with eggs |
|---|---|---|---|---|---|---|
| Control | 7 | 1,677 | 166,023 | 81,655 | 19,528a | 19,528a |
| 60[d] | 14 | 1,385 | 137,115 | 364 | 0c | 0b |
| 60 | 3 | 757 | 74,943 | 17,133 | 860b | 0b |
| 80 | 3 | 551 | 54,549 | 17,034 | 30c | 1b |
| 100 | 6 | 2,027 | 200,673 | 36,516 | 7c | 0b |
| 120 | 3 | 716 | 70,884 | 10,276 | 14c | 0b |
| 140 | 3 | 984 | 97,416 | 18,392 | 31c | 0b |
| 200 | 3 | 274 | 27,126 | 0 | 0c | 0b |

Means within a column followed by different letters were significantly different by a Tukey's test ($P < 0.05$).

[a] Irradiation applied to adults with eggs and newly hatched crawlers unless otherwise noted.

[b] Estimated number of eggs irradiated assumes 99 eggs per female.

[c] Scale count data in the $F_1$ generation were divided by the numbers of individuals in the parent generation (treated by irradiation) and transformed using log $(x + 1)$, before ANOVA and means separations.

[d] Irradiation applied to adults with eggs only (none hatching).

**Table 4. Large-scale validation tests irradiating *A. destructor* adult females with eggs and newly hatched crawlers**

| Target dose (Gy) | No. pumpkins | Measured doses | No. adult scales irradiated | No. eggs treated | No. $F_1$ generation second-stage nymphs | No. $F_1$ generation adults | No. $F_1$ generation adults with eggs |
|---|---|---|---|---|---|---|---|
| Adults without and with eggs | | | | | | | |
| Control | 15 | | 3,162 | 313,038 | 144,352 | 2,865 | 20,911 |
| 100 | 47 | 100–110 | 15,506 | 1,535,094 | 22,958 | 0 | 0 |
| 150 | 19 | 144–150 | 3,316 | 328,284 | 14,687 | 0 | 0 |
| Adults with eggs and newly hatched crawlers | | | | | | | |
| Control | 5 | | 765 | 75,735 | 47,567 | 19,492 | 17,971 |
| 100 | 8 | 100–110 | 2,016 | 199,584 | 91,570 | 0 | 0 |
| 150 | 23 | 144–148 | 8,151 | 807,282 | 118,767 | 351 | 0 |

eggs and hatching crawlers, at irradiation doses of 100 or 150 Gy resulted in no successful development of $F_1$ adults with eggs (Table 4). In the 100-Gy treatment, no eggs or crawlers matured to the $F_1$ adult stage, whereas in the 150-Gy treatment 4.3% of irradiated eggs or crawlers matured to the $F_1$ adult stage (Table 4). This apparent contradiction again is a reflection of variation in the maturity of eggs and crawlers under the female at the time of treatment. Nevertheless, no $F_1$ adults produced eggs at either 100 or 150 Gy. The highest dose measured during validation testing becomes the minimum dose for a quarantine treatment. Therefore, reproduction and generation turnover can be stopped at irradiation doses above 150 Gy.

Historically, the USDA has used 99.9968% efficacy (probit 9) as the basis for approving many quarantine treatments, particularly for tephritid fruit flies (Follett and Neven 2006). To achieve probit 9 mortality at the 95% confidence level, a minimum of 93,613 insects must be tested with no survivors (Couey and Chew 1986). A probit 9 treatment usually provides adequate quarantine security, and developing the treatment frequently proves to be the quickest and most easily accepted method for overcoming phytosanitary restrictions. In recent years, the USDA has been flexible in approving quarantine treatments with less than probit 9 numbers of insects, particularly if the potential economic and environmental impact of the pest, should it be introduced, is low (Follett and Neven 2006). Other countries (Japan, Australia, and New Zealand) accept quarantine treatment efficacy at 99.99% (at the 95% confidence level), which is obtained by treating a minimum of 29,956 insects with no survivors (Couey and Chew 1986). Japan requires a total of 30,000 individuals in three to four trials, New Zealand requires three replicates of 10,000 test insects, and Australia accepts a cumulative total of 30,000 treated insects with no survivors (Follett and Neven 2006). During validation testing, 28,989 coconut scale adults in total were irradiated at 100 or 150 Gy with no reproduction in the $F_1$ generation (Table 4). In dose–response tests, another 3,727 adult scales were treated at doses of 100–140 Gy with no reproduction in the $F_1$ generation (Table 3). Therefore, in total 32,716 adult coconut scales with eggs were irradiated at doses between 100 and 150 Gy with no survivors. Treatment of coconut scale with a minimum dose of 150 Gy satisfies the 99.99% quarantine treatment efficacy requirement.

The dose uniformity ratio for commercially irradiated banana in Hawaii is ≈1.5, meaning some fruit receive a dose 1.5 times the target dose. Some bananas receive a dose of 600–650 Gy during irradiation treatment to meet the current requirement for a minimum absorbed dose of 400 Gy. Research indicates that bananas irradiated at 600 Gy occasionally show scald damage on the skin (M. Wall, personal communication). Lowering the dose for banana to 150 Gy would result in commercial doses up to 250–300 Gy, which would minimize any deleterious effects on quality.

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