Acute Exposure to Low-Dose Radiation Disrupts Reproduction and Shortens Survival of *Wasmannia auropunctata* (Hymenoptera: Formicidae) Queens

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ABSTRACT Irradiation is a postharvest quarantine treatment option to control ants and other hitchhiker pests on fresh horticultural products exported from Hawaii. The radiotolerance of the invasive little fire ant, *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae: Myrmicinae), was studied to determine a dose sufficient for its control. Queens from each of five laboratory microcolonies started from five geographic locations in Argentina were irradiated at 20, 50, 70, or 100 Gy or left untreated as controls and then followed for 13 wk to observe colony growth. In general, queen survivorship, and the number of eggs, larvae, and pupae observed in the microcolonies decreased with increasing irradiation dose. In the 50-, 70-, and 100-Gy treatments, the number of eggs observed was reduced by 68, 66, and 76%, respectively, compared with untreated control microcolonies. The number of larvae in the 50-, 70-, and, 100-Gy treatments was reduced by 99.6%, and only one pupa was observed in the 50-Gy treatment and none in the 70- and 100-Gy treatments during the 13-wk experiment. Queens in the 100-Gy treatment had significantly reduced longevity compared with queens in the other treatments. Radiation doses ≥70 Gy stopped reproduction in *W. auropunctata* queens and should be sufficient as a phytosanitary treatment. Information from additional invasive ants in Myrmicinae and other subfamilies is needed before recommending a generic irradiation treatment for ants.

KEY WORDS little fire ant, quarantine pest, irradiation, phytosanitary treatment, invasive ant

Low dose ionizing radiation is used as a postharvest treatment to control quarantine pests in fresh agricultural commodities (Follett and Griffin 2006, Wall 2008). Hawaii is currently exporting ≈15 million pounds of fresh fruits and vegetables annually using irradiation to control tephritid fruit flies and other regulated pests (Follett and Weinert 2012). The presence of hitchhiking ants on exported products from Hawaii can cause rejection and return shipment (Costa et al. 2005, Follett and Taniguchi 2007). Ants are particularly problematic on exported rambutan, *Nephelium lappaceum* L.; longan, *Dimocarpus longan* Lour; and sweet potato, *Ipomoea batatas* (L.). Rejection of a single export shipment due to ants can be devastating financially to the small farmer. Although most interceptions are sterile workers that do not pose a risk, if a substantial number of workers are found in a small sample of boxes, the risk of having a reproductive female (queen) somewhere in the shipment may be significant.

The little fire ant, *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae: Myrmicinae), is an invasive ant probably originating from tropical South American forests that has spread throughout tropical and subtropical zones of the world, particularly in the Pacific (Fabres and Brown 1978, Wetterer and Porter 2003, Krushelnycky et al. 2005, Foucaud et al. 2010), and has recently invaded temperate areas of the Mediterranean region (e.g., Israel, Vonshak et al. 2009). In North America it is found in the West Indies, warmer parts of Mexico, and the southeastern United States (i.e., Florida). It was first reported in Hawaii in 1999 and has rapidly spread to a variety of agricultural sites such as nurseries, pastures, and orchards (Souza et al. 2008). In orchards, *W. auropunctata* tends mealybugs and soft scales for their honeydew (Hölldobler and Wilson 1990) and also may nest in protected sites in trees (Souza et al. 2008), leading to increased prevalence on fruit. This ant is becoming increasingly common in produce destined for export from Hawaii to the U.S. mainland, particularly California where it is an actionable pest. The small size of *W. auropunctata* (1.5 mm in length) makes it particularly problematic because it can escape from insect proof boxes that are designed to prevent the entry or exit of tephritid fruit flies of quarantine concern; ants inside the box are assumed to be irradiated, but the origin and therefore treatment status of ants found on the outside of the box is uncertain. Queens are much larger than workers and

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therefore may be more easily contained or excluded by using insect-proof boxes. *W. auropunctata* is unicolonial and polygynous (Wetterer and Porter 2003, Souza et al. 2008), characteristics that may increase the risk of introduction by way of infested commodities.

We studied the tolerance to radiation of *W. auropunctata* to determine the dose sufficient for its control. Information from *W. auropunctata* may give us an idea of how tolerant ants in general are to irradiation. Unlike other disinfestations 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 2009). The objective of an irradiation quarantine treatment is to prevent reproduction and thereby prevent the insect's introduction and establishment into new areas. Ant workers and rarely queens have been intercepted in fresh fruits and vegetables exported from Hawaii. The desired response with irradiation treatment of ants therefore is to sterilize reproductive females (queens).

Materials and Methods

Cooperative research on *W. auropunctata* has been conducted between the U.S. Department of Agriculture (USDA); Agricultural Research Service (ARS); Pacific Basin Agricultural Research Center (PBARC) in Hilo, HI; and the ARS–South American Biological Control Laboratory (SABCL) in Buenos Aires, Argentina since 2008. The research reported here was conducted entirely in Argentina due to the availability of clonal populations of *W. auropunctata* (Chifflet et al. 2011) in culture at SABCL.

Collection of Colonies. During January–March 2011, five clonal colonies of *W. auropunctata* were collected from five distinct geographic locations separated by 100–1,300 km in eastern and northwestern Argentina: Zárate (34° 06′ S, 58° 60′ W) and San Nicolas de los Arroyos (33° 21′ S, 60° 13′ W) in Buenos Aires Province; San Javier (30° 35′, 59° 56′ W) in Santa Fe Province; and Volcán (23° 55′ S, 65° 28′ W) and El Carmen (24° 22′ S, 65° 15′ W) in Jujuy Province. The colonies were dug up from the ground, brought to the laboratory in covered buckets, and separated from soil by flotation (Banks et al. 1981). Stock colonies were held individually in white plastic trays (25 by 40 by 7 cm) coated with talc, with a permanent source of water and sugar water, and fed twice a week alternating with peanut butter, corn meal, hard boiled eggs, and sausage. Fifteen queens from each colony were placed individually in small plastic containers (9 by 9 by 3 cm) with 50–100 workers (microcolony) to care for and feed the queen and its progeny. Within each microcolony, the queen was confined in a 12-ml round-bottomed centrifuge polycarbonate tube (7.7 by 1 cm; Nalgene, Rochester, NY) with a moist cotton ball in the bottom, and with four 1-mm-diameter holes in the tube cap to allow worker movement in and out. The microcolonies were kept in an incubator at 25°C, 60% RH, and a photoperiod of 12:12 (L:D) h. Microcolonies were fed as described above.

Fecundity Before Irradiation. Before irradiation, the 75 queens from all microcolonies were checked weekly for a 3–5-wk period with a 40× stereomicroscope for egg production and larval growth to confirm that they were actively reproducing. The traceability of the queens was strictly maintained to compare the fecundity of individuals before and after irradiation. The number of new eggs, larvae, and pupae per queen were calculated weekly, understanding that some individuals remained in the same stage from the previous week and others developed to the next stage. Some underestimation could have been introduced since emergence of adults was not possible to record, and several eggs, larvae, and pupae did not develop or were predated by workers.

Data on the number of eggs, larvae, and pupae per microcolony were square-root ($x + 0.5$) transformed and subjected to one-way analysis of variance (ANOVA), with colony source as the main effect and the microcolonies as replicates. A Welch ANOVA was used when a Levene test for equal variances revealed that the group variances were significantly different. For significant effects, means separation were done using a Tukey test. The monitoring time before irradiation for queens assigned to each treatment ranged from 3.6 ± 0.3 to 4.6 ± 0.4 wk, which was not significantly different (Kruskal–Wallis test, $H = 4.63$, $n = 75$, df = 4, $P = 0.33$). Thus, the period of estimation of colony fecundity before irradiation was similar for all treatments.

Irradiation Treatment. Following Follett and Taniguchi (2007), the 75 queens were randomly assigned to an irradiation treatment: 0 (control), 20, 50, 70, or 100 Gy. Queens were irradiated individually in the centrifuge tubes where they were reared or in 1-dram glass vials, the latter with a wet cotton ball in the base to provide them a water source, and a metallic net on top to prevent their escape. Irradiation was carried out at the Comisión Nacional de Energía Atómica (Centro Atómico Ezeiza, Buenos Aires, Argentina) by using a Gammacell-220 cobalt-60 irradiator (MDS Nordion, Ottawa, ON, Canada) with a dose rate of 0.42 Gy/min (transit dose, 0.03 Gy). For each treatment, four dosimeters were placed in separate empty glass vials to estimate the absorbed dose received by ants. Measured dose extremes for the 20-, 50-, 70-, and 100-Gy treatments were 20.1–21.6 Gy, 50.5–52 Gy, 71.1–72.4 Gy, and 98.5–103.6 Gy, respectively. Eggs and brood, which have the potential to develop into reproductive females, have not been found in exported commodities during inspection and therefore were not tested. Early ant life stages are likely to be more susceptible to radiation than later stages, as is invariably the case in other insects (Follett 2006, Follett and Griffin 2006).

Fecundity and Survivorship After Irradiation. After irradiation, the queens were placed in new artificial nests and reared as above, although this time with ≈40–50 workers to decrease the risk of egg and larval ant predation by workers. The mean number of eggs, larvae, and pupae per microcolony was recorded

Table 1. Fecundity and production of larvae and pupae (mean ± SE) of *W. auropunctata* queens from five localities and reared for 3–5 wk before irradiation

Locality	No. queens	No. eggs/wk	No. larvae/wk	No. pupae/wk
Zárate	15	30.58 ± 3.19a	13.16 ± 3.36a	0.44 ± 0.22a
San Nicolás	15	10.42 ± 1.04b	3.99 ± 0.52b	0.55 ± 0.25a
San Javier	15	7.16 ± 0.71b	1.97 ± 0.33b	0b
El Volcán	15	6.75 ± 1.24b	3.83 ± 1.02b	0.24 ± 0.13a
El Carmen	15	6.01 ± 0.68b	2.29 ± 0.37b	0.42 ± 0.19a

Means within a column followed by different letters are significantly different at $\alpha = 0.05$ by Tukey's test.

weekly for 13 wk for each queen. Queen survivorship was also recorded weekly to determine the effect of radiation treatment on residual longevity. Data for the number of eggs, larvae, and pupae per queen and queen survivorship were square-root ($x + 0.5$) transformed and analyzed using an ANOVA with radiation treatment as the main effect and colony source as a blocking factor. A Welch ANOVA was used when a Levene test for equal variances revealed that the group variances were significantly different. For significant effects, means separation were done using a Tukey test. Linear regression on untransformed data was used to predict the dose that would prevent oviposition.

Results

Fecundity Before Irradiation. The fecundity of the queens was significantly different among colonies ($F_{4,70} = 37.4$; $P < 0.00001$) before irradiation. The mean number of eggs per week was highest in the colony from Zárate (Table 1). The percentage of larvae developing from eggs was also significantly different among colonies (Welch F-test: $F_{4,34} = 6.7$; $P < 0.0005$), but the percentage of pupae developing from larvae was not significantly different ($F_{3,56} = 0.34$; $P = 0.78$). The success rate of development from egg to larvae and from larvae to pupae ranged from 36 to 54% and from 0 to 29%, respectively (Table 1). Mean weight of queens also differed among colonies ($F_{4,75} = 6.0$; $P < 0.0005$), and heavier queens tended to lay more eggs than lighter queens ($r = 0.23$; $P = 0.05$).

Fecundity After Irradiation. In general, the mean number of eggs, larvae, and pupae observed in the microcolonies decreased with increasing irradiation dose (Table 2). The effect of radiation dose on egg production was significant (Welch F-test: $F_{4,34} = 20.9$; $P < 0.0001$), and the mean number of eggs per week was 66–76% lower in the 50-, 70-, and 100-Gy treatments compared with the control (Table 2). Queens treated with a radiation dose of 100 Gy laid eggs only during the first week (Fig. 1); only one larva hatched from these eggs and it did not develop to the pupal stage. The linear regression equation describing the effect of dose on the number of eggs laid is $y = -0.04x + 5.1$ ($R^2 = 0.38$) ($F_{1,73} = 44.6$; $P < 0.0001$), and the predicted radiation dose to prevent any oviposition is 112 Gy (94.9–142.5; 95% confidence limits).

The production of larvae and pupae was strongly reduced after irradiation treatment (Welch F-tests: $F_{4,34} = 21.2$; $P < 0.0001$ and $F_{4,34} = 15.6$; $P < 0.0001$, respectively); only three larvae were observed in each of the 50-, 70-, and 100-Gy treatments, and only one pupa was observed in the 50-Gy treatment. No pupae were observed at the 70- and 100-Gy radiation treatments. The relative fecundity in the five untreated source colonies during the experiment (0-Gy control) was similar to that observed before irradiation ($r = 0.9$; $P = 0.038$; Table 1). Radiation treatment at 70 and 100 Gy sterilized queens in all five source colonies, suggesting that different clonal populations or biotypes of *W. auropunctata* will have similar susceptibility to irradiation.

Survivorship After Irradiation. Queen survivorship also decreased with increasing irradiation dose. In total, 14, 15, 10, 2, and 0 queens survived at the end of the experiment at radiation doses of 0, 20, 50, 70, and 100 Gy, respectively (Fig. 2). All queens treated with a radiation dose of 100 Gy were dead at week 11 (2 wk before the end of the experiment), and they had significantly lower survivorship than queens in the other four treatments (Welch F-test: $F_{4,28} = 12.7$; $P < 0.0001$).

Discussion

Oviposition by *W. auropunctata* fluctuated in the irradiated and unirradiated (control) treatments which may have been due to laboratory rearing conditions. The number of eggs laid declined from week 1 to week 2 in all treatments but rebounded in the control and 20-Gy treatments in week 5 and again in week 12 to levels similar to week 1 (Fig. 1). There was little or no oviposition in the 50-, 70-, and 100-Gy treatments after week 1 reflecting irradiation effects.

Table 2. Residual longevity and reproduction (mean ± SE) of *W. auropunctata* queens reared during 13 wk after acute exposure to ionizing radiation

Irradiation dose (Gy)	No. queens	Survivorship (wk)	No. eggs/wk	No. larvae/wk	No. pupae/wk
0 (control)	15	12.40 ± 0.60a	4.96 ± 0.79a	2.56 ± 0.52a	0.73 ± 0.16a
20	15	13.00 ± 0.00a	5.12 ± 0.73a	1.46 ± 0.26a	0.43 ± 0.09a
50	15	11.00 ± 0.80a	1.60 ± 0.19b	0.01 ± 0.01b	0.01 ± 0.01b
70	15	8.13 ± 0.82a	1.71 ± 0.19b	0.01 ± 0.01b	0b
100	15	5.93 ± 0.83b	1.11 ± 0.24b	0.01 ± 0.01b	0b

Means within a column followed by different letters are significantly different at $\alpha = 0.05$ by Tukey's test.

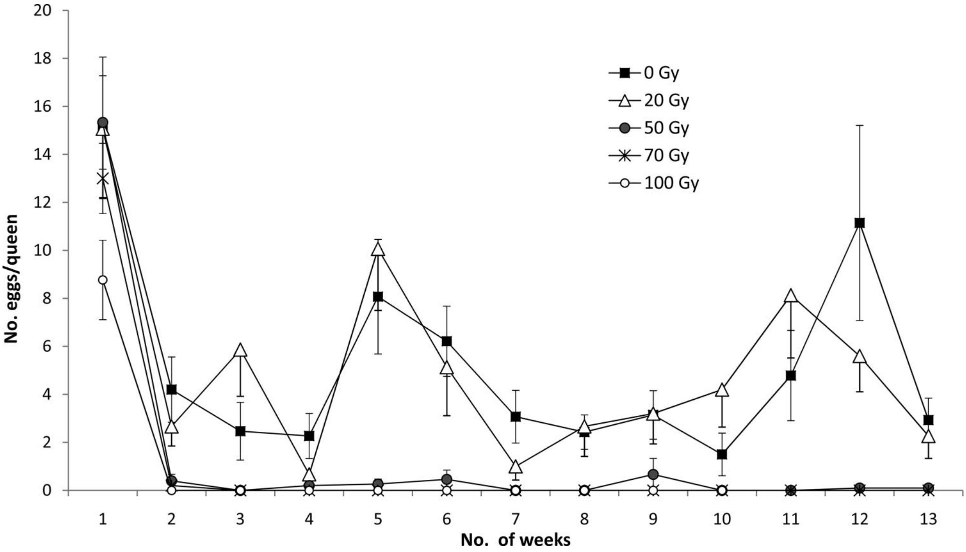

Fig. 1. Mean (±SE) number of eggs laid per queen after acute exposure to ionizing radiation.

The numbers of eggs observed may not reflect the true numbers of offspring produced by the queens in each treatment. Chang (1985) reported that only 38% of bigheaded ant, *Pheidole megacephala* (F.), eggs laid by queens became larvae; some eggs did not hatch and others were eaten by newly hatched larvae or workers in the nest. In our experiment, *W. auropunctata* survivorship in controls from egg to larvae was 43% before the start of the experiment and 51% during the experiment. The higher survival during the experiment could be due to the lower number of workers placed in the microcolonies after irradiation. The number of workers in the *W. auropunctata* microcolonies was maintained at equal levels, and equal amounts of food were supplied each week, which should have minimized the chance for differences in cannibalism between treatments. Hence, the numbers of eggs observed should reflect real radiation treatment effects.

In 2006, 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 (which may require a higher dose). These generic radiation treatments apply to all fresh horticultural commodities (Follett and Neven 2006, USDA–APHIS 2006). The 400-Gy default dose was based on radiotolerance information for a wide variety of insect taxa. The rationale for generic doses was that information on radiotolerance for a limited number of species could be extrapolated to related species to arrive at an effective generic dose. The 400-Gy dose is assumed to be effective against ants, although there was no information on radiation tolerance of ants at the time. Studies on the radiotolerance of ants and other poorly studied regulatory insects such as thrips, mealybugs, and scale insects are important to confirm the validity of the generic dose approach (Follett 2009).

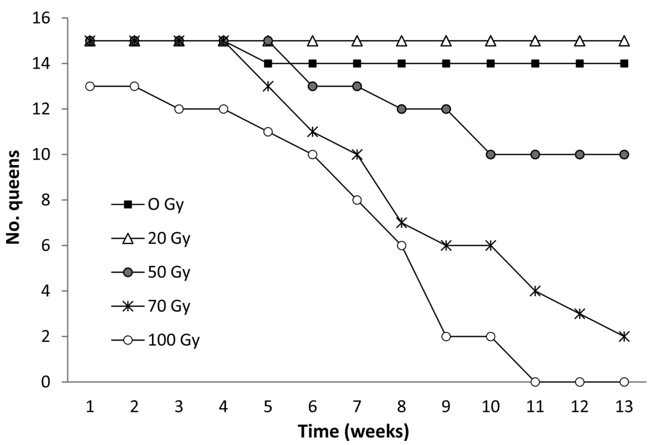

Fig. 2. Number of live queens during 13 wk after acute exposure to ionizing radiation.

Fresh tropical fruits currently traded between Hawaii and the United States, and between the United States and foreign countries (e.g., India, Thailand, Vietnam, and México), are treated using the 400-Gy generic dose due to the diversity of insect pests and the absence of information on radiotolerance for each specific pest (Follett 2009). Many of the pests associated with the tropical fruits are surface pests and therefore can be detected by visual inspection. This high-dose approach provides insurance against rejections due to the presence of surface pests or hitchhikers.

The generic radiation doses can be lowered for specific pests and commodities if this is practical. Lowering the dose will reduce costs and minimize any quality problems, and the capacity of the treatment facility may be increased owing to shorter treatment time (Follett 2009). The insurance against random hitchhiker pests, however, will be lost. The radiation treatment for sweet potatoes exported from Hawaii to the U.S. mainland was lowered from 400 to 150 Gy when it was shown that this dose was sufficient to control three internally feeding quarantine pests (Follett 2006). Ants are occasionally found on sweet potatoes during USDA–APHIS inspection before export. If a significant number of ants are found, the product may be returned to the grower to remove the ants, treated at the higher 400-Gy dose, or rejected. Information for ants showing that radiation treatment at a dose $\leq$150 Gy is effective for control may help avoid rejection or interruption of export shipments.

In this study, progeny of *W. auropunctata* queens receiving radiation doses of 70 and 100 Gy did not successfully develop to the pupal stage; thus, these doses prevented the development of reproductive queens. During export, ants traveling with the commodity would be under additional stress due to limited food and cold storage temperatures. Queens surviving irradiation and shipment conditions would have to find a suitable nest site to found a colony. In our experiment, placing irradiated queens back into the colonies with workers and food improved their chance of survival and reproduction, which is a best-case scenario.

Follett and Taniguchi (2007) showed that big-headed ant queens were sterilized at 90 Gy. This study and the current study suggest that ants are relatively susceptible to irradiation. By comparison, Lepidoptera are sterilized at irradiation doses between 150 and 450 Gy (Ignatowicz 2004, Follett 2009). *W. auropunctata* and *P. megacephala* are classified within the subfamily Myrmicinae, a diverse group that contains nearly 50% of all known ant species (Bolton et al. 2006). Research on *W. auropunctata* and *P. megacephala* suggests a radiation dose of 100 Gy may be sufficient to control at least ants in this subfamily. The numbers of queens used in these two ant studies are low by quarantine treatment standards (Follett and Neven 2006); therefore, caution should be exercised in extrapolating the results to ants in general. Other important invasive ant species such as Argentine ant, *Linepithema humile* (Mayr); crazy ant, *Paratrechina longicornis* (Latreille); *Nylanderia pubens* (Forel); and *Anoplolepis longipes* (Jerdon) belong to other subfamilies, and their radiotolerance should be studied before recommending a generic irradiation treatment for the Formicidae to regulatory authorities.

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