# Effect of Irradiation on the Longevity and Reproduction of *Pheidole megacephala* (Hymenoptera: Formicidae) Queens

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**Abstract.** Irradiation is a quarantine treatment option to control ants and other hitchhiker pests on fresh horticultural products exported from Hawaii. The radiotolerance of the bigheaded ant, *Pheidole megacephala* (F.), was studied to determine a dose sufficient for its control. This ant was chosen as a representative species because it is a common hitchhiker and rearing methods in the laboratory have been developed. The desired response with irradiation treatment of ants is sterility of reproductive females. Queens from micro-colonies were irradiated at 60, 90, 120, or 150 Gy or left untreated as controls, then followed for 19 weeks to observe colony growth. In general, queen longevity, and the number of eggs, larvae, and pupae observed in the micro-colonies decreased with increasing irradiation dose. In the 60 Gy treatment, the number of eggs observed was reduced by 89.6% compared with the untreated controls. In the 120 Gy and 150 Gy treatments, the number of eggs observed was reduced by 99.5% and 98.5%, respectively, and no eggs were found after the first observation date at 7 days after treatment. No larvae or pupae were observed in the 90, 120, or 150 Gy treatments, suggesting these irradiation doses sterilized queens. This study suggests the USDA-APHIS-approved generic irradiation dose of 400 Gy is sufficient for the Formicidae. Information is needed on the radiotolerance of additional species of ants to confirm our findings.

**Key words:** Bigheaded ant, quarantine treatment, generic doses, phytosanitary treatment

## Introduction

Ionizing radiation (irradiation) is used as a postharvest quarantine treatment to disinfest fresh and durable agricultural commodities of quarantine pests (Follett and Griffin 2006). Ionizing energy breaks chemical bonds within DNA and other molecules, thereby disrupting normal cellular function in the insect. Insect response to irradiation varies with the insect species and life stage, and the absorbed dose received by the insect.

In 2006, USDA-APHIS published a landmark rule providing generic irradiation quarantine treatments for fresh fruit and vegetable and other horticultural commodities (USDA APHIS 2006, Follett and Neven 2006). The rule approved irradiation 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). 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. Information is minimal or lacking, however, for many important insect groups such as thrips, mealybugs, scale insects, and ants; information on the radiotolerance of these groups would be valuable to confirm the validity of the generic dose approach.

Hawaii is currently exporting approximately 10 million pounds of fresh fruits and vegetables annually using irradiation to control quarantine pests. The presence of hitchhiking ants can interrupt exports of irradiated fresh fruit from Hawaii. Ants are particularly problematic on exported rambutan, as ants may nest in fruit clusters. Although most interceptions are sterile workers, if a substantial number of workers are found in a small sample of boxes, the likelihood of a having a reproductive female somewhere in the shipment may be significant. The most common species of ants intercepted on Hawaiian produce are the bigheaded ant, *Pheidole megacephala* (F.) white-footed ant, *Technomyrmex albipes* (Fr. Smith) and long-legged ant, *Anoplolepis longipes* (Jerdon). Other invasive ants such as the little fire ant, *Wasmannia auropunctata* (Roger), are spreading rapidly and may become increasingly common in exported produce.

We studied the radiotolerance of *P. megacephala* to determine the dose sufficient for its control. This ant was chosen as a representative species because it is a common hitchhiker and rearing methods in the laboratory have been developed (Chang 1985). Information from *P. megacephala* may give us an idea of how tolerant ants in general are to irradiation. Unlike other disinfestation techniques, irradiation does not need to kill the pest immediately to provide quarantine security, and therefore live (but non-viable 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. The desired response with irradiation treatment of ants is sterility of reproductive females.

## Materials and Methods

Eight *Pheidole megacephala* colonies were collected from pineapple fields in Waialua, HI in February 2006. Colonies were dug up with a shovel, brought into the laboratory at the University of Hawaii at Manoa, and separated from soil using the water displacement method (Chang 1985). Laboratory rearing methods were modified from Chang (1985). Stock colonies were held individually in heavy plastic 'bus boy' tubs (37 cm x 50 cm x 10 cm; length x width x height) until use for testing. The inner sides of the tubs were coated with Fluon AD-1 (Northern Products Inc., Woonsocket, RI) to prevent ant escape. Ants were given water, food, and sucrose weekly. An artificial nest was provided consisting of a plastic Petri dish (25 mm x 140 mm, height x diameter) with four 5 mm diameter holes drilled in the side of the dish to allow ant movement in and out of the nest. The inside of the dish was lined with several layers of moist paper towel to provide moisture and a place to hide. An assortment of food items was used including egg yolks and whites, corn meal, and tephritid fruit fly larvae and adults. Each colony was transferred periodically to a clean tub when debris built up.

When a stock colony started to produce winged reproductives, as many as 20 females, 3 males and approximately 200 workers, were transferred to a smaller (22 cm x 10 cm x 16 cm) plastic tub (Gladware food container, The Clorox Company, Oakland, CA) for mating. The inner sides of the plastic food containers were again coated with Fluon AD-1 to prevent ant escape, and ants were given food, water and sucrose weekly.

Mated females were removed and placed individually into a micro-colony with approximately 100 workers. The reproductive female (queen) and workers were given food, water and sucrose weekly, and a micro-nest consisting of a 15mm x 60mm plastic Petri dish with four 5 mm diameter holes drilled on the side of the dish. The inside of the dish was lined with layers of moist paper towel to provide moisture and a place to hide.

The micro-colony was checked weekly for egg production and larval growth. When enough actively reproducing queens had been collected to conduct a replicate of the study, individual queens were removed and placed in a plastic snap cap vial (50 mm x 25 mm, length x diameter) with a moist cotton ball and subjected to irradiation treatment. Queens

**Table 1. Effect of irradiation on the longevity and reproduction (means ± SE) of *Pheidole megacephala* queens.**

| Target dose (Gy) | Reps | Total no. queens | Longevity[a] (days) | No. eggs observed[b] | No. larvae and pupae observed[b] |
|---|---|---|---|---|---|
| 0 (control) | 3 | 9 | 133.0 ± 0.0 a | 223.5 ± 38.4 a | 69.5 ± 64.0 a |
| 60 | 3 | 9 | 97.2 ± 14.4 a | 23.3 ± 5.9 b | 0.4 ± 0.9 |
| 90 | 3 | 9 | 91.0 ± 15.5 a | 10.2 ± 4.1 bc | 0.0 |
| 120 | 3 | 9 | 34.2 ± 10.1 b | 1.0 ± 0.8 c | 0.0 |
| 150 | 3 | 8 | 21.8 ± 9.1 b | 3.6 ± 2.0 c | 0.0 |

[a]Maximum possible longevity = duration of the experiment = 133 days.
[b]Data were log transformed before analysis.
Means within a column followed by different letters are significantly different, Tukey's test ($P<0.05$).

were treated at target doses of 60, 90, 120, or 150 Gy or left untreated as controls. Irradiation treatment was carried out using a Gammacell 220E cobalt-60 irradiator (MDS Nordion, Ottawa, Canada) with a dose rate of 200 Gy per minute. Dosimeters (Opti-chromic detectors, FWT-70-83M, Far West Technology, Goleta, CA) were placed in a separate vial without ants at each dose in each replicate. The dosimeters were read with a FWT-200 reader (Far West Technology, Goleta, CA) at 600-nm absorbance to verify the minimum absorbed dose in each replicate. After irradiation, treated and control queens were given a new micro-colony and artificial nest with 100 new workers and followed for 19 weeks to observe colony growth. The artificial nest, a 15 mm x 60 mm Petri dish, was layered with a thin layer of plaster of Paris (moistened weekly with a few drops of water) colored with Nile blue dye. The blue background facilitated viewing of eggs, larvae, and pupae. Four 5 mm diameter holes were drilled just above the plaster line. Micro-colonies were inspected weekly and new workers were added as old workers died throughout the course of the study to maintain approximately 100 workers per colony.

Each week, the micro-colony was checked for the presence of eggs, larvae, and pupae. The micro-colony was chilled in a refrigerator at 2°C for 30 min to slow ant movement before making observations. The experiment included three replicates with three queens treated at each dose in each replicate. Replicates were run in sequence about 5 months apart, and observations for each replicate were terminated after 19 weeks. Data on mean queen longevity and the mean number of eggs, larvae, and pupae observed were subjected to ANOVA, and mean separation tests were done using a Tukey's test at the 0.05% level of probability (SAS Institute 2002).

## Results

In general, queen longevity, and the number of eggs, larvae, and pupae observed in the micro-colonies decreased with increasing irradiation dose (Table 1). All untreated queens survived for the duration of the experiment (133 days); in the 120 and 150 Gy treatments, queens lived for a mean of 34.2 and 21.8 days, respectively, and no queens were alive at the end of the experiment. Irradiation treatment significantly reduced the number of eggs laid; in the 60 Gy treatment, the number of eggs observed was reduced by 89.6% compared with the untreated controls. In the 120 Gy and 150 Gy treatments, the number of eggs observed was reduced by 99.5% and 98.5%, respectively, and eggs were observed only on the first sampling date. No larvae or pupae were observed in the 90, 120, or 150 Gy treatments, suggesting these irradiation doses are sufficient to sterilize queens.

## Discussion

Insect pests such as thrips, mealybugs, scale insects, and ants are common surface pests on exported Hawaiian fruits irradiated using the generic dose of 400 Gy. Little or no information on radiotolerance is available for these groups (Follett and Griffin 2006). The sample sizes used in our study were small due to the difficulty in collecting colonies and establishing them in the laboratory. *P. megacephala* queens were sterilized at an irradiation dose of 90 Gy, suggesting this ant is relatively susceptible to irradiation. By comparison, Lepidoptera are typically sterilized at irradiation doses between 150-200 Gy (Follett and Lower 2000, Follett 2006a). The International Database of Insect Disinfestation and Sterilization (IDIDAS) lists irradiation studies for only six species in the order Hymenoptera, including the honey bee and several parasitic wasps (Bakri et al. 2005, IDIDAS 2007). No information exists in IDIDAS for the disinfestation or sterilization of ants.

Chang (1985) reported that only 38% of *P. megacephala* eggs laid by queens became larvae; some eggs did not hatch and others were eaten by newly hatched larvae or workers in the nest. Thus, the numbers of eggs observed in our study may not reflect the true numbers of offspring produced by the queens in each treatment. The number of workers in the 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 irradiation treatment effects.

Assuming that the radiotolerance in *P. megacephala* is representative of Formicidae in general, the 400 Gy generic dose approved for insects will safely control this group. For some commodities, research has been conducted on specific regulated insect pests to lower the dose below 400 Gy if this will reduce treatment costs or help preserve fruit quality (Follett and Griffin 2006). For example, papayas (*Carica papaya*) exported from Hawaii to the U.S. mainland are routinely irradiated at a minimum dose of 400 Gy to control white peach scale (*Pseudaulacaspis pentagona* [Targioni-Tozzetti]) in addition to fruit flies because no information was available on the radiotolerance of white peach scale. Recent studies demonstrated that this scale is controlled at 150 Gy (Follett, 2006b). Hence, an irradiation treatment with a minimum absorbed dose of 150 Gy provides quarantine security for white peach scale in addition to fruit flies on exported papaya. Lowering the dose will significantly reduce costs of treatment and increase capacity of the treatment facility. The information in this paper on radiotolerance of *P. megacephala* may be useful to regulatory agencies if ants are found on papayas or other commodities treated at doses below 400 Gy. Our data suggest that ants are relatively susceptible to irradiation and doses <150 Gy may be sufficient for ant control. Information is needed on the radiotolerance of additional species of ants to confirm our findings.

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