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ORIGINAL CONTRIBUTION

# Fecundity and longevity of Argentine ant (Hymenoptera: Formicidae) queens in response to irradiation

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## Abstract

Irradiation is a post-harvest quarantine treatment option to control ants and other hitchhiker pests on fresh horticultural products traded between countries. As little is known about irradiation effects on ants, radiotolerance of the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae: Dolichoderinae), was studied to determine a dose sufficient for its control. Queens collected from Buenos Aires, Argentina, were irradiated with 30, 60, 90 Gy or left untreated as controls, and then followed for 8 weeks to evaluate their survival and fecundity. Overall queen survival and brood viability decreased with increasing irradiation dose. The number of eggs was reduced by 50%, 69% and 56% in the 30, 60 and 90 Gy doses, respectively, compared with untreated control queens. The percentage of eggs that developed into larvae decreased from 41.1% in the control to 22.5%, 1.4%, and 0% in the 30, 60, and 90 Gy treatments, respectively. Thus, the number of larvae was reduced by 69% in the 30 Gy treatment compared with the control, only one larva was observed in the 60 Gy treatment, and none in the 90 Gy treatment. Only one pupa was observed in the 30 Gy treatment and none in the 60 and 90 Gy treatments during the 8-week experiment. Queens irradiated with 60 and 90 Gy had significantly reduced longevity compared with queens treated with lower doses or untreated queens. Radiation dose ≥90 Gy stopped brood development in Argentine ant queens and should be sufficient as a phytosanitary treatment. The radiotolerance of Argentine ant appears to be similar to that of two other important invasive ants.

## Introduction

Low-dose ionizing radiation is used as a post-harvest treatment to control quarantine pests in fresh agricultural commodities (Follett 2009). Ants can be problematic hitchhiker pests that cause rejection or return shipment (Costa et al. 2005; Follett and Taniguchi 2007). Although most interceptions are sterile workers, 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. Determining an effective radiation dose to control ants may help avoid rejection or interruption of export shipments.

The Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae: Dolichoderinae), is a small invasive ant native to northern Argentina, Uruguay, Paraguay and southern Brazil. This ant has been inadvertently spread to many areas with a 'Mediterranean' climate including South Africa, New Zealand, Japan, Easter Island, Australia, Hawaii, Europe, and the United States (Suarez et al. 2001). Genetic studies suggest that the source of introduced populations is the southern Rio Parana in Argentina (Tsutsui et al. 2001). Argentine ant was first discovered in the continental United States in 1891 and in Hawaii in 1940. Between 1995 and 2003, a total of 451 ant interceptions were made by the Hawaii Department of

Agriculture, with Argentine ant making up 43.7% of all interceptions (Krushelnycky et al. 2005). This ant has been intercepted on bananas, guavas, peppers and pineapples imported into the United States. The small size of Argentine ants (2–3 mm in length) makes them particularly problematic because they may escape from insect proof boxes that are designed to prevent the entry or exit of quarantine tephritid fruit flies in irradiated produce exported between countries; 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 are uncertain. Queens are much larger than workers and therefore may be contained or excluded by screens and sealing of box lids that do not exclude or contain workers. As in many other ant species, Argentine ant workers are unable to lay reproductive eggs but can direct the development of eggs into reproductive females (Aron 2001). Thus, if eggs and workers present in the box survive irradiation treatment, eggs promoted by the workers could eventually replace the queens giving rise to a new colony. The Argentine ant is unicolonial and polygynous (Tsutsui et al. 2001), and may form supercolonies, which increases its risk of introduction by way of infested commodities.

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; Follett 2009). The generic radiation treatments apply to all fresh horticultural commodities. The International Plant Protection Commission has also approved the 150 Gy generic dose for tephritid fruit flies (IPPC 2007). These first-ever generic doses have spurred interest in the use of phytosanitary irradiation, and Hawaii, Mexico, Thailand, India, Vietnam and several other countries have begun exporting irradiated fresh fruit to the United States. Australia is exporting irradiated fruit to New Zealand using similar generic doses (Follett and Neven 2006).

Most studies of radiation effects on arthropods have focused on Diptera, Lepidoptera and Coleoptera pests, and consequently, generic radiation doses are based on effective doses for those insect groups. The generic 400 Gy dose is assumed to be effective against ants, although there was no information on radiation tolerance of ants at the time it was approved. There is a need for additional studies with other groups of quarantine insects and hitchhiker pests such as thrips, mealybugs and ants, to define their specific radiation dose. Lowering the dose for commodities associated with specific pest groups would help reduce treatment costs, minimize any radiation effects on commodity quality and increase the capacity of the treatment facilities owing to shorter time (Follett 2009). Radiation doses for specific groups, such as the 150 Gy dose approved for tephritid fruit flies, would only be applied for commodities with no other associated quarantine pests (which may require higher doses).

We studied the tolerance to radiation of the Argentine ant to determine the dose sufficient for its control. Information from the Argentine ant, combined with recent information with two other invasive species, little fire ant, *Wasmannia auropunctata* (Roger), and big-headed ant, *Pheidole magacephala* (F.) (Follett and Taniguchi 2007; Calcaterra et al. 2012), will give us a better idea of how radiotolerant ants are compared with other insects. 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 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 imported fresh fruits and vegetables. The desired response with irradiation treatment of ants therefore is to sterilize reproductive females (queens) or prevent brood development.

## Materials and Methods

The research was conducted at the Fundación para el Estudio de Especies Invasivas (FUEDEI) in Buenos Aires, Argentina due to the availability of a laboratory culture of Argentine ant that is genetically similar to the populations established in the United States (Tsutsui et al. 2001).

### Collection of colonies

In November–December 2012, queens and workers from a supercolony of Argentine ant were collected near the coast of the La Plata River at the Ciudad Autónoma de Buenos Aires (CABA), Argentina: (34°32′24″S, 58°26′49″W). The colony was collected by digging up part of the nests including queens, sexuals, workers and brood from the ground and placing soil and ants into covered buckets coated with talc (to prevent escape). In the laboratory, ants were separated from soil by flotation (Banks et al. 1981) and transferred to plastic trays (25 by 40 by 7 cm) coated with talc, from where queens and workers were captured.

 

Each year, Argentine ant queens are executed by workers in late winter (Markin 1970), enabling the development of new alate females which mate with males in mid-spring (Markin 1970; Keller et al. 1989). As the collection took place during late spring, the colony contained mostly newly mated queens, which are supposed to reach the maximum oviposition rate in <2 weeks from their emergence (Keller and Passera 1990). Forty-eight of these presumably newly mated queens were placed individually in plastic containers with 15–20 workers (referred to hereafter as a microcolony) to care for and feed the queen and its progeny, with a permanent source of water and sugar water, and fed twice a week alternating with peanut butter and hard boiled eggs. Each microcolony included a transparent plastic tube (10 cm length × 1.5 cm diameter) half-filled with water and sealed with a cotton plug (to provide humidity) leaving a 4 by 1.5 cm space for the queen and its progeny to settle, and allowing free access for workers. All microcolonies were kept in an incubator at 28°C as in Abril et al. (2008), 12:12 (L:D) h., and 60% RH, and fed as described above in an attempt to provide optimal conditions for oviposition and development.

### Fecundity before irradiation

Before irradiation, the 48 queens from all microcolonies were checked weekly for 2–4 weeks with a 40× stereomicroscope for egg production and larval growth to confirm that they were actively reproducing. As in Calcaterra et al. (2012), after irradiation treatment, the number of new eggs, larvae and pupae per queen was calculated weekly assuming that the number of brood in each stage was the sum of the new brood and the brood from the previous week which had not developed to the next stage. Some underestimation could have been introduced because emergence of adults was not possible to record, and all eggs, larvae and pupae did not develop or were predated by workers. However, this underestimation would have similarly operated in all treatments, and thus, it would not have affected the result of the experiment. The period of estimation of colony fecundity before irradiation was similar for all treatments (Welch ANOVA test, $F_{.} = 0.65$; d.f. = 3, 23.63; $P = 0.59$), ranging from 2.3 to 2.6 weeks.

### Irradiation treatment

Following Calcaterra et al. (2012) and Follett and Taniguchi (2007), the 48 queens were randomly assigned to an irradiation treatment: 0 (control), 30, 60, or 90 Gy. Queens together with a pair of workers were irradiated individually in the tubes where they were reared (see above). Queens from the control treatment received the same manipulation and transportation as irradiated queens to avoid differences between treatments. Irradiation was carried out at the Comisión Nacional de Energía Atómica (Centro Atómico Ezeiza, Buenos Aires, Argentina) using a Gammacell-220 cobalt-60 irradiator (MDS Nordion, Ottawa, ON, Canada) with a dose rate of 0.35 Gy per min (transit dose = 0.02 Gy). For each treatment, four dosimeters were placed in separate empty glass vials to estimate the absorbed dose received by ants. Measured doses (mean ± SE) for the 30, 60 and 90 Gy treatments were 29.1 ± 0.4, 61.7 ± 0.9 and 90.1 ± 3.4 Gy, respectively. Eggs and brood, which have the potential to develop into reproductive females, have not been found in imported commodities during inspection and therefore were not tested. Early ant life stages are likely more susceptible to radiation than adults, as is invariably the case in other insects (Aldryhim and Adam 1999; Follett 2006; Follett and Griffin 2006).

### Fecundity and survivorship after irradiation

Less than 24 h after irradiation, all queens were placed in new artificial nests and reared as above. The number of eggs, larvae and pupae per microcolony was recorded weekly for 8 weeks. Queen survivorship was also recorded weekly to determine the effect of radiation treatment on residual longevity.

### Data analysis

The total number of eggs laid per queen (microcolonies) during the two previous weeks before irradiation was analysed by one-way ANOVA with the microcolonies as replicates. For significant effects, means separation was performed using a Tukey's HSD test. After irradiation, the number of eggs laid per queen (microcolonies) and per week was analysed by repeated measures ANOVA based on general linear mixed model using the R software package version 3.0.1 for Windows (R Development Core Team 2013) in which 'treatment', 'week' and 'treatment × week' were fixed effects and 'queens' was the random effect (function glmm). Variance was modelled using the function varIdent. Comparisons between treatments were conducted using Fisher's LDS test. Total number of larvae and pupae produced per queen and survivorship data, which did not show a normal distribution, was analysed using a Kruskal–Wallis test. Linear regression on ln $(x + 1)$-transformed data was used to predict the dose that would prevent egg laying.

## Results

The total number of eggs laid during the last 2 weeks before irradiation was similar among queens randomly assigned to treatments (F = 0.40; d.f. = 3, 44; P = 0.75), ranging from 19 ± 0.30 to 24 ± 0.29 eggs. Thus, the queens chosen to be irradiated in each treatment showed similar fecundity.

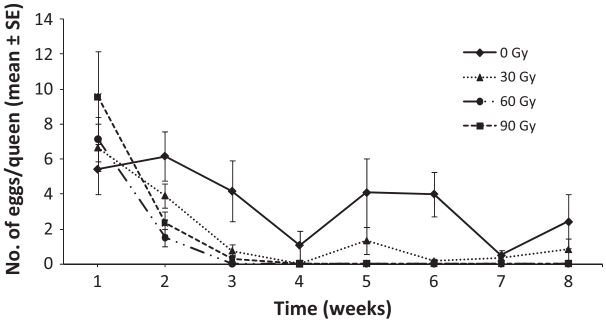


**Fig. 1** Mean (±SE) number of eggs laid per queen after acute exposure to ionizing radiation.

### Effect of irradiation on fecundity

The mean number of eggs, larvae and pupae in the microcolonies decreased after irradiation (Table 1). Radiation had a significant effect on the mean number of eggs laid per queen (F = 5.13; d.f. = 3, 341; P = 0.003), which was 50%, 69% and 56% lower in the 30, 60 and 90 Gy treatments compared with the control (Table 1). Although differences on the number of eggs laid per queen between treatments were visually observed at the second week after irradiation (mainly between the control and 60 and 90 Gy treatments, Fig. 1), no significant interaction was found between treatments and time (F = 1.53; d.f. = 21, 341; P = 0.064). Queens treated with a radiation dose of 90 Gy laid eggs only until the second week, except one queen, which laid eggs on the third week as well (Fig. 1); however, no larvae hatched from these eggs. The percentage of eggs that developed into larvae linearly decreased with irradiation dose ($r = -0.96$, $P < 0.05$), averaging 41.1%, 22.5%, 1.4% and 0% in the 0, 30, 60 and 90 Gy treatments, respectively. The linear regression equation describing the effect of dose on the mean number of eggs laid per queen $y = -0.01x + 1.22$ ($R^2 = 0.18$; F = 10.6; d.f. = 1, 46; P = 0.0027), and although the model had a poor fit to the data, the predicted radiation dose to prevent any egg laying is 122 Gy (101–144, 95% Cl).

The production of larvae decreased markedly with irradiation treatment. The number of larvae developing in the control was significantly higher than that from radiated queens, as well as for the number of larvae in the 30 Gy treatment in comparison with the 60 and 90 Gy treatments ($H = 23.48$; n = 48, d.f. = 3; $P < 0.0001$); only one larva was observed in the 60 Gy treatment, while no larvae were observed in the 90-Gy treatment. No significant differences were found between treatments in pupa production ($H = 4.29$; n = 48, d.f. = 3; P = 0.23); eight pupae were produced in the control treatment, while only one was observed in the 30 Gy treatment. No pupae were observed at the 60 and 90 Gy doses.

### Irradiation effect on survivorship

Queen survivorship decreased with increasing irradiation dose. By the end of the 8-week experiment, a total of 11, 12, 6 and 0 queens survived at the radiation doses of 0, 30, 60 and 90 Gy, respectively (Fig. 2). At 6 weeks, all queens treated with a dose of 90 Gy were dead, which was significantly lower survivorship than queens in the other three treatments ($H = 24.12$, $P < 0.0001$).

**Table 1** Residual longevity and reproduction (mean ± SE) of *Linepithema humile* queens reared during 8 weeks after acute exposure to ionizing radiation

| Irradiation dose (Gy) | No. of queens | Survivorship (weeks) | No. of eggs/week | Total no. of larvae | Total no. of pupae |
|---|---|---|---|---|---|
| 0 | 12 | 7.83 ± 0.17 ab | 3.48 ± 0.87 a | 11.83 ± 2.58 a | 0.67 ± 0.26 a |
| 30 | 12 | 8.00 ± 0.00 a | 1.75 ± 0.29 b | 3.67 ± 0.86 b | 0.08 ± 0.08 a |
| 60 | 12 | 5.83 ± 0.72 b | 1.08 ± 0.18 b | 0.08 ± 0.08 c | 0 a |
| 90 | 12 | 3.83 ± 0.21 c | 1.52 ± 0.33 b | 0 c | 0 a |

Different letters indicate significant differences between treatments ($P < 0.05$).

 

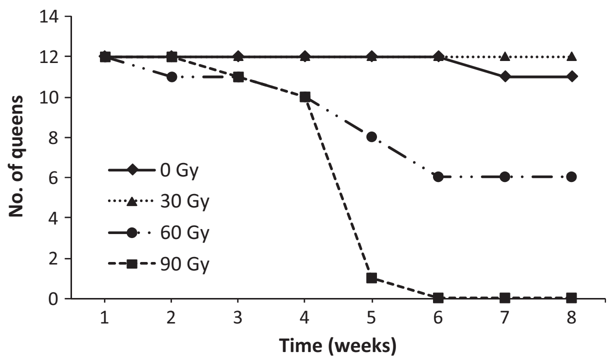


**Fig. 2** Number of live queens during 8 weeks after acute exposure to ionizing radiation.

## Discussion

Irradiation lowered Argentine ant fecundity and shortened its longevity. At a radiation dose of 90 Gy, queens were unable to produce either larvae or pupae and they died shortly after treatment, suggesting that this dose is sufficient to stop reproduction. Thus, this radiation dose would be enough to control *L. humile*, as eggs and larvae (the only alternative for new colony founding in the absence of the queen) should be non-viable at the 90 Gy dose or even in lower doses (Aldryhim and Adam 1999; Follett 2006; Follett and Griffin 2006).

The number of eggs observed under experimental conditions in this study did not represent the real number of offspring produced by Argentine ant queens in nature. In laboratory colonies, some eggs do not hatch or are eaten by larvae or workers from the same microcolony, besides, variation between queens also occurs, experienced in other previous works on the Argentine ant (Abril et al. 2008) and associated with genetic and physiological differences as suggested by Keller (1988). However, the success rate of egg to larvae recorded in this study was similar to that obtained in other works. On average, 41.1% of Argentine ant eggs developed to larvae in untreated queens, which was similar to that observed in the invasive ants *W. auropunctata* (43–51%) (Calcaterra et al. 2012) and *P. megacephala* (38%) (Chang 1985).

After irradiation, egg production fluctuated but was always higher in untreated control queens than in the irradiation treatments, except for the first week when oviposition by treated queens was greater. Queens irradiated at 60 and 90 Gy laid the highest number of eggs during the first week post-treatment, followed by a steep drop and then no oviposition after the third week. Similar behaviour was also observed in irradiated queens of *W. auropunctata* (Calcaterra et al. 2012); this species showed highly variable oviposition in the untreated control and 20 Gy treatments, and a high oviposition rate in the first week after irradiation at 70 and 100 Gy, and no oviposition after the third week. Thus, higher doses of radiation (>60 Gy) should stop reproduction after 3 weeks. This helps explain the poor fit of the linear regression line describing the relationship between oviposition and radiation dose, which may result in an overestimation of the effective dose. In fact, when the procedures with Argentine ant were repeated at higher doses of radiation (135 and 160 Gy), a higher mortality rate was obtained, but with similar oviposition values in the first 2 weeks after irradiation among treated and control queens (C. Coulin, L.A. Calcaterra, P.A. Follett, unpublished data). Therefore, the development from egg to larva and from larva to pupa, and queen mortality, should be better estimators of the radiation tolerance of Argentine ant to determine an effective dose for this invasive ant.

Based on this study and previous studies, a dose of ≥90 Gy should be enough to sterilize three of the world worse invasive species belonging to two ant subfamilies: *L. humile* in the Dolichoderinae, and *W. auropunctata* (Calcaterra et al. 2012) and *P. megacephala* (Follett and Taniguchi 2007) in the Myrmicinae. Therefore, a general dose of 100 Gy may be sufficient as a general treatment to control invasive ants. Irradiation tolerance tests in other invasive ants, such as the Myrmicinae red imported fire ant *Solenopsis invicta* Buren and *Nylanderia pubens* (Forel), *Paratrechina longicornis* (Latreille), and *Anoplolepis longipes* (Jerdon) belonging to the Formicinae subfamily, would provide useful information to support a general dose for the Formicidae.

Irradiation is a post-harvest quarantine treatment option for exported commodities such as fruits and vegetables to prevent movement of viable invasive ants. Irradiation treatment is effective against insect pests at doses that typically do not harm the quality of fresh commodities (Wall 2008). While the majority of taxa (e.g. Coleoptera, Diptera, Lepidoptera, Hemiptera) have shown high variability in the tolerance dose to radiation, Hymenoptera seems to be the insect order with less variation in the required dose for sterilization, ranging from 80 to 100 Gy (Bakri et al. 2005; Mastrangelo and Walder 2011). Our results agree with radio tolerance values reported for Hymenoptera. Thus, effective doses in other invasive ant species are expected to fall within this range (80 and 100 Gy). Radiation treatments applied to control tephritid fruit flies (150 Gy) in traded fresh commodities should also control any invasive hitchhiker ants.

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