

Phytosanitary irradiation of the invasive herbivorous terrestrial snail *Cornu aspersum* (Stylommatophora: Helicidae)

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Abstract

Phytosanitary measures are needed to prevent the transport of snails and slugs during trade in regulated articles. Ionizing radiation is increasing in use as a phytosanitary treatment against insect pests. Irradiation has the potential to be a treatment against snails and slugs, but little research has been done on radiosensitivity of terrestrial herbivorous gastropods. Adult *Cornu aspersum* (Müller) (Stylommatophora: Helicidae) were subjected to ionizing radiation at 25–100 Gy. Irradiation decreased the number of eggs laid, and those laid by adults irradiated with ≥ 75 Gy did not hatch. This research suggests that gastropod reproduction can be prevented with relatively low doses of radiation. A phytosanitary irradiation dose for *C. aspersum* should also serve to allow export of live snail species as food to areas that quarantine against them.

Key Words: escargot; invasive species; prevention of reproduction; radiosensitivity; regulated articles

Resumen

Se necesitan medidas fitosanitarias para prevenir el transporte de caracoles y babosas durante el comercio en artículos reglamentados. La irradiación ionizante es un tratamiento fitosanitario cuyo uso está incrementando; sin embargo, se han realizado pocos estudios sobre la radio-sensibilidad en gasterópodos terrestres fitófagos. Adultos de *Cornu aspersum* (Müller) (Stylommatophora: Helicidae) fueron expuestos a radiación ionizante entre 25–100 Gy. La irradiación redujo la cantidad de postura de huevos, y los huevos puestos por adultos irradiados con ≥ 75 Gy no emergieron. Este estudio sugiere que es posible prevenir la reproducción de gasterópodos con dosis relativamente bajas de radiación. Una dosis de irradiación fitosanitaria para *C. aspersum* también debe servir para permitir la exportación de caracoles vivos como alimento a las áreas que la cuarentena.

Palabras Clave: escargot; especies invasivas; la prevención de la reproducción; radiosensibilidad; artículos reglamentados

Snails and biologically similar slugs (Class Gastropoda) may be found on a wide variety of traded commodities from fresh vegetables to ceramic tiles. Some are significant crop pests (Barker 2002). With increased globalization of trade, the risk of the introduction of invasive species of snails and slugs, organisms that can also be intermediate hosts to parasites and infective agents of significance to humans and animals, increases. Disinfestation of gastropod quarantine pests is often achieved by methyl bromide fumigation, but alternatives are sought because methyl bromide is a significant stratospheric ozone depleting substance and its use is being increasingly restricted (Heather & Hallman 2008).

Doses of ionizing radiation to control most quarantine pests do not damage most fresh fruits and vegetables (Hallman 2011). Currently irradiation is used in several countries for disinfestation of insect quarantine pests. The objective of phytosanitary irradiation (PI) is to prevent development or reproduction of regulated pests; acute mortality of pests usually is not achieved by doses tolerated by fresh commodities.

Some studies to determine radiation doses needed to prevent reproduction of snails have been done. Fujita & Egami (1984) found that eggs laid by the adult aquatic snail *Physella acuta* (Draparnaud) (Physidae) up to 15 d after irradiation with 100 Gy did not hatch. After 15 d some eggs hatched, with the hatch rate increasing in a linear fashion

for the 50 d that observations were made until it reached almost 70%. The only other dose they tested for its effect on reproduction was 500 Gy, and at that dose eggs were only laid up to 15 d after irradiation and none hatched. The number of eggs laid declined upon irradiation at 100 Gy to $\sim 10\%$ of the non-irradiated control within 15 d. Twenty d after irradiation the number began to increase until reaching control levels 60 d after irradiation.

Several radiation studies have been done with the medically important aquatic snail *Biomphalaria glabrata* (Say) (Planorbinae). This species has been extensively studied because it is an intermediate host for the trematode *Schistosoma mansoni* Sambon (Strigeidida: Schistosomatidae), a causative agent for the disease schistosomiasis in humans. A dose of 112 Gy prevented egg production, while eggs were laid and some hatched at 84 Gy, the next lowest dose tested by Perlowagora-Szumlewicz (1964b). Cantinha et al. (2009) found similar results: prevention of egg hatch at 90 Gy while 0.1% hatch occurred at 80 Gy. A dose of 20 Gy reduced egg hatch to 5.9% compared with 98.8% in the non-irradiated control. Fertility was not recovered after 65 d of observation. Liard et al. (1968) found that adults laid eggs at the 3 highest doses studied (160, 320, and 640 Gy) for only 3 wk following irradiation although no eggs hatched. Eggs laid for 8 wk after irradiation with the next lowest dose, 80 Gy, did not hatch, but starting at the 9th week some hatched.

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The effect of irradiation on egg laying and egg hatching for 9 wk was studied in adults of the terrestrial snail, *Zonitoides arboreus* (Say) (Gastrodontidae), by Hollingsworth et al. (2003) who found that no F_1 eggs hatched when ~70 adults were irradiated with 69–79 Gy. At 43–51 Gy 0.6% of eggs hatched. Egg laying for snails irradiated with 69–79 Gy was reduced to 15% of the control during this time period. After 9 wk mortality of the irradiated snails (9.8–21.1%) was lower than the control (32.5%). It was noted that in the laboratory some *Z. arboreus* lived > 2 yr.

The above studies observed reproduction up to 50–65 d after irradiation when many irradiated snails were still alive. When developing irradiation phytosanitary treatments, the irradiated organisms should be held until they die in order to observe possible recovery of reproduction, such as was observed for *B. glabrata* (Liard et al. 1968). Therefore this research examined a cohort of snails over their lifetime.

The objective of this study was to observe fecundity, F_1 generation egg hatch, and adult longevity of a terrestrial, herbivorous gastropod species during the remainder of their lifespan after irradiation. Thus this study was conducted on the brown garden snail; *Cornu aspersum* (Müller) (Stylommatophora: Helicidae). It is native to the Mediterranean and western European regions and has become naturalized in parts of Africa, Australia, and the Americas (CABI 2014). It is consumed as *petit-gris* escargot, which has aided its geographical spread. For example, in the 1850s it was brought into California for human food and subsequently became a serious plant pest.

Previous studies have found that snail radiotolerance increases as they develop (Bonham & Palumbo 1951, Perlowagora-Szumlewicz 1964a, b); the same trend was found in an extensive review of insect radiosensitivity (Hallman et al. 2010). Therefore, only adults were studied in this research because a PI dose must be efficacious against all life-stages that might occur on exported host commodities, and an irradiation dose efficacious against adults would also be efficacious against immatures.

Materials and Methods

Mature-sized *C. aspersum* were collected in Fresno County, California in 2003, 2005 and 2006 where they were defoliating *Citrus* sp. trees. They were sent to the quarantine facility of the USDA, Agricultural Research Service in Weslaco, Texas. *Cornu aspersum* in California reaches reproductive age in ~2 yr, lives another 1–3 yr, and lays a few to several egg masses of a mean of 50–85 eggs/mass each year (Basinger 1931; Herzberg & Herzberg 1968; CABI 2014).

The snails were fed carrot roots and citrus foliage in plastic containers (0.25 × 0.38 × 0.16 m) with solid lids. Approximately 3 cm-deep moist potting soil that had been heated in a microwave oven to ~85 °C to kill parasites and predators was placed in the bottom of the containers for egg laying. The containers were kept in an environmental chamber at 20 ± 0.5 °C, 70 ± 5% RH and 12:12 h L:D because the plastic containers had solid lids and the soil was kept moist, RH within the containers was assumed to be close to 100%. Voucher specimens were deposited in the Kansas State University Entomology Department collection (voucher number 232).

The irradiation source used in this study (Husman model 521A, Isomedix, Inc., Whippany, New Jersey) emits gamma rays from ^{137}Cs , and is located at the USDA-APHIS Mexican Fruit Fly Rearing Facility at Mission, TX. The dose rate was ~40 Gy/min during the time of the study. Reference standard dosimetry was done in 1996 with the Fricke system, and routine dosimetry was performed at the time of sample treatment with radiochromic film (Gafchromic MD-55, ISP Technologies, Inc., Wayne, New Jersey) placed in areas of the load (center and

edges) with the most extreme dose readings. Dosimeters were read 24 h after exposure with a spectrophotometer (Milton Roy Spectronic 401, Ivyland, Pennsylvania) at 600 nm.

Adult *C. aspersum* were irradiated in 0.5-L cardboard containers with food. In 2003 one cohort of 20 snails was irradiated at 100 Gy, and a non-irradiated control consisted of 10 snails. In March 2005 snails were irradiated in 3 equal replicates, with total numbers irradiated being 45, 90, 180, 360, and 480 at target doses of 0 (control), 25, 50, 75, and 100 Gy, respectively. In 2006 snails were irradiated in 3 similar-sized replicates with a total of 106, 240, 320, and 397 snails with 0 (control), 25, 50, and 75 Gy, respectively.

The measure of efficacy used was prevention of hatch of F_1 generation eggs. After snails were irradiated they were placed back in the plastic containers in the environmental chamber. The containers were inspected weekly for egg masses, which were placed in petri dishes (90 mm) over moist filter paper until they had either hatched or died, as evidenced by decomposition. Dead snails were removed from the containers.

Results

Absorbed doses recorded from routine dosimetry ranged from 22–28, 45–59, 73–90, and 96–124 Gy for target doses of 25, 50, 75, and 100 Gy, respectively. No measurable radiation was recorded in the dosimeters that were included with the control snails, which also were brought to the irradiation facility but were not irradiated.

The 20 *C. aspersum* irradiated in 2003 with 100 Gy laid a mean of 145 ± 16.7 eggs per snail, while the average number of eggs laid per snail in the control group was 254 ± 26.1. None of the eggs from irradiated snails hatched, while hatching in the control was 88.8 ± 1.8%.

From the snails collected in 2005, the control snails laid a mean of 165 ± 18.9 eggs/snail and 93.9 ± 1.3% of the eggs hatched. Mean numbers of eggs/snail for 25, 50, 75, and 100 Gy were 173 ± 19.2, 110 ± 14.6, 62 ± 11.2, and 39 ± 7.5 eggs, respectively. Mean hatch rates of eggs laid from snails irradiated with 25, 50, 75, and 100 Gy were 25.0 ± 5.7, 10.3 ± 6.9, 0, and 0%, respectively. Adults irradiated with a target dose of 75 or 100 Gy did not recover the ability to reproduce before they had died.

In 2006 eggs that hatched were counted, but not total eggs. None of the eggs laid by irradiated (25–75 Gy) snails hatched, while hatching in the control was 40.3 ± 6.3%. The relatively low level of egg hatch in the control of adults irradiated in 2006 casts some doubt upon the results with irradiated snails for that year. Evidently there was a source of stress that affected egg hatch independently of radiation.

The age of the snails upon collection was unknown. The post-irradiation longevity of adult *C. aspersum* was not appreciably affected by irradiation except perhaps at 100 Gy (Table 1). However, the last irradiated snails (2 at 25 Gy) died 3.7 yr after having been irradiated, and one other snail irradiated with 25 Gy died 3.0 yr after irradiation. The last of the snails irradiated with 50 and 75 Gy died 2.4 yr after irradiation while the last of the control snails died 1.9 yr after the experiment was initiated.

Table 1. Percentage survival ± SEM of *Cornu aspersum* at 90, 180, and 360 d post irradiation.

Dose (Gy)	90 d (%)	180 d (%)	360 d (%)
Control	37.9 ± 3.3	8.9 ± 1.3	6.5 ± 1.5
25	38.0 ± 2.0	11.5 ± 2.4	7.8 ± 1.0
50	38.4 ± 2.5	10.8 ± 1.6	3.3 ± 0.86
75	40.2 ± 4.3	7.1 ± 1.1	1.0 ± 0.66
100	7.6 ± 2.9	2.1 ± 0.56	0.83 ± 0.43

The dose to prevent hatching of eggs laid by irradiated *C. aspersum* seems to be ~75 Gy, which is roughly in agreement with studies of other snails (Perlowagora-Szumlewicz 1964b; Cantinha et al. 2009; Liard et al. 1968; Hollingsworth et al. 2003). Large-scale testing with many thousands of snails would need to be conducted before irradiation could be approved as a phytosanitary treatment (Heather & Hallman 2008). A PI dose for *C. aspersum* should also serve to permit the export of live (fresh) snails as food to areas that quarantine against it with the snail shell being potentially useful to verify irradiation treatment using laboratory measurements (Yordanov et al. 2001).

Acknowledgments

Beth Grafton-Cardwell and Janet McLain, Univ. of California, Kearney, are thanked for providing *C. aspersum*. Crissy Gutierrez and Maura Rodriguez, formerly with USDA-ARS, Weslaco, TX, are thanked for technical support. John Díaz-Montano, USDA-ARS, Manhattan, KS, is thanked for correcting the resúmen. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U. S. Department of Agriculture. USDA is an equal opportunity provider and employer. This work was part of the FAO/IAEA Coordinated Research Project D62008 on Development of Generic Irradiation Doses for Quarantine Treatments.

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