Ann. appl. Biol. (2003), **143**:395-399
Printed in UK

Effects of irradiation on the reproductive ability of *Zonitoides arboreus*, a snail pest of orchid roots

By ROBERT G HOLLINGSWORTH*, PETER A FOLLETT and JOHN W ARMSTRONG

US Pacific Basin Agricultural Research Center, USDA-Agricultural Research Service, PO Box 4459, Hilo, Hawaii 96720, USA

(*Accepted 19 June 2003; Received 19 June 2002*)

Summary

The effect of irradiation on the reproductive ability of the orchid snail, *Zonitoides arboreus*, a serious pest of potted orchids in Hawaii, was investigated. Weekly egg production averaged between 0.8 and 1.9 over a 9-wk period for snails not exposed to irradiation, and egg hatch averaged 61%. In comparison to untreated controls, irradiation of snails at the lowest dose tested (34-37 Gy) reduced egg production and egg hatch by 63% and 94%, respectively over a 9-wk period. None of the snails treated with levels of irradiation ≥ 69 Gy produced viable eggs. This is the first study measuring the effect of ionising irradiation on a terrestrial snail or slug species using sterilising doses. Overall, the results show that the reproductive ability of this snail species is affected by irradiation in a similar manner as for *Biomphalaria glabrata,* an aquatic snail for which the effects of irradiation have been studied in detail.

Key words: *Zonitoides arboreus,* bush snail, orchid snail, fecundity, fertility, irradiation, sterilisation

Introduction

Between 1990 and 2000, the value of fruit and vegetable exports worldwide rose from ~ US $25.5 to 38.5 billion, a 51% increase (Anon., 2002). With this increase in international trade, there has been a concomitant increase in the risk of invasion by alien pest species, including pest species of slugs and snails (Robinson, 1999). Port inspectors find "hitch-hiker" species of slugs and snails not only on agricultural and horticultural commodities, but also on shipping containers, household tiles, military hardware and aquarium supplies (Robinson, 1999). Commodities found infested with quarantine pests must either be cleaned, subjected to an approved quarantine treatment, sent back or destroyed. Methyl bromide fumigation is the standard quarantine treatment used for many exported agricultural and horticultural commodities found to be infested at their destination. In the US, most uses of methyl bromide are being phased out because of health and environmental concerns, and the cost of this chemical is rising dramatically (Ross, 1999). Therefore, alternatives to methyl bromide are urgently needed.

Irradiation has proven useful as an alternative quarantine treatment for control of insect pests infesting horticultural commodities. Since 1995, irradiation at 250 Gy has been used commercially to provide quarantine security against tephritid fruit flies on papaya and other tropical fruits shipped from Hawaii to the US mainland (Moy & Wong, 2002). Higher doses (400 Gy) are used if surface pests, such as mealybugs, are present (Follett *et al.,* 1999; Moy & Wong, 2002; Hallman, 1999, 2000). Unlike other quarantine treatments, the objective of irradiation treatment is to prevent emergence of the adult stage of the pest and/or prevent adults from producing viable progeny (Hallman, 1999). Most fresh commodities are damaged (changes in appearance, taste, color, shelf life, texture or chemical content) at levels of irradiation required to produce acute mortality of pests (Hallman 1998, 2001*a,b*). Quarantine species of slugs and snails are commonly intercepted on commodities that are suitable for irradiation, such as cut flowers and foliages, fruits, vegetables, and herbs (Robinson, 1999). However, there are no published studies relating to the reproductive sensitivity of terrestrial molluscs to irradiation. Data for aquatic molluscs are limited to two species in the genus *Biomphalaria* (Liard *et al.* 1968; Perlowagora-Szumlewicz, 1964*a-d*; da Motta & Melo, 1997). In this study, our objective was to determine the minimum absorbed dose of irradiation that would prevent egg laying and egg hatching in *Zonitoides arboreus* (Say), a snail pest that feeds on the roots of orchids and other ornamental plants.

Materials and Methods

Z. arboreus is not classified as a quarantine pest in the USA. However, we chose this species for our studies because we have developed methods for its culture and because it is similar in size to many other

*Corresponding Author E-mail: rholling@pbarc.ars.usda.gov

terrestrial molluscs that might travel undetected in commodity shipments. Adult shells of *Z. arboreus* are 3.5 mm in diameter, and 2-2.5 mm in height with a very slight conical spire (Godan, 1983). Eggs are white, semi-spherical in shape, and have a maximum diameter of about 1.0 mm. At 25°C, average time to hatching is about 13 days. When infesting potted orchids, eggs are laid within the potting medium in close proximity to orchid roots (unpublished data). Studies by Bartsch & Quick (1926) show that *Z. arboreus* is hermaphroditic; in addition, we have discovered that *Z. arboreus* can produce viable eggs if held in isolation beginning at the egg stage (unpublished data). The lifespan of this species has not been reported. Snails held individually in our lab have lived more than 2 yr.

Studies were carried out between March 2001 and May 2002. Snails were obtained from used orchid media from a commercial greenhouse in Kurtistown, Hawaii. Snails were held in the laboratory within ventilated plastic containers on a medium of moist coir (chipped coconut husk) and provided cabbage leaves for food until needed for bioassays.

Adult snails were placed individually within 9-cm Petri dishes provisioned with moist filter paper, several pieces of coir, a small piece of lettuce and several pieces of moss. Fresh plant material, coir and filter paper were replaced as necessary to ensure enough food and to prevent the growth of mould. Dishes were checked 1-2 times per week for eggs. Egg clutches (usually 3-5 per clutch) were generally laid within cracks and crevices of the coir. Any eggs found were transferred to a Petri dish (4.5 cm diameter) holding moist filter paper. Eggs were transferred using a brush or by moving the piece of coir holding eggs to the new dish. A small percentage of snails did not produce eggs during the 2-4 wk observation period prior to the scheduled irradiation date, and were therefore excluded from bioassay treatments. Shell diameter and weight was measured 1-2 days prior to the irradiation date, and eggs were removed from all dishes at this time.

Adult snails were exposed to irradiation treatments individually within the Petri dishes described above. Three replications (= experimental "blocks") of treatments were carried out over time. Each replication included a total of 115-120 snails, with 23-24 snails (= one replicate) assigned to each of the five radiation levels (= treatments). Irradiation was done with dishes arranged in four stacks of five or six dishes each within a cardboard box that was carried by a conveyor belt at speeds of 1.5 to 6.0 m min^{-1} past an X-ray (bremsstrahlung) source used commercially for quarantine disinfestation (Hawaii Pride, LLC, Keaau, Hawaii). Dose level (a function of the speed of the conveyor belt) was measured by averaging readings from two Opti-chromic dosimeters (FWT-70-40M, Far West Technology, Inc., Goleta, CA) placed in the central area between the stacks of dishes. Four doses (absorbed) were targeted: 35, 50, 75 and 100 Gy. Average dose levels in different blocks ranged from 34-37, 43-51, 69-79, and 94-102 Gy, respectively. The range within each dose level was due primarily to unavoidable variability in the output of the irradiator source. An untreated control comprised the fifth treatment group.

Data on total egg production, percentage egg hatch and percentage mortality of adult snails over 63 days in each block were analysed by ANOVA using the GLM (General Linear Models) procedure of the SAS statistical package (Anon., 1988) using "block" as a "class" (categorical) variable and "irradiation dose" as a continuous variable. Before analysis, percentage data were changed to proportions and square-root arc-sine transformed (Steel & Torrie, 1980). The model used was "dependent variable" = "block effects" (2 df) plus "dose effects" (1 df). Curve fitting was accomplished using Excel software (Version 7.0, Microsoft Corporation, Portland, OR). The intervals between egg counts varied slightly among the replications of the experiment. To standardise egg count data among replications for purposes of analysis and graphing, egg production was expressed on a weekly basis. This required redistributing eggs count data associated with intervals that overlapped the end of a week, using the formula: (no. eggs produced during interval) ÷ (no. days in interval) × (no. days of a particular week occupied by the interval).

Results

Weekly egg production by untreated snails averaged between 0.8 and 1.9 eggs per snail over a 9-wk period (Fig. 1). Irradiation caused reduced fecundity of snails, with the effect being more pronounced at higher doses. Egg production by snails exposed to 34-37 Gy dropped from 1.6 to 0.7 between the first and second week following irradiation. Thereafter, egg production declined more gradually until it reached a level of 0.1 eggs per snail during the final week (Fig. 1). Egg production by snails exposed to 94-103 Gy, the highest level of irradiation, dropped from 1.1 to 0.2 eggs per snail between the first and second weeks, and production remained at ≤ 0.02 eggs per snail from the end of the fifth week until the end of the trial (Fig. 1).

Over 9-weeks, total egg production by snails in the three replications of the control varied between 0 and 28 per snail, with an average of 11.2 (SD = 6.3). Only six snails in the control group produced no eggs; all of these zero counts were associated with snails that died during the observation period. A total of 266 eggs were produced by snails in the control group; relative to the control, total egg production by snails exposed to 34-37, 43-51, 69-79, and 94-102 Gy was associated with reductions of 63%, 77% 85%and 88%, respectively (Table 1). ANOVA results indicated a significant negative relationship between irradiation dose and total egg production over the nine-week period ($P < 0.0001$, df = 1, 11; Table 1). An exponential curve provided a good fit to the data, with an R^2 of 0.77 (Fig. 2).

Percentage egg hatch associated with snails in the control group averaged between 48% and 77% over the 9-wk sampling period (Table 1). Percentage egg hatch was sharply reduced by irradiation, even at the lowest dose tested. The reduction in hatching associated with irradiation was immediate: during the first week following irradiation, 68 of the 113 eggs produced by snails in the control group hatched, while hatching was limited to only one of the 113 eggs produced by snails exposed to the lowest dose level of 34-37 Gy. Of the 101 eggs produced by snails exposed to 43-51 Gy over the same period, none hatched. There was a significant negative relationship between dose and percentage egg hatch ($P < 0.0003$, df = 1, 11).

Levels of irradiation used in our tests were apparently too low to cause acute mortality in adult snails. In contrast, irradiation was associated with a significant decrease in natural adult mortality over the nine-week observation period (Table 1; $P < 0.03$, df = 1, 11).

Discussion

Our results show that irradiation at absorbed doses ≥ 69 Gy was sufficient to prevent successful reproduction of snails for a period of 9 weeks after treatment. Levels of irradiation currently approved as quarantine treatments for fruit flies and other pests range from 150 to 400 Gy (Hallman, 1999). Thus, we would expect adult *Z. arboreu*s to be completely sterilised if these snails were infesting commodities commercially treated with irradiation.

Although this is the first study published on the effects of irradiation on a terrestrial mollusc, there have been a number of detailed studies regarding the effects of irradiation on the survival and reproductive ability of *Biomphalaria glabrata,* a fresh water snail species (Perlowagora-Szumlewicz 1964*a-d*; Perlowagora-Szumlewicz & Berry, 1964; Liard *et al.,* 1968). In addition, there are several studies dealing with the effects of irradiation on other species of *Biomphalaria* (da Motta & Melo, 1997; Nabih & Rizk, 1984).

Studies with *B. glabrata* were motivated by an interest in setting up "irradiation barriers" in water-distribution systems to control this vector of schistosomiasis (Perlowagora-Szumlewicz, 1964*a*).

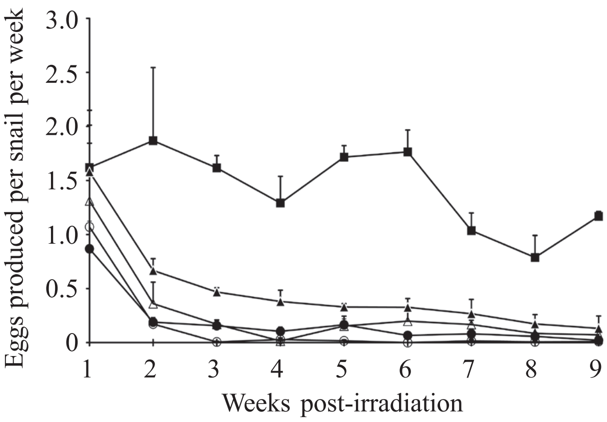

Fig. 1. Fecundity of *Z. arboreus* as affected by irradiation dose during nine week sampling period following irradiation. Error bars show + 1 SEM. ■ = Control; ▲ = 34-37 Gy; △ = 43-51 Gy; ● = 69-79-Gy; ○ = 94-103 Gy.

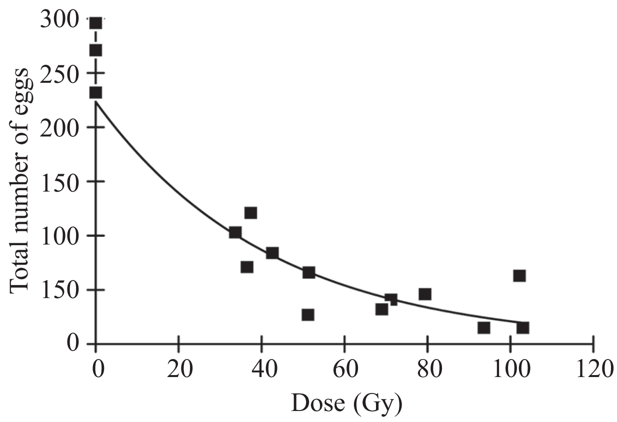

Fig. 2. Total fecundity in each experimental replicate as a function of irradation dose over nine-week period following irradiation.
$y = 223.39e^{-0.236x}$
$R^2 = 0.7681$

Table 1. *Number of eggs produced, percentage egg hatch and percentage mortality of adult snails over 9-wk period as a function of irradiation dose*

Treatment	Total eggs	% egg hatch	% mortality of adults
Control	266 (18.6, 232-296)	61.2 (8.6, 48-77)	32.5 (6.5, 21-43)
33-37 Gy	98 (14.6, 71-121)	3.7 (1.8, 0.8-7)	21.1 (2.2, 17-25)
43-51 Gy	59 (16.8, 27-84)	0.6 (0.5, 0-1.5)	9.8 (1.3, 8-13)
69-79 Gy	40 (4.1, 32-46)	0	9.8 (6.0, 0-21)
94-103 Gy	31 (16.0, 15-63)	0	14.1 (1.3, 13-17)

Data represents average of three replicates of 23-24 snails each; SE and ranges for replicates shown in parentheses

Using sexually mature snails that were 14-16 mm in diameter, Liard *et al.* (1968) found that irradiation at 40 Gy (the lowest dose studied) was associated with a 75% reduction in egg laying. The decline started between the second and third week after treatment and reached a minimum after five weeks. Egg production gradually returned to normal levels between weeks seven and 10. A similar pattern (but more extreme in extent) occurred in snails exposed to 80 Gy, except the minimum was reached 1 wk earlier and recovery began 1 wk later. In both groups, percentage egg hatch fell to almost zero immediately following irradiation treatment, although percentage egg hatch for snails treated with 40 Gy gradually returned to normal levels between weeks 7 and 10. Snails treated with ≥ 160 Gy remained sterile throughout the 11-week observation period, and all snails treated with ≥ 320 Gy died within 60 days (Liard *et al.,* 1968).

Perlowagora-Szumlewicz (1964*d*) also investigated the effects of irradiation on *B. glabrata* and found that the dose level required for sterilisation was between 80 and 160 Gy for sexually mature snails and between 100 and 150 Gy for eggs of this species.

In comparison to *B. glabrata*, the reproductive ability of *Z. arboreus* adults was affected to a somewhat greater degree by comparable doses of irradiation. In our study, there was a significant reduction in fecundity between the first and second weeks following irradiation with 34-37 Gy. Irradiation of *B. glabrata* with 40 Gy caused a similar effect, but not until 1 wk later. However, the most important difference in the responses of these two species to irradiation is that irradiated *B. glabrata* snails gradually recovered their reproductive ability beginning 7 wk after irradiation, while the reproductive ability of irradiated snails in our study remained at depressed levels, or continued to decline throughout the 9-wk study period (12 wk in one replication; additional data not shown). Nevertheless, we cannot rule out the possibility that such a recovery would have occurred eventually.

Based on the pattern with *B. glabrata*, the critical measures for determining the minimum sterilising dose of a snail species are: (1) a permanent cessation of egg production by snails that were treated as young adults and (2) 100% mortality (prior to sexual maturity) of snails treated while in the older egg stage. While we did not definitively determine either of these endpoints for *Z. arboreus*, our results are important because they show that fecundity in *Z. arboreus* is affected by irradiation at least as much as *B. glabrata*, a species for which the irradiation sensitivity has been documented in much detail. Our results with *Z. arboreus* provide a useful comparison for *B. glabrata* because these two mollusc species which are unrelated phylogenetically also differ greatly in their biology: *Z. arboreus* is a small, terrestrial species (3.5 mm diameter) that produces an average of one egg every 5-6 days; *B. glabrata* is an aquatic, fresh-water species up to 24 mm in diameter that produces an average of 25 eggs per day. Together these two species provide a first glimpse into the radiation sensitivity of molluscs.

Acknowledgments

We thank John Ross and Robert Lower (USDA-ARS) for technical assistance; Robert Cowie (University of Hawaii, Honolulu) for confirming identity of snail species used in tests; and John Clark of Hawaii Pride, LLC for use of the electron beam irradiator. We thank Robert Cowie (University of Hawaii, Honolulu) and Guy Hallman (USDA-ARS, Weslaco, TX) for their critical reviews on an earlier draft of this manuscript.

References

Anon. 1988. *SAS/Stat User's Guide*, Release 6.03 Edition. Cary, NC, USA: SAS Institute Inc.

Anon. 2002. *U.S. Agricultural Trade: global agricultural trade*. U.S. Department of Agriculture, Economic Research Service. URL:http://www.ers.usda.gov/briefing/agtrade/commoditytrade.htm

Bartsch P, Quick M E. 1926. An anatomic study of *Zonitoides arboreus* Say. *Journal of Agricultural Research* **32**:783-791.

da Motta M A, Melo A M M A. 1997. Fecundity changes induced by low-doses of gamma radiation on *Biomphalaria straminea* (Dunker, 1848). *Memórias do Instituto Oswaldo Cruz (On-line)* **92**:559-561.

Follett P A, Armstrong J W, Hara A H, Yalemar J. 1999. Irradiation treatments for quarantine pests in Hawaii other than fruit flies. In *Proceedings, The Use of Irradiation as a Quarantine Treatment of Food and Agricultural Commodities*, pp. 19-25. Eds J H Moy and L Wong. Honolulu, USA: University of Hawaii at Manoa, workshop held 10-12 November, 1997.

Godan D. 1983. *Pest Slugs and Snails: Biology and Control*. New York: Springer-Verlag. 445 pp.

Hallman G J. 1998. Ionizing radiation quarantine treatments. *Anais da Sociedade Entomologica do Brasil* **27**:313-323.

Hallman G J. 1999. Ionizing radiation quarantine treatments against tephritid fruit flies. *Postharvest Biology and Technology* **16**:93-106.

Hallman G J. 2000. Expanding radiation quarantine treatments beyond fruit flies. *Agricultural and Forest Entomology* **2**:1-11.

Hallman G J. 2001*a*. Irradiation as a quarantine treatment. In *Food Irradiation: Principles and Applications*, pp. 113-130. Ed. R A Molins. New York: John Wiley & Sons, Inc.

Hallman G J. 2001*b*. Ionizing irradiation quarantine treatment against sweetpotato weevil (Coleoptera: Curculionidae). *Florida Entomologist* **84**:415-417.

Liard F, Chiriboga J, Pellegrino J, Colón J, Silva R M. 1968. Effect of radiation on the reproductive potential of *Biomphalaria glabrata. Revista Brasileira de Pesquisas Medicas e Biologicas* **1**:157-162.

Moy J H, Wong L. 2002. The efficacy and progress in using radiation as a quarantine treatment of tropical fruits - a case study in Hawaii. *Radiation Physics and Chemistry* **63**:397-401.

Nabih I, Rizk M. 1984. Genetic studies on fresh water snails, specific intermediate hosts for schistosomiasis-VII. Effect of gamma radiation as a physical mutagen on histones in *Biomphalaria alexandrina* snails. *Cellular and Molecular Biology* **30**:27-31.

Perlowagora-Szumlewicz A. 1964*a*. Schistosomiasis: age of snails and susceptibility to X-irradiation. *Science* **144**:302-304.

Perlowagora-Szumlewicz A. 1964*b*. Effect of ionizing radiation on the population kinetics of the snail *Australorbis glabratus*: age at exposure and immediate and late effects of X-rays. *Radiation Research* **23**:377-391.

Perlowagora-Szumlewicz A. 1964*c*. Effect of ionizing radiation on the population kinetics of the snail *Australorbis glabratus*: age at exposure and the effects on reproduction. *Radiation Research* **23**:392-404.

Perlowagora-Szumlewicz A. 1964*d*. Survival, growth, and fecundity of *Australorbis glabratus*: snails developed from eggs exposed to ionizing irradiation. *Experimental Parasitology* **15**:232-241.

Perlowagora-Szumlewicz A, Berry E G. 1964. Effect of ionizing radiation on *Australorbis glabratu*s eggs. *Experimental Parasitology* **15**:226-231.

Robinson D G. 1999. Alien invasions: the effects of the global economy on non-marine gastropod introductions into the United States. *Malacologia* **41**:413-438.

Ross R. 1999. Current status of domestic and international controls for methyl bromide and the status of alternatives. In *Proceedings, The Use of Irradiation as a Quarantine Treatment of Food and Agricultural Commodities*, pp. 4-10. Eds J H Moy and L Wong. Honolulu, USA: University of Hawaii at Manoa, workshop held 10-12November, 1997.

Steel R G D, Torrie J H. 1980. *Principles and Procedures of Statistics*. New York: McGraw-Hill.