AM19002 Building Capacity in Irradiation

Technical Report

Effect of irradiation dose on survival and sterility of vineyard snail *Cernuella virgata*

SARDI Entomology

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Author(s): Nancy Cunningham, Humayra Akter

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Contents

INTRODUCTION

With increased globalization of trade, the risk of introducing invasive species of snails or slugs has increased (Robinson, 1999). Commodities infested with quarantine pests not subjected to an approved quarantine treatment before export will be sent back or destroyed to prevent exotic pest propagation in importing countries (Robinson, 1999; Hollingsworth et al. 2003). Methyl bromide fumigation is an effective means of disinfesting produce of gastropod quarantine pests. However, methyl bromide is a significant stratospheric ozone depleting substance, and its use is becoming increasingly restricted (Heather & Hallman 2008). Snails are found on a wide variety of fresh commodities some of which are significant crop pests (Barker 2002). Produce containing foliage, fruits, vegetables, and herbs are all routinely inspected for slugs and snails of quarantine significance. However, snails are cold resistant and thus relatively hard to disinfest with standard phytosanitary cold treatments. Irradiation has been an effective phytosanitary treatment for certain quarantine species of snail (Hallman, 2016).

Vineyard snail, or common white snail *Cernuella virgata* (Da Costa) is an invasive species and an agricultural pest in many parts of Southern Australia. They are considered a serious pest as they contaminate grain during harvest and can clog and damage harvest machinery. They aestivate in vine canopies, stubble, and on fence posts from late spring through summer—often to escape the heat. In high-value exportable commodities such as table grapes, the presence in the canopy can cause major contamination issues during harvest and there is a risk that importing countries could restrict trade from the region if no available disinfestation treatments are available.

Doses of ionizing radiation to control most quarantine pests does 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, as standard doses tolerated by fresh commodities usually don't achieve acute mortality. However, phytosanitary irradiation treatment has the potential to be more effective against snail infestations than other regular phytosanitary treatments.

Little research exists on the radiosensitivity of terrestrial herbivorous gastropods. Studies with the orchid snail *Zonitoides arboreus* (Say) (Stylommatophora: Gastrodontidae) showed at 70 Gy reproduction can be prevented (Hollingsworth et al. 2003). Another terrestrial species citrus tree defoliator, the brown garden snail*, Cornu aspersum* (Müller) became sterile after irradiation with ≥ 75 Gy (Hallman, 2016). A dose of > 150 Gy prevented the establishment of viable populations of semi-slug *Parmarion martensi* Simroth (Stylommatophora: Ariophantidae), a quarantine pest of fresh sweet potatoes and other fruits and vegetables in Hawaii (Follett et al., 2021). Follett et al. (2021) suggested a generic dose of 150 Gy as an effective irradiation dose against many slugs and snail pest species to prevent and control snail reproduction.

Research Objectives

The main objective of this study was to select an effective irradiation dose that will either kill vineyard snails or prevent egg laying and/or egg hatching. The study aimed to:

- Observe for survival/mortality in response to different irradiation doses.
- Record the weight of snails to detect dietary effects from various dose/doses.
- Observe egg deposition and hatching rate of eggs at different irradiation doses.
- Analyze data to establish the overall effects of irradiating snails.
- Establish a potential use of irradiation as a commercial phytosanitary treatment against vineyard snails.

METHOD

Sample collection

Vineyard snails *Cernuella virgata* were collected from Wauraltee, Yorke Peninsula, SA 5573 between May, and October 2022 and west of Port Wakefield in March 2023. The gap between the initial 2 collections varied from 2 weeks to 4 weeks depending upon the weather. In total, 8 field collections were completed and locations for collections can be seen in Appendix A and B.

We also collected soil from the same area to ensure the snails' initial environment remained stable. We used this soil as a medium for the snails after the irradiation treatments (Appendix C, Figure 1).

Snails were collected between autumn to spring. On collection day snails were collected from 11am to 3 pm by hand and transported to SARDI Entomology laboratories, a 2–3-hour drive from the collection site.

After collection, snails were kept in 5L plastic containers. The floor of each container was covered with moist paper towel and mesh lids ensured adequate air flow. After arriving at SARDI laboratories, fresh cabbage leaves were provided as food and water sprayed continuously to maintain humidity.

In total, 8 batches of vineyard snails were collected from the field to study the efficacy of various irradiation doses.

Treatments

The day after field collection, snails were sorted, packed and sent to Steritech facilities in Melbourne for irradiation for a small pilot study. Treatment doses in later batches were dependent upon the results from this pilot study.

Batch 1 and 2 (Group 1-pilot study)

Snails were sorted into two sizes (small and large) and into four treatments:

1. Control (no irradiation dose).
2. Transport-control (no irradiation doses but sent with samples to be irradiated to observe transport effect).
3. 150 Gy.
4. 400 Gy.

Batch 3-7 (Group 2)

After results were observed for the pilot study, the remaining batch of snails were not sorted by size and randomly assigned seven treatments:

1. Control (no irradiation dose).
2. Transport-control (no irradiation doses but sent with samples to be irradiated to observe transport effect).
3. 150 Gy.
4. 400 Gy.
5. 500Gy.
6. 750 Gy.
7. 1000 Gy.

Batch 8 (Group 3)

Another batch of snails was collected and tested, like batches 3-7 they were not sorted by size and randomly assigned the same seven treatments:

1. Control (no irradiation dose).
2. Transport-control (no irradiation doses but sent with samples to be irradiated to observe transport effect).
3. 150 Gy.
4. 400 Gy.
5. 500Gy.
6. 750 Gy.
7. 1000 Gy

Treatment preparation involved placing snails into a 1L rectangular shaped transparent plastic container with 7x14cm mesh opening in the middle of lid to ensure aeration. To restrict movements of snails, paper towels were placed in

between snail layers and water sprayed to provide moisture inside and maintain relative humidity for transport. Lids were closed and sealed with tape to avoid accidental escape. Each container was labeled with the required dose rate.

As the number of snails per batch varied, the initial number of snails was recorded in lab book.

The container with control treated snails was kept in SARDI Entomology laboratories at room temperature 21^{o}C ± 1°C, 98% RH, and 12:12 light:dark cycle.

The remaining treatment containers were placed in a paper carton along with a request for irradiation (RFI) form where all the details of samples and irradiation dose rates were written for the irradiation facility technicians.

The carton was then sealed with tape and couriered to Steritech, Victoria, a commercial irradiation facility designed to apply low-dose irradiation for phytosanitation of fresh agricultural produce.

Post-treatment

All snails, including the control snails kept at SARDI laboratories, were placed in containers for data collection. For each treatment, snails were contained in a 5L rectangular transparent plastic container with a 20X12cm lid that has a mesh opening. A 5cm layer of thick soil was added to the bottom of each container, allowing an adequate oviposition surface for the adult snails to lay eggs. This soil, from field collection sites, provided snails with an environment for acclimatization to lab conditions and minimized effects on their survival and oviposition behavior from treatment and transportation. We sprayed water daily to provide adequate moisture and maintain relative humidity.

As a source of nutrition 2 whole fresh cabbage leaves, 3-5 slices of carrot, 1 teaspoon of oats and 1/4th teaspoon of Calcium carbonate ($CaCO_3$) were provided on top of the soil. Snails were then placed onto the soil and sprayed with water. The container was covered with a fine mesh lid, labelled by treatment and placed on a laboratory bench at room temperature. Each container had an average of 100 snails.

Images of post-treatment containers, medium and food sources used can be seen in Appendix C, Figure 2.

Data collection

Survival

Snails that clung to the wall of the plastic container or the mesh lid, were recorded as alive and removed to a corresponding container. Other snails were poked gently with forceps to observe their reaction. Snails where no reaction was recorded were considered dead. Dead snails were separated, counted, and recorded. Snails that were not clearly categorized as dead or alive (moribund), were recorded as ‘unsure’ and kept in a separate container for further observation. We also provided these snails with a small piece of cabbage leaf and sprayed them with water. The following day, the ‘unsure’ container was checked. Snails that clung to the leaf, wall or mesh of the container were recorded as alive and if no such attributes were further observed, we labeled the snail as dead. Alive snails from the ‘unsure’ group were transferred to the experiment container with other living snails and the dead snails discarded. We recorded all data with data from each treatment and replicates pooled from all snails within that treatment/replicate group.

Weight

On assessment day, snails were weighed by group treatment and recorded. After recording mortality/survival data, live snails, ‘unsure’ snails, and dead snails were weighed separately, recorded, and later added together as a total weight of snails of that treatment on that assessment day. We collected weight related data once a week.

Egg deposition and hatching

After observing mortality/survival and weight, old food (cabbage leaves, oats, and carrots) was removed, and the soil checked thoroughly by digging with a small spatula to observe egg laying. Eggs are white transparent round shaped and usually laid in groups. Found eggs were collected with a fine brush and placed on a piece of mesh cloth over a thin layer of soil (also collected from Yorke peninsula) within a petri dish (Appendix C, Figure 3). The eggs were then sprayed with a fine mist of water, covered, and kept in a dark chamber. The petri dishes were observed twice a week and hatching data was recorded. We transferred data to excel for analysis.

Food replacement

After collecting and removing eggs, we replenished food and water within the experimental containers and returned the adult snails.

Statistical analysis

Data has been sent to Rho Environmetrics, and preliminary data analysed. Further experimental work to be conducted will also be sent to Environmetrics with the results finalised and presented in the final report.

- Effect of dose on survival, the LD50 value of the adult snails, is there any differences within doses, survival curve
- Effect of dose on weight, is there any differences within doses.
- Effect of dose on egg deposition (fecundity) and hatching, is there any differences within doses.

RESULTS

Group 1 – Batches 1-2

Effect of irradiation on survival

The majority of large snails died between week 4 and 8 (Figure 1) with gradual mortality observed in smaller snails (Figure 2). The survival rate of snails in Batches 1 and 2 is shown in Table 1 and illustrated in Figure 3. The effect of the treatment increased over time and it was statistically significant at Week 12. After 12 weeks with 400Gy 7.4% of the snails were still alive. When the two control treatments were combined, there was a statistically significant ($p < 0.05$) of the effect of dose of radiation on the survival of snails at the 12-week measurement.

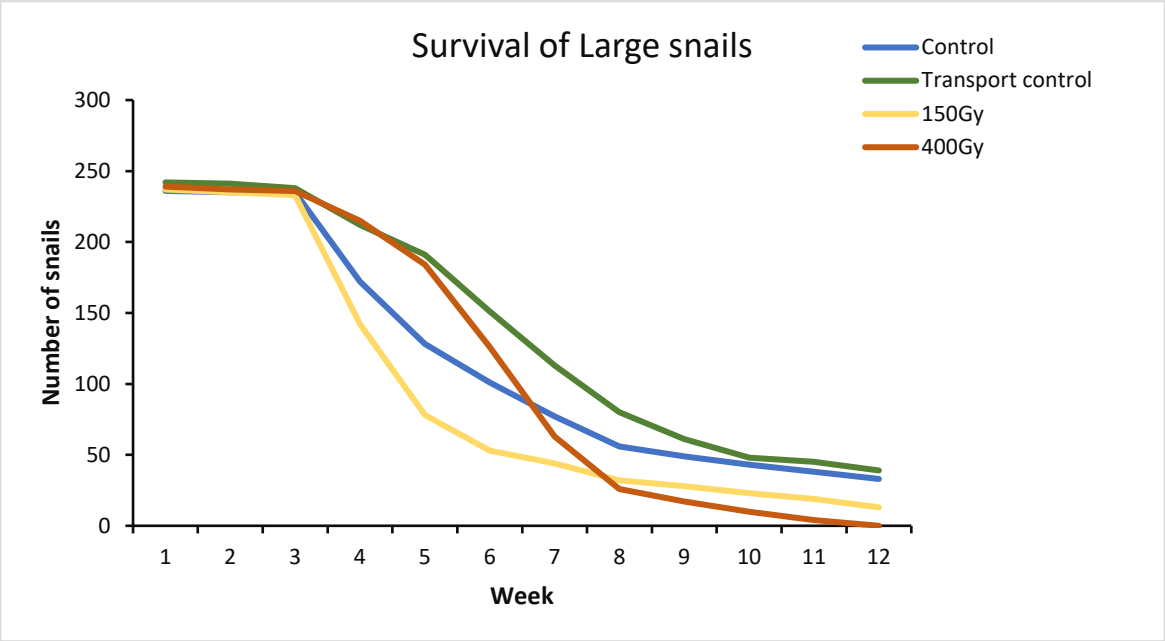

Figure 1: Effect of irradiation on survival of large snails in group 1 study.

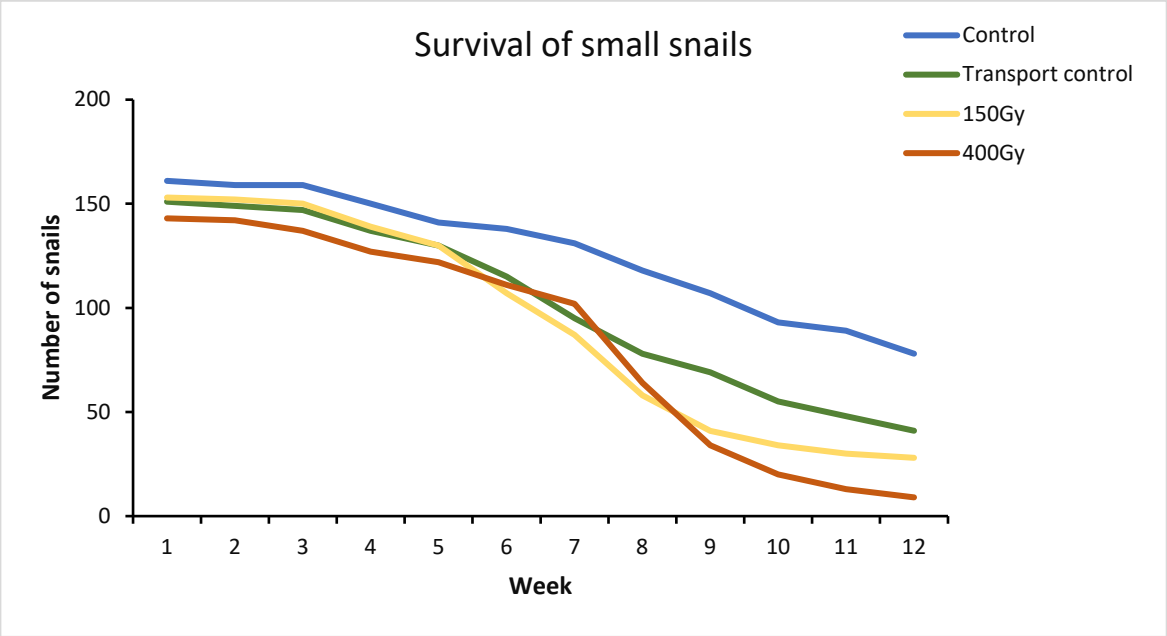

Figure 2: Effect of irradiation on survival of small snails in group 1 study.

Table 1. Cumulative survival rates for each treatment across 12 weeks of observations together standard errors

	Control	Transport	150 Gy	400 Gy
Week 1	1±0	1±0	1±0	1±0
Week 2	0.987±0.009	0.986±0.01	0.996±0.003	0.987±0.008
Week 3	0.987±0.009	0.979±0.009	0.989±0.006	0.976±0.012
Week 4	0.896±0.068	0.929±0.031	0.861±0.106	0.930±0.031
Week 5	0.827±0.117	0.891±0.054	0.768±0.178	0.866±0.052
Week 6	0.790±0.147	0.798±0.087	0.693±0.205	0.775±0.113
Week 7	0.739±0.168	0.698±0.124	0.635±0.215	0.673±0.175
Week 8	0.648±0.165	0.584±0.139	0.529±0.22	0.469±0.172
Week 9	0.603±0.163	0.545±0.161	0.483±0.231	0.363±0.182
Week 10	0.562±0.165	0.477±0.166	0.446±0.23	0.245±0.139
Week 11	0.549±0.171	0.451±0.165	0.400±0.211	0.143±0.102
Week 12	0.505±0.165	0.422±0.172	0.328±0.184	0.074±0.069

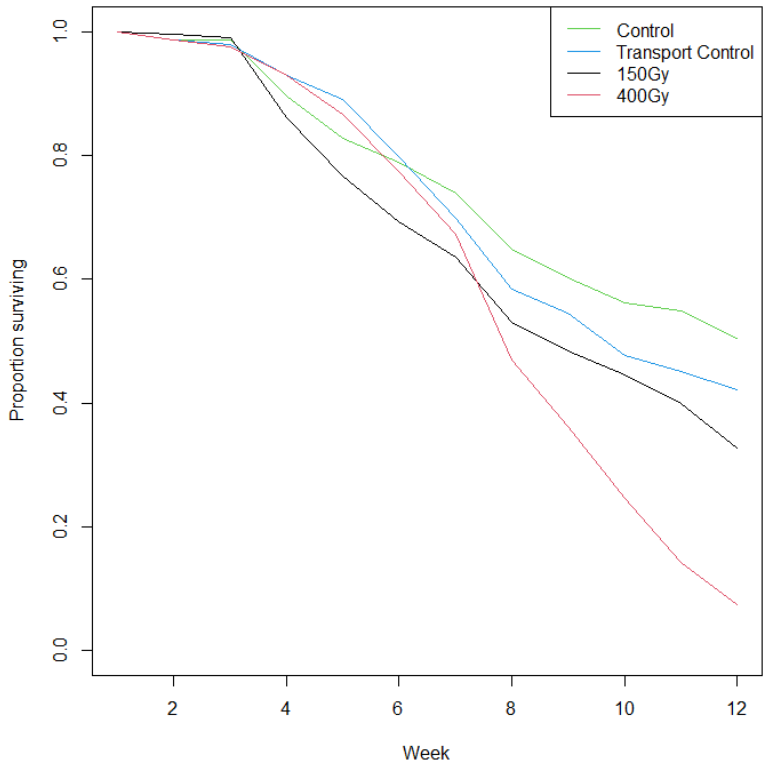

Figure 3: Cumulative survival rates for snails subjected to radiation treatments.

Effect of irradiation on weight

We observed more weight loss in irradiated snails compared to unirradiated snails over time in Group 1 (Batches 1 and 2). However these differences were not statistically significant (Figure 4 and 5).

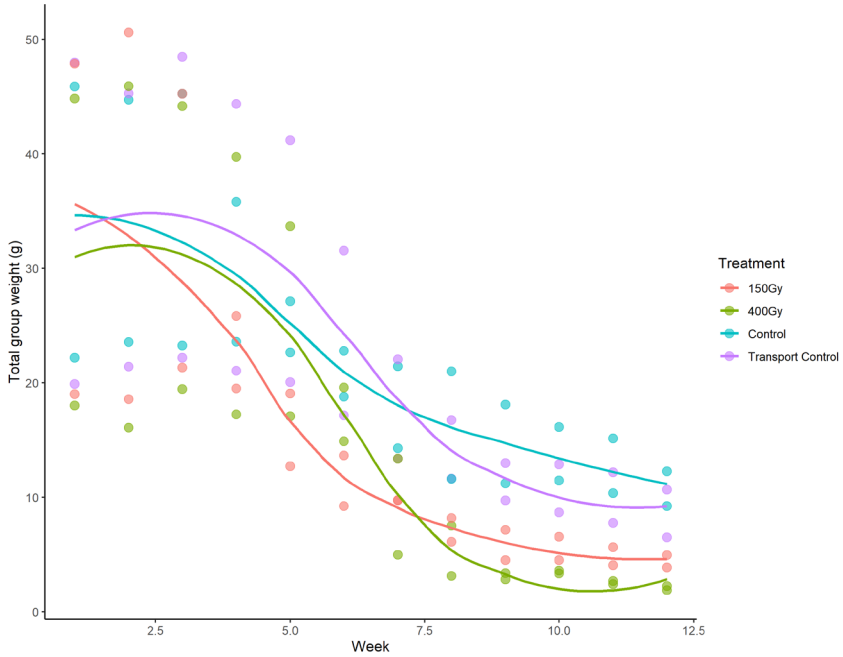

Figure 4: Effect of irradiation treatment on weight of snails in group 1 study.

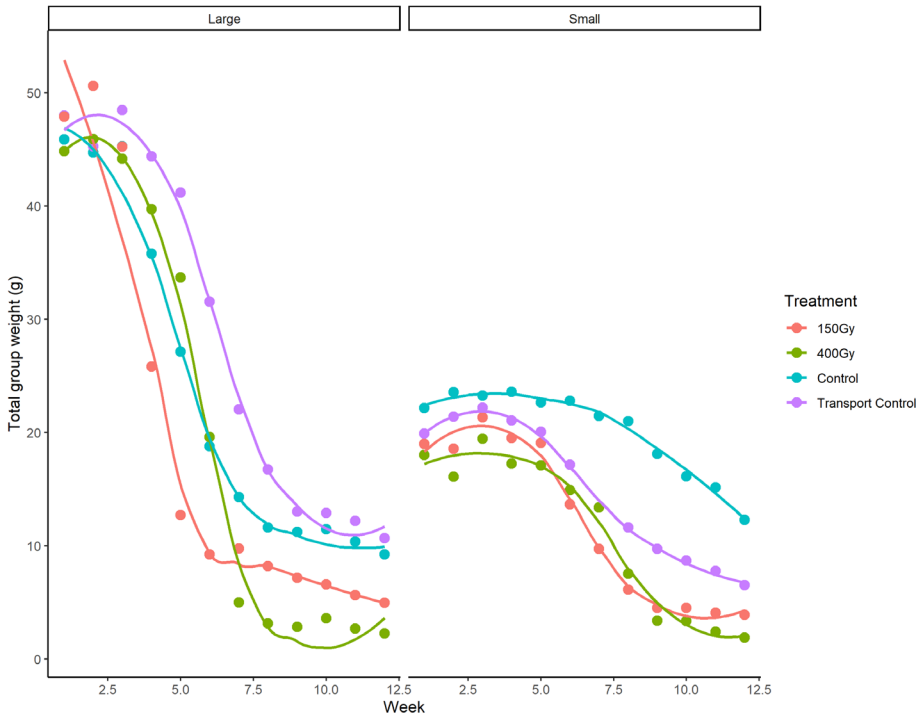

Figure 5: Effect of irradiation and size on weight of snails in group 1 study.

Egg Production

Egg production continued over the experimental period, but the rate of production varied between weeks (Figure 6). The larger snails produce more eggs than the smaller snails but the effect was only marginally (0.1%) significant. Although there was an apparent effect of dose on egg production, that effect was not statistically significant.

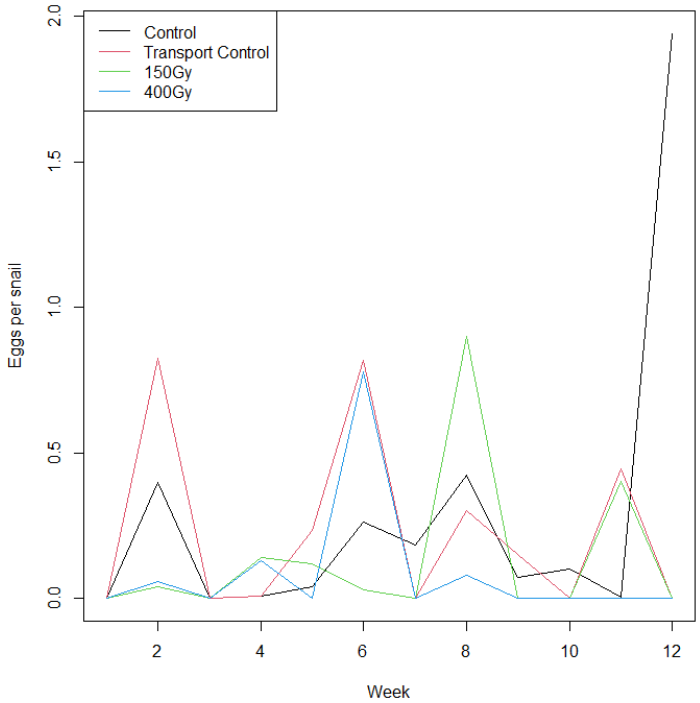

Figure 6: Egg production per snail for each period and each treatment averaged across sizes.

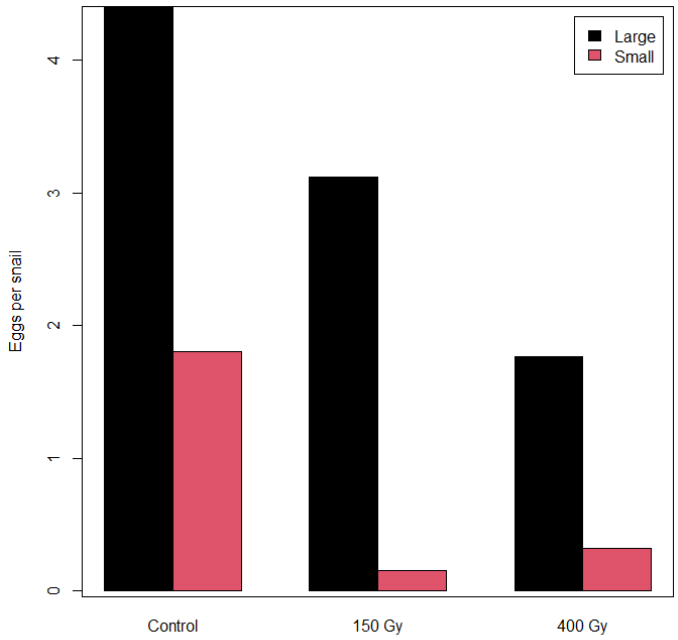

Figure 7: Effect of treatment on egg production in Batches 1 and 2

Effect of irradiation on fecundity and percent egg hatching

In group 1 differences were observed between unirradiated and irradiated snails in egg deposition. No eggs were hatched from irradiated snails (Table 2).

Table 2: Egg deposition, and percent hatching of snails treated with irradiation dose in group 1

Treatment	**Control (0Gy)**		**Transport-control (0Gy)**		**150Gy**		**400Gy**	
	Large (236 snails)	Small (161 snails)	Large (242 snails)	Small (151 snails)	Large (237 snails)	Small (153 snails)	Large (239 snails)	Small (143 snails)
Total eggs laid	825	231	591	477	259	38	153	76
Number of neonates	564	103	258	310	0	0	0	0
% hatching	68.36	44.59	43.65	64.99	0	0	0	0

Group 2 – Batches 2-7

A summary of the available data for batches 3 – 7 is given in Table 3. Some samples were split for laboratory convenience. In some cases, there were no survivors by that week.

The dose of radiation applied was zero for the control and transport control, and 150, 400, 500, 750 and 1000 Gy.

Table 2. Available data for batches 3 - 7

Batch	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7
Week 1	14	14	14	7	7
Week 2	14	14	14	7	7
Week 3	14	14	14	7	7
Week 4	7	14	14	7	7
Week 5	2	14	14	7	7
Week 6	2	14	14	7	0
Week 7	2	14	14	0	0
Week 8	2	14	14	0	0
Week 9	0	14	0	0	0

Effect of irradiation on survival

A summary of the differences between treatments on snail survival is given in **Table 3** and the standard errors of the estimates is given in **Table 3**. The statistical significance is given in **Table 5**. **Table 5** shows first the differences between the 6 different doses (after combining the control and transport controls), followed by a trend of survival rates related to the dose of radiation. Only in week 1 was the trend negative (greater the dose the lower the survival rate) and in that case the slope did not differ from zero as in the 3rd column. There were significant differences between the batches indicating that there was power in the experiment.

If there was an effect of the radiation on snail survival, the effect must have been very small – and possibly not biologically significant.

Table 3. Survival rates of snails in batches 3 - 7 following radiation treatment

	Control	Transport	150Gy	400Gy	500Gy	750Gy	1000Gy
Week 1	0.633	0.584	0.809	0.771	0.683	0.692	0.598
Week 2	0.206	0.188	0.313	0.327	0.160	0.240	0.241
Week 3	0.037	0.045	0.096	0.064	0.018	0.038	0.121
Week 4	0.014	0.020	0.059	0.032	0.004	0.015	0.085
Week 5	0.014	0.013	0.014	0.026	0.004	0.014	0.083
Week 6	0.009	0.005	0.007	0.024	0.004	0.014	0.083
Week 7	0.009	0.005	0.007	0.024	0.004	0.014	0.083
Week 8	0.007	0.005	0.006	0.024	0.004	0.014	0.083
Week 9	0.007	0.005	0.006	0.024	0.004	0.014	0.083

Table 4. Standard errors of survival rates from Batches 3 - 7

	Control	Transport	150Gy	400Gy	500Gy	750Gy	1000Gy
Week 1	0.070	0.088	0.037	0.056	0.153	0.105	0.115
Week 2	0.082	0.098	0.111	0.133	0.085	0.057	0.094
Week 3	0.011	0.022	0.038	0.030	0.009	0.004	0.083
Week 4	0.006	0.012	0.026	0.022	0.004	0.005	0.079
Week 5	0.006	0.009	0.007	0.021	0.004	0.005	0.079
Week 6	0.006	0.002	0.006	0.019	0.004	0.005	0.079
Week 7	0.006	0.002	0.006	0.019	0.004	0.005	0.079
Week 8	0.004	0.002	0.006	0.019	0.004	0.005	0.079
Week 9	0.004	0.002	0.006	0.019	0.004	0.005	0.079

Table 5. Statistical significance of difference between treatments, the slope of a trend term and its significance and the significance between batches. Colours indicate statistical significance.

	Treatment P value	Slope	Trend P value	Between batches P value
Week 1	0.261	-0.023	0.792	0.009
Week 2	0.113	0.010	0.863	0.000
Week 3	0.201	0.036	0.328	0.025
Week 4	0.334	0.032	0.317	0.059
Week 5	0.426	0.046	0.141	0.129
Week 6	0.393	0.054	0.091	0.251
Week 7	0.393	0.054	0.091	0.251
Week 8	0.379	0.056	0.083	0.242
Week 9	0.379	0.056	0.083	0.242

By week three, batch 3, 4, 5 and 7 snails began to die off, with most snails dead by week 4-5. Batch 6 showed that the fraction surviving snails were much higher than other batches (Figure 7). For some reason snails treated at 1000Gy in batch 6 had higher survivability than other treatments in that same batch (Figure 8).

Effect of irradiation on fecundity and percent egg hatching

In group 2 differences were observed between unirradiated and irradiated snails in egg deposition. Only ten eggs were laid from irradiated snails (Table 7).

Table 7: Egg deposition, and percent hatching of snails treated with irradiation dose in group 2

Treatment	**Control (0Gy)**	**Transport control (0Gy)**	**150Gy**	**400Gy**	500Gy	750Gy	1000Gy
	(198 snails)	(200 snails)	(200 snails)	(200 snails)	(200 snails)	(200 snails)	(200 snails)
Total eggs laid	115	106	0	10	0	0	0
Number of neonates	108	88	0	0	0	0	0
% hatching	93.91	83.02	0	0	0	0	0

Group 3 – Batch 8

For the 2023 experiments there was a similar number of treatments as group 2 batches - 0 (control), 150, 400, 500, 750 and 1000 Gy. However, it did not result in the production of any eggs in either the controls or irradiated treatments.

Table 8. Survival rates of snails in batch 8 following radiation treatment

	Control	Transport	150Gy	400Gy	500Gy	750Gy	1000Gy
Week 1	0	0.66	0.578	0.578	0.66	0.66	0.573
Week 2	0.523	0.469	0.482	0.417	0.469	0.469	0.412
Week 3	0.345	0.312	0.32	0.28	0.312	0.312	0.28
Week 4	0.198	0.18	0.236	0.207	0.18	0.184	0.207
Week 5	0.081	0.074	0.095	0.129	0.074	0.074	0.129
Week 6	0	0.026	0.033	0.044	0	0.026	0

Table 9. Standard errors of survival rates from Batch 8

	Control	Transport	150Gy	400Gy	500Gy	750Gy	1000Gy
Week 1	0	0.079	0.108	0.108	0.079	0.079	0.108
Week 2	0.127	0.132	0.125	0.136	0.132	0.132	0.136
Week 3	0.155	0.149	0.148	0.144	0.149	0.149	0.144
Week 4	0.155	0.146	0.15	0.142	0.146	0.145	0.142
Week 5	0.127	0.117	0.139	0.139	0.117	0.117	0.139
Week 6	0	0.087	0.108	0.131	0	0.087	0

After week 5 more than 50% of control snails were dead and just under 70% of snails for control-transport snails had died (Table 10).

Table 10. Cumulative Percentage Dead for batch 8.

Week	Control	Control Transport	150Gy	400Gy	500Gy	750Gy	1000Gy
Week 1	0.00	2.50	1.49	1.01	0.50	1.00	3.50
Week 2	2.47	6.50	4.46	34.33	3.02	18.50	28.50
Week 3	3.45	37.50	54.01	55.50	8.55	63.50	80.50
Week 4	6.40	68.00	69.01	58.00	14.57	89.50	99.5
Week 5	50.58	70.50	99.50	82.00	69.30	98.50	100
Week 6	93.53	100.00	100.00	95.50	97.99	98.50	100

Analysis of variance for cumulative percentage dead for the entire six-week period showed that there were some differences between control and treated snails, particularly 150, 400, 750 and 1000Gy. However, no difference was recorded for control snails that had been on the same transport as the irradiated snails. Although there is a difference in cumulative data, week to week there was no statistically significant differences between controls and any of the treatments. Control transport was generally higher than control standard, with the result that transport may have influenced snail death. For cumulative data, only treated snails at 500 Gy showed similar mortality to control snails (See Table 11).

Table 11. Cumulative percentage differences over six weeks

Treatment	Mean Cumulative % Mortality	
Control	26.155	a
Control Transport	52.75	b
150Gy	54.809	b
400Gy	54.271	b
500Gy	32.28	a
750Gy	62.083	b
1000Gy	59.667	b

Rows with the same number are not significantly different from one another. $F=4.58_{(6,41)}$ p=0.0021 (Data back transformed from Arcsin percentage data)

Combined batch data analysis for egg laying and hatching

An attempt at Probit analysis was conducted to see whether a dose could be confirmed for snail egg sterility. However, all doses tested (150 Gy and up) either had no eggs hatching or produced no eggs at all. Therefore, although analysis showed only very low doses of radiation would be needed to ensure sterility, because the program struggled with the data (no 95% CI could be generated for LC99), a true dose could not be determined. The experiment could be repeated which includes rates of irradiation well below 150 Gy, to determine LC_{50} – LC_{99} levels.

DISCUSSION

The radiation treatment was not very effective on snail survival. There was no significant negative correlation between the amount of radiation and the number of snails surviving. The observed decline in live snails was matched by the decline in the controls.

In Batch 3, the egg production was less than one per snail – that would not be viable in nature. The data obtained cannot therefore be representative of what occurs in the field.

With Batches 1 -2, there were less eggs from snails that had a radiation treatment, but that effect was not statistically significant. In Batch 3, there was a statistically significant effect of radiation on eggs. Overall, it was concluded radiation decreased egg production.

There were no neonates produced from eggs from snails that had radiation treatment. However, there was sufficient data to indicate that radiation decreased the chance of eggs producing neonates. Where eggs were produced by snails, only the controls hatched. This echoes earlier studies on terrestrial gastropods (Hallman (2016) where levels of 75 Gy were able to prevent egg hatching in the invasive snail *Cornu aspersum* (Müller) (Stylommatophora: Helicidae). Although in that study, low control hatching was noted whereas in our experiments high control hatching was observed (83-94%).

No eggs were laid in batch 8. Several factors may have influenced egg laying across all batches tested including humidity fluctuation, inappropriate soil depth for egg laying and general age and health of the snails collected. The major issue was keeping snails out of aestivation. This was done by maintaining constant humidity, providing variety in food sources and for snails that can reproduce after irradiation treatment and offering appropriate egg laying material/opportunities. An initial experiment in 2023 (between batch 7 and 8) where humidity was maintained over 90% failed due to the increased amount of mould present and the detrimental effect it had across treatments (all control snails perished at an early stage due to the increase of fungi within experimental chambers.) That batch was not included in further data analysis.

There were statistical challenges with the data. The experimental unit was the sample of snails, both for survival and for the egg component. The number of eggs followed a very skewed distribution that was best matched using a generalised linear model with a gamma distribution. There was also a challenge with an outlier in egg viability with one sample that had received 400Gy produced some viable eggs. A simplification in the data that the viability was none or complete except in two cases where it was 23/28 or 18/20. Egg samples were therefore considered viable or not. Preliminary dose data suggested a much lower dose of radiation could affect hatching. Repetition of this experiment at a lower test range (<150 Gy) and with a larger number of eggs (eg over two thousand eggs were produced by combined control treated snails) could easily give industry the confidence that irradiation treatments over the lowest ranger tested her would be and acceptable commodity treatment for ensuring snail sterility.

RECOMMENDATIONS

Currently an irradiation rate of 400 Gy and above is an acceptable treatment for control of Vineyard Snails and has been used for shipments of table grapes to New Zealand (Benjamin Reilly, pers comm). Although the dose is likely to have limited effects on adult snail mortality, the effect on snail egg production and viability was clearly evident from this study.

Suggestions for further research in this area:

Vineyard Snail:

- Study the effect of selected irradiation dose in-situ (on grape vines) particularly on effects on commodity absorption effects and snail sterility.
- Test lower dose irradiation (<150Gy) to see reproductive ability of snails and get true dose mortality curves for snail sterility.
- Once a lower dose for LC_{50} has been established research into reproductive recovery can commence

Other species of arthropods:

Several studies have been conducted on both preharvest and postharvest controls of Fuller's Rose Weevil (*Pantomorus cervinus*) including irradiation of eggs. Research showed that rates of 150 Gy were needed to stop egg hatch with some batches of FRW (Johnson et al, 1990).

Work in Australia could concentrate on:

- Confirming dose rates
- Looking at in-situ eggs for FRW on citrus and corresponding effects on fruit quality (tie in with other researchers focusing on fruit quality after irradiation treatment).
- Examination of reproductive recovery.

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APPENDICES

Appendix A:

Table 1: Field collection and irradiation schedule

Batch	Collection date	Sending to Steritech for irradiation	Irradiation date	Receival date of irradiated snail at SARDI	Experiment set up at SARDI
1	12/05/2022	13/05/2022	17/05/2022	18/05/2022	18/05/2022
2	08/06/2022	09/06/2022	14/06/2022	15/06/2022	15/06/2022
3	08/08/2022	09/08/2022	11/08/2022	12/08/2022	12/08/2022
4	23/08/2022	24/08/2022	26/08/2022	01/09/2022	01/09/2022
5	06/09/2022	07/09/2022	09/09/2022	13/09/2022	13/09/2022
6	19/09/2022	20/09/2022	27/09/2022	29/09/2022	29/09/2022
7	11/10/2022	12/10/2022	21/10/2022	25/10/2022	25/10/2022
8	14/03/2023	22/03/2023	23/03/2023	24/03/2023	24/03/2023

Appendix B:

Figure 1. Vineyard snail collection site 2022, Yorke Peninsula (dark lined area in map).

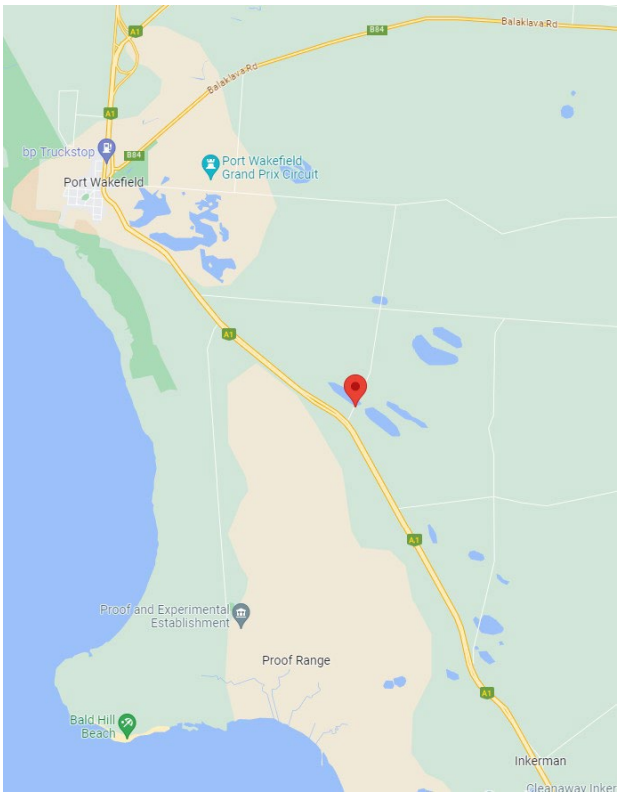

Figure 2. Vineyard snail collection site 2023, Port Wakefield (Red pin)

Appendix C: Photo gallery

Photo credit: Peter Osborne Idea, Instruction and Editing: Humayra Akter, Nancy Cunningham

A **B** **C**

Figure 1. Field collection of Vineyard snails; A. Snail stick to stalk B. Snails on soil, C. Collected snails

A **B**

Figure 2. Typical set up of irradiation efficacy experiment on vineyard snail, each such 5L cage contains 100 snails at best; A. Snail set up showing leaves and oats as food source for snail, carrots were also added later (not showing) B. Container with mesh lid

A **B** **C**

Figure 3. Sterility test of Vineyard snail; A. Egg hatching device: a thin layer of soil in a petri dish with mesh on top. Eggs are placed onto mesh, sprayed water, covered with lid and kept in dark place at room temperature B. 53 days old eggs of irradiated snail, no hatching occurred C. Neonates hatched from eggs of control snail.