



The lethal effects of gamma irradiation on larvae of the Huhu beetle, *Prionoplus reticularis*: a potential quarantine treatment for New Zealand export pine trees

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Accepted: November 11, 1999

Key words: Insecta, *Prionoplus reticularis*, gamma irradiation, postharvest disinfestation

Abstract

Gamma irradiation was investigated as a possible method for disinfestation of huhu beetle larvae, *Prionoplus reticularis* White, in *Pinus radiata* D. Don. Larvae of four representative size classes were irradiated at six doses, and the lethal dose (LD₉₉) calculated from mortality data 3 days and 10 days post treatment. All larval size classes showed a similar sensitivity to gamma irradiation and required 3677 Gray (Gy) and 2476 Gy for a LD₉₉ 3 and 10 days post-treatment, respectively. The penetration of gamma irradiation into pine wood was found to be lowest in freshly cut logs, and decreased linearly at a rate of 0.698 Gy mm⁻¹ of wood. The penetration was greatest in wood that had been stored for 2 years, and decreased 0.512 Gy mm⁻¹ of wood. These results are likely to be correlated with wood moisture content. Gamma irradiation appears to be a potential alternative method to fumigation for quarantine treatment of *P. reticularis*.

Introduction

Huhu beetle larvae, *Prionoplus reticularis* White (Coleoptera: Cerambycidae), are common in the dead wood of New Zealand soft wood trees including radiata pine *Pinus radiata* D. Don (= Monterey pine) (Edwards, 1961b). While not attacking living trees, eggs can be laid on freshly felled timber or on cut logs awaiting export. Larvae will hatch from these eggs after 16 to 25 days, followed by a larval period of at least 2 to 3 years which includes an over-wintering, non-feeding final larval instar. After a subsequent 25-day pupation period, adults chew their way out of the logs to mate and females oviposit approximately 250 to 350 eggs (Edwards, 1961a; Hosking, 1978; Morgan, 1960). Edwards (1959) found huhu had a wide host range including a variety of New Zealand native and non-native tree species. Therefore, *P. reticularis* is likely to be able to establish in countries such as the

United States where similar timber species are present (Anonymous, 1992). Currently, *P. reticularis* is considered of quarantine importance in countries such as the United States (Anonymous, 1992), and consequently logs are currently fumigated with methyl bromide prior to entry. New Zealand also exports logs and timber products to a variety of North Asian markets where substantial volumes are fumigated upon arrival with methyl bromide to control insect pests (Maud, 1995). However, methyl bromide has been classified as a ozone depleting chemical (UNEP, 1992) and researchers are searching for alternatives to this chemical control method. Gamma irradiation is a possible alternative, non-chemical disinfestation treatment to methyl bromide.

Research on gamma irradiation disinfestation has generally been limited to fresh horticultural and agricultural produce (Burditt, 1994). However, these crops have a limited tolerance to gamma irradiation and of-

ten cannot withstand the high doses required to kill insects (Kader, 1986), limiting the use of gamma irradiation for quarantine purposes to sublethal effects such as sterilization (Toba & Burditt, 1992; Lester & Petry, 1995; Lester & Barrington, 1997). Most countries require that quarantine treatments result in insect mortality, usually within the range of Probit 9. Gamma irradiation may not damage logs and timber, which may tolerate doses sufficient to kill insects for quarantine purposes. In New Zealand and overseas, gamma irradiation has been suggested as a possible alternative disinfestation treatment to methyl bromide (Anonymous, 1991, 1995; Morrell, 1995).

Our aims in this study were to determine the gamma irradiation dose required to kill huhu beetle larvae, assessed 3 and 10 d after treatment, and to examine gamma irradiation penetration of pine. Larvae were examined here primarily because of their availability. Different life stages of arthropods have significant differences in susceptibility to gamma irradiation (e.g. Lester & Petry, 1995), and our aim is to examine other *P. reticularis* life stages after the development of a laboratory rearing method.

Materials and methods

Larval collection and treatment. Huhu beetle larvae were collected from pine logs and stumps in Riverhead Forest, Kumeu, New Zealand, during 1995 and 1996. Logs and stumps in a variety of decompositional stages were split open with an axe, and undamaged larvae were sorted into four representative size classes 0–15, 15–30, 30–40 and 40+ mm in length. These size classes were used because instar determination for this species has not been described, and measurements such as head capsule width are not indicative of instars (P.J. Lester, unpublished data). During collection, small larvae (<30 mm) were individually placed into 12 × 45 mm holes drilled into 250 × 140 × 50 mm blocks of untreated pine wood, and larger larvae (>30 mm) into glass tubes (100 × 25 mm), because larger larvae tended to eat their way out of the pine blocks. Cellulose paper was added to the glass tube to absorb any external moisture.

Larvae were collected and treated on the same day. A cobalt-60 gamma irradiation source (Gammacell) was used to irradiate the larvae with a dose rate of 47.60 ± 0.32 Gray (Gy) min^{-1} as determined using the Fricke method (Spinks & Woods, 1964). Twenty to 45 larvae were removed from the blocks and tubes

using soft forceps, placed in a plastic container (length 110 mm, diameter 40 mm) with tissue paper, and irradiated at doses of 700, 1300, 1600, 2000, 2500 and 3000 Gy. Three replicates were used for each dose and stage combination. A control was used for each size class, and was treated similarly to the irradiated larvae. The total insect numbers irradiated for each size class ranged from 402–644, with 63–170 in the control treatment (Table 1). Differences in insect numbers between irradiations were due to variation in the availability of larvae from collections on different days. After treatment, the larvae were placed back in the collection containers and stored at $20 \pm 0.5^\circ\text{C}$ and 60–80% r.h. until assessment, 3 and 10 d after treatment. These times were chosen because in a commercially operating treatment facility, the shortest time period for wood to be treated and shipped to a foreign market would be ~3 d, or a more reasonable time would be ~10 d. During assessment larvae were removed from the blocks and recorded as 'live' (movement) or 'dead' (no movement) when gently probed with blunt forceps.

Statistical analysis. Dose-mortality data were analyzed using the complementary loglog (cloglog) model (Preisler & Robertson, 1989). This transformation gave approximate linearity, and the line was used to estimate the dose required to obtain 99% mortality (LD₉₉). A model was fitted using a robust version of the generalized linear model analysis available in S-PLUS (Statistical Sciences Inc., 1991). Variance was assumed proportional to a binomial distribution. Non-parametric Kruskal–Wallis analysis of variance (ANOVA) was used to compare LD₉₉'s from different larval size classes, and times until assessment.

Pine dosimetry. Rough sawn *Pinus radiata* was irradiated at the Mallinkroft Veterinary cobalt-60 gamma radiation source (Gammacell) at Upper Hutt, New Zealand, with a dose rate of 10.61 ± 0.32 Gy min^{-1} . The timber was obtained from a local Auckland (New Zealand) sawmill, from trees cut approximately 1 month, 6 months and 2 years before sampling. The timber had the bark removed, and was stored outdoors for this period. Two 200 × 200 × 300 mm blocks of each age were used for the penetration tests. Blocks were cut in half longitudinally, and in one half six holes cut in a diagonal across the timber to fit 3.5 ml vials filled with Fricke solution, to determine the irradiation dose received in that part of the wood (Spinks & Woods, 1964). The halves of the

Table 1. Mean lethal dose (Gy) required to kill 99% (LD₉₉) of four size classes of huhu larvae, by day 3 or day 10 after irradiation. CI, 99% confidence intervals

Size class (mm)	n (treated)	n (controls)	3 d after treatment		10 d after treatment	
			LD ₉₉	99% CI	LD ₉₉	99% CI
0–15	644	99	3677	(3206–4218)	2272	(1981–2607)
15–30	740	170	3250	(2833–3728)	2274	(1983–2609)
30–40	434	63	2956	(2577–3391)	2476	(2159–2840)
40+	402	69	3295	(2872–3779)	2386	(2080–2737)

blocks were then joined back together using plastic bolts through holes drilled at either end of the blocks. We ensured that only solid wood was between the vials and the irradiation source. Vials with Fricke solution were also attached to the front and back of the blocks, and the blocks rejoined together with the other half before being exposed to the gamma irradiation dose of approximately 230 Gy. This dose was used, rather than higher doses approximating those used for larval mortality estimates, because the Fricke solution cannot withstand doses greater than 500 Gy (Spinks & Woods, 1964).

Moisture content was assessed immediately after the penetration tests by sawing the pine blocks in half, and removing four 42–120 g samples, approximately 5 mm in width. These samples consisted of an entire cross-section of the blocks. The samples were weighed and then heated in an oven at 100°C for 48 hours, and repeatedly weighed until a constant weight was obtained.

Results and discussion

When mortality was assessed 3 days after treatment, 3677 Gy was the estimated dose required to kill 99% of huhu beetle larvae, but the dose needed to cause similar mortality when measured 10 days post-irradiation was significantly reduced to 2476 Gy ($\chi^2 = 16.67$, $df = 1$, $P < 0.001$) (Table 1, Figure 1). There was no significant difference in the mortality response of each of the size classes within each assessment time ($\chi^2 = 1.33$, $df = 3$, $P = 0.721$). The mean mortalities in the controls on day 10 were 12.0%, 4.8%, 1.3% and 5.8% for the size classes 0–15 mm, 15–30 mm, 30–40 mm and 40+ mm, respectively, which were not significantly different ($\chi^2 = 2.00$, $df = 1$, $P = 0.157$). The control mortality did not change significantly between the 3 and

10 d assessment for any of the four larval size classes ($P \geq 0.10$).

The dose necessary for disinfestation will thus vary according to the time between treatment and assessment. If logs are treated in New Zealand before being shipped to their destination, which is likely to take at least 10 days for New Zealand trees (Anonymous, 1992), then a dose of 2476 Gy is required to kill 99% of any huhu beetle larvae. If logs are treated in the importing country, the larger dose of 3677 Gy may be necessary. These doses are much higher than those necessary to kill other beetles in trees. For example, Yoshida et al. (1975) found an LD₉₉ of 730 Gy for the ambrosia beetle *Xylosandrus compactus* (Coleoptera: Scolytidae), 910 Gy for *X. germanus*, and 1300 Gy for *Xyleborus semiopacus*, as determined 12 days post-treatment.

As a quarantine treatment, irradiation would need to be applied to insects inside the log. In our experiments, the penetration of gamma irradiation through pine decreased in a linear fashion with wood depth (Figure 2). A linear model was fitted to the data for moisture content, incorporating a quadratic term to test for non-linearity. In all cases, the quadratic term was unnecessary ($P > 0.2$). In wood cut one month prior to irradiation the dose received decreased at a rate of 0.698 Gy mm⁻¹ of wood, or 0.311% mm⁻¹ of the initial dose received immediately outside of the wood (Figure 2). In wood cut 2 years prior to irradiation the dose received decreased at a rate of 0.512 Gy mm⁻¹ of wood, or 0.222% mm⁻¹ of the initial dose applied. Irradiation penetration in 6-month old wood was slightly lower than that of the 2 year, at 0.656 Gy mm⁻¹ of wood, or 0.299% mm⁻¹ of the initial dose applied (Figure 2).

The penetration of irradiation appeared dependent on the age of the logs, being lowest through those freshly cut. This effect may be due to the different amounts of water in the timber associated with each

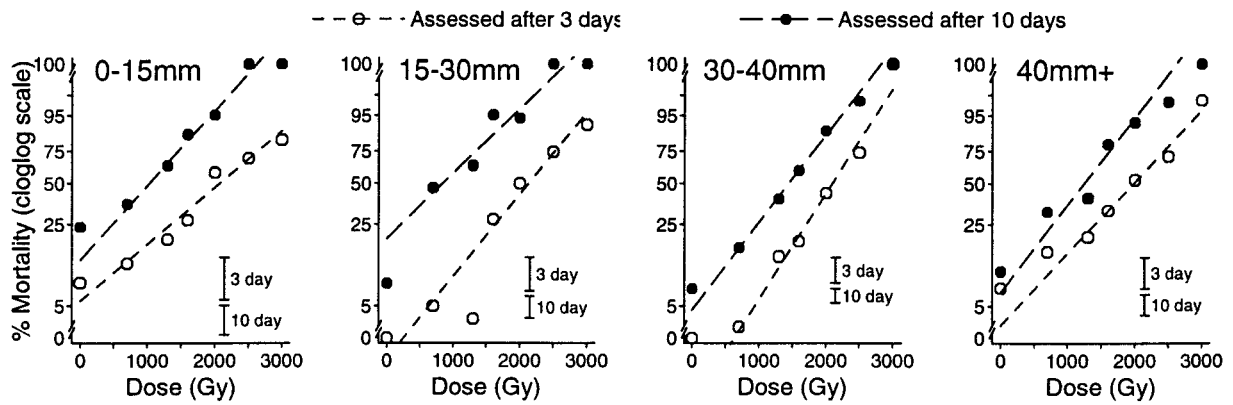


Figure 1. Average observed mortality of huhu larvae for each larval length size class and irradiation dose tested, 3 and 10 d after treatment. Fitted lines were used to predict the LD₉₉. Error bars are 1 SEM, *n* = 3.

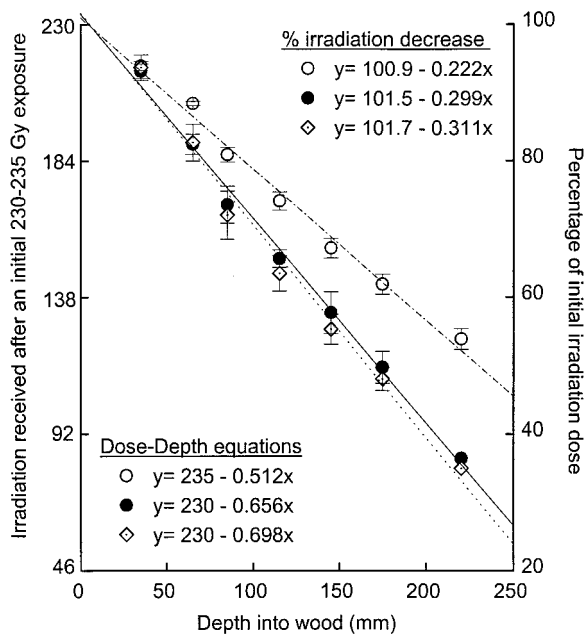


Figure 2. Gamma irradiation penetration of pine blocks. On the left y-axis is the dose of irradiation received in the specific depth of wood after an initial exposure to 230 or 235 Gy. On the right y-axis is the percent of the total dose received as a function of wood depth. The mean water content of the wood was 16.1%, 29.5% and 41.0% from the trees cut approximately 2 yr (○), 6 mo (●) and 1 mo (◇) before sampling, respectively. Error bars are standard deviation; *n* = 2. All fits were significant (*P* < 0.001), with *r*² values for the fitted lines are in excess of 0.98.

harvest date. The water content of the wood (expressed as a percentage of the original weight) was, 41.0% (range= 36.9–44.6), 29.5% (27.9–30.4) and 16.1% (14.3–17.4) from the trees cut approximately 1 month, 6 months and 2 years before sampling, respectively. From these results it appears that older, drier, logs

provide the best irradiation penetration. By the time logs are dry, however, any larvae within the logs could be bigger and may have penetrated deeper due to the longer period since oviposition. With some source-dose combinations such as those used in the present study, the penetration is limited to a certain point, after which it is no longer effective. Further research is required to determine how deeply the larvae penetrate the logs after felling, how penetration is altered with water content, and how higher doses of irradiation penetrate pine. With this knowledge we would be able to determine the optimal time after felling for treatment and export to kill *P. reticularis*.

Sublethal effects of gamma irradiation may also be considered an effective quarantine treatment. *Xylosanders compactus*, *X. germanus*, and *Xyleborus semiopacus* were sterilized within lumber by doses between 20–40 Gy, and adult emergence inhibited by 10–30 Gy (Yoshida et al., 1975). Other beetle larvae have been shown to be sterilized by gamma irradiation dosages as low as 70 Gy (Brower & Tilton, 1985), and 500 Gy inhibited adult emergence (Milne et al., 1977). Thus, gamma irradiation as a quarantine treatment is likely to sterilize any adults developing from larvae not killed by a dose such as 2476 Gy, discussed above. A much-reduced dose to sterilize the insects may be a suitable quarantine treatment for some countries. A gamma irradiation treatment that sterilizes insects such as *P. reticularis*, would be much cheaper to perform. However, lethal quarantine treatments are required in many countries. To estimate the dose of gamma irradiation to achieve a Probit 9 level of mortality (99.99683%), we extended the regression line of our data. In both 3 and 10-d analyses, all data for the size classes were combined as no significant

differences in the mortality response was observed in the LD₉₉ analysis between size classes. To achieve a Probit 9 level of quarantine security 3 days after irradiation, we estimate a mean dose of 3792 Gy (95% CI: 3479–4134) would be required. To achieve a Probit 9 level of quarantine security 10 days after irradiation, we estimate a mean dose of 2873 Gy (95% CI: 2635–3132) would be required. Such an extension of the regression line used to calculate the LD₉₉ from the current data is not an accurate method of predicting a Probit 9 level of mortality, which probably requires far more than 30,000 insects (Robertson et al., 1994).

Gamma irradiation may also be useful for disinfestation of other New Zealand forestry pests of quarantine importance including *Sirex noctilio* F. (Hymenoptera: Siricidae), which can penetrate deeply into pine trees. Current practices of methyl bromide fumigation may not be effective in controlling this pest in pine, as the fumigant does not penetrate fresh pine sufficiently to be lethal (Cross, 1991). The penetration profile of logs may be more variable than that of the blocks we used, but our results suggest that gamma irradiation can penetrate pine that has been cut one month prior to the irradiation treatment.

Gamma irradiation can be effective in killing coleopteran pests other than *P. reticularis* (Yoshida et al., 1975), nematodes (Eichholz et al., 1991), bacteria and fungi in forestry products (Sharman & Smith, 1970; Schoeman et al., 1994). It may also reduce the attractancy of wood for insect oviposition after irradiation (French et al., 1982). Gamma irradiation thus appears to be a viable alternative to chemical fumigation for a range of forest pests and diseases. Further work is required to confirm that *P. reticularis* can be killed when treated within timber, as well as the economic viability of irradiation as a quarantine treatment of forestry products. To this end, importing countries will need to decide on the level of quarantine security required for specific pests; specifically, will lethal or sterilising sublethal irradiation doses be a sufficient quarantine treatment?

Acknowledgements

We thank A. Barrington, P. Dentener, M. Lay-Yee and two anonymous reviewers for comments which significantly improved this manuscript; John Maindonald for statistical advice.

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