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Tolerance of *Hylurgus ligniperda* (F.) (Coleoptera: Scolytinae) and *Arhopalus ferus* (Mulsant) (Coleoptera: Cerambycidae) to ionising radiation: a comparison with existing generic radiation phytosanitary treatments

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Abstract

Background: Irradiation is accepted as a phytosanitary treatment for horticultural products by many countries. Irradiation is a potential alternative to chemical fumigation for wood products; however, data supporting its efficacy against potential forestry pests is limited.

Methods: Irradiation efficacy data were obtained experimentally for *Arhopalus ferus* (Mulsant) and *Hylurgus ligniperda* (F.). The results are compared to existing ionising-radiation treatment data for various bark and wood-boring beetles species as found in the International Database on Insect Disinfestation and Sterilization (IDIDAS) and the published literature.

Results: Existing IDIDAS records suggest that the effective dose required to sterilise insects in the families Cerambycidae and Scolytinae is < 150 Gy. Estimated LD_{99} obtained here for the sterility of adult *A. ferus* were 44. 1 Gy (LD_{99}, ± 15.3, 95% CI) and eggs 40.4 Gy (± 9.8, 95% CI). Our results suggest that an effective sterilisation dose for *A. ferus* eggs will be from 20 to 40 Gy; however, LD_{99} dose estimates were not obtained for other life stages. Adult *H. ligniperda* were more tolerant of radiation with 1.6% of adults producing viable eggs at doses of between 100 and 150 Gy despite 100% sterility being recorded at 75 and 175 Gy.

Conclusions: Our results are consistent with existing studies of other bark and wood-boring beetles. The doses tested here were equivalent to, or lower than, those used in previous studies. *Arhopalus ferus* adults were less tolerant to ionising radiation than the published literature for other Cerambycidae. Further studies with adult *H. ligniperda* are recommended as 150 Gy represents the upper limit currently reported in IDIDAS for other Scolytinae. An assessment of the potential cost-effectiveness of irradiation as a phytosanitary treatment at the range of doses identified in this study should be conducted before committing to further efficacy testing.

Keywords: Phytosanitary treatment, *Pinus radiata*, Quarantine, Irradiation

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Background

The use of ionising radiation as a phytosanitary treatment for horticultural commodities is expanding worldwide, in part due to the acceptance of the treatment of produce on arrival in the USA (Bustos-Griffin et al. 2015). The development and approval of generic treatment doses for groups of insect pests by the International Atomic Energy Agency and the US Department of Agriculture over the past 10 years have helped to increase both awareness and acceptance of phytosanitary irradiation treatments (Follett 2009; Hallman 2012).

Many countries require wood and wood products to undergo phytosanitary measures/treatment before entry, or at the border, to mitigate potential biological risks. Chemical fumigants, insecticidal sprays, heat treatment, and debarking, or a combination of several measures, are approved for use by various countries (Pawson et al. 2014). Ionising radiation has been used to disinfest specific wooden artefacts (Fan et al. 1988) and is an accepted phytosanitary treatment for bulk wooden commodities, e.g. logs and timber in Australia at 25,000 Gy (Anon 2016). Despite its acceptance for bulk wood commodities, we are unaware that this has been implemented as an operational solution by any country. Given its acceptance for other commodities, irradiation is a potential non-chemical alternative to the current phytosanitary treatments for wood products.

The International Plant Protection Convention guidelines specify that the efficacy of ionising-radiation treatments must be scientifically demonstrated for the regulated pest(s) of concern and the required treatment response (IPPC 2003). Treatment responses to irradiation are dose-dependent whereby relatively low doses can cause sterility or prevent development and higher doses cause death. This dose-dependent effect is the foundation of the sterile-insect control technique (Klassen and Curtis 2005). Lower doses that prevent successful reproduction are adequate to mitigate phytosanitary risks except for pests known to vector plant pathogens (IPPC 2003). The use of a sublethal dose that leaves pests alive but sterile can challenge the traditional method of verifying treatment efficacy, where complete mortality is currently expected (Follett 2009). For example, the generic radiation dose currently approved by the USDA for all pests (except for lepidopteran pupae and adults) on horticultural commodities is 400 Gy (Follett 2009).

New Zealand exported 16 million m^3 of logs (predominantly *Pinus radiata* D.Don) in the year ending June 2015 (MPI 2015). These logs were treated prior to export with phosphine (52.4%) and methyl bromide (22.8%) or were debarked (6.0%); the remaining 18.8% were shipped untreated to countries that apply phytosanitary treatments on arrival (Ministry for Primary Industries, Plant Export Group, 15 January 2016). Development of a new phytosanitary treatment to augment the existing toolbox requires confirmation of treatment efficacy for relevant life stages of quarantine pests, identified by a formal pest risk assessment process. To circumvent the laborious and expensive process of testing each target species to accumulate complete mortality data, initial screening tests are done to identify the species and life stage that is most tolerant to the treatment, followed by more detailed studies of those that are most tolerant. To begin our study of relative tolerance to irradiation, we chose two species, *Hylurgus ligniperda* (F.) and *Arhopalus ferus* (Mulsant), that are potentially found on or beneath the bark of *P. radiata* logs after harvest in New Zealand (Pawson et al. 2014). Determining an effective dose for these two species is a first step towards evaluating the potential of ionising radiation as an alternative phytosanitary treatment for wood exports. We report the initial results of bioassays to determine the relative tolerance to irradiation treatment by the life stages of *H. ligniperda* and *A. ferus* and compare the doses required for controlling these two species with similar species found in the International Database on Insect Disinfestation and Sterilization (IDIDAS 2013) and other published literature.

Methods

Collection of dose-response data for related species

A search for existing data on the effect of ionising radiation on other species of bark and wood-boring beetles was conducted by examining the IDIDAS (2013) database. An additional search of published scientific literature from 1960 to 2015 was conducted using the Scopus database (Elsevier™) with each of the search terms "Cerambycidae", "Scolytinae", "wood borer", and "bark beetle" in combination with either "radiation" or "irradiation".

Experimental irradiation of *H. ligniperda* and *A. ferus*

H. ligniperda

Adult *H. ligniperda* were collected using ethanol and alpha-pinene baited flight intercept panel traps following Meurisse and Pawson (2017), in recently harvested *P. radiata* stands in Ashley (−43.203, 172.567), Bottle Lake (−43.460, 172.697), and West Melton (−43.466, 172.415) plantation forests (Canterbury, New Zealand). Twenty adult *H. ligniperda* were placed in artificial bark habitats that comprised two thin 12 cm × 7 cm pieces of bark held phloem-side together with rubber bands (Clare and George 2016). The beetles were not sexed at this stage but eggs were laid in each case, which indicated that either females were already mated or there were sufficient males in each group of 20 to mate with the females. Bark habitats were placed on a moist cloth inside a vented plastic 750-mL plastic container that was incubated at 20 °C for 12 to 14 days. To arrest development and achieve synchronised egg hatch and subsequent larval development, eggs (and larvae where necessary) collected from the bark habitats were stored at 10 ± 1.5 °C as needed. Once

synchronised, batches of stored eggs were incubated at 20 ± 1.5 °C. Upon emergence, neonate larvae were transferred in batches of 20 to Petri dishes of artificial diet (a modification of Rogers et al. (2002) as described by Romo et al. (2015)) and reared at 20 ± 1.5 °C until treatment. Pupae were held in Petri dishes of artificial diet at a constant 10 ± 1.5 °C until treatment.

A T-shaped plastic insert was placed in a 90-mm-diameter Petri dish to create a customised container that housed individually sexed wild-caught adult females and artificially reared larvae, pupae, and eggs of *H. ligniperda* during irradiation treatments. The adult females were placed in a ventilated 1-mL plastic vial in one quarter of the dish, larvae were placed into holes made in artificial diet in one quarter, and pupae were placed in shallow impressions in artificial diet in the remaining half of the Petri dish. The eggs were placed on filter paper on the surface of diet on top of the pupae. Each Petri dish contained 20 adults, 20 eggs, 20 larvae, and 10 pupae. Following irradiation, as described below, individual adult female *H. ligniperda* were paired with an untreated "wild-caught" male on a bark disc in a 90-mm ventilated Petri dish and checked for egg-laying between 25 and 37 days after treatment. Larval emergence from any eggs laid indicated female adults had not been sterilised. Treated *H. ligniperda* eggs were monitored every second day for 32 days, and successful hatch was deemed indicative of non-sterility. Treated *H. ligniperda* larvae and pupae were reared on artificial diet. Each emerging female adult was paired with a "wild-caught" male, and each emerging male adult was paired with a laboratory-reared virgin female on bark discs in 90-mm ventilated Petri dishes to monitor egg production and subsequent hatch for up to 50 days. Again, successful egg hatch was considered indicative of non-sterility.

A. ferus

Although approximately 5% of the adult *A. ferus* used in our study were collected from panel traps in the forest, most were collected at night by hand at the SRS Rolleston sawmill (–43.587, 172.379) where they are attracted to building lights (Pawson et al. 2009). Eggs were then obtained by placing groups of 10 to 20 adults at ambient room temperature (~ 18 to 20 °C) in plastic containers (L300 mm × W210 mm × H80 mm) that were lined with wax paper to provide a substrate for egg deposition (Van Epenhijusen et al. 2012). *Arhopalus ferus* lay large egg batches; hence, no periods of chilling were required to synchronise the availability of sufficient individuals for rearing. Once hatched, 10 neonate *A. ferus* larvae were placed in each Petri dish of artificial diet (Romo et al. 2015) and reared at 20.0 ± 1.5 °C until they were used in tests. Logs are exported from New Zealand within 5 months of harvest, and laboratory studies at a range of temperatures have shown that *A. ferus* does not pupate in this time (Romo et al. unpublished data). Therefore, as only adult, egg, or larval life stages can be present on (or in) logs when preshipment phytosanitary treatments are applied, we did not test pupal tolerance of *A. ferus* to radiation.

For treatment, adult *A. ferus* were placed individually in 10-mL plastic vials sealed with cotton wool and laid horizontally. For the purposes of estimating the likely upper boundary of an effective treatment dose, it was not considered effective to rear virgin *A. ferus* females (as artificial rearing methods were not developed at the time, but are now available (Barrington et al. 2015)) in order to test separate effective doses for male individuals. As such, male/female pairs of treated adults were placed in 100-mL plastic containers with moistened paper towels and a square of bark. Egg production and subsequent viability was then monitored for 47 days. *Arhopalus ferus* larvae were placed in groups of 10 in a 90-mm Petri dish by removing a wedge of diet, inserting larvae, and then recapping with diet. Clusters of *A. ferus* eggs cut from the waxed paper were placed on moistened filter paper on the surface of a diet-filled Petri dish along with the *A. ferus* larvae. Treated *A. ferus* eggs were subsequently left on moist filter paper in a ventilated Petri dish and observed every second day for 40 days to assess viability. Many *A. ferus* larvae moved from their individual prepared holes and attacked each other during radiation treatment (which took place overnight) resulting in high levels of mortality. Surviving individuals were transferred to individual 10-mL diet tubes, but, during rearing, some chewed through the plastic lids between weekly checks and became mixed in a communal container. Mixing made it impossible to determine the dose received by many individuals and a formal analysis of *A. ferus* larval data was not possible.

Irradiation treatments

For both species, a control replicate comprising the same number of individuals of each life stage was used to assess the efficacy of irradiation treatments. Individuals used in the control treatment were subjected to the same pre- and post-treatment conditions and monitoring as the test subjects. During the test, the control replicate was placed on a table outside the irradiation testing enclosure and was subjected to the same transport, temperature, and humidity levels as experienced by the treated individuals.

Initial range-finding tests were conducted for both *H. ligniperda* and *A. ferus* adults. Adults were selected as they are the most readily available life stage, and adults of a range of species have been shown to be more tolerant of irradiation than other life stages (Follett 2008; Tilton et al. 1966; Yoshida et al. 1974). Twenty *H. ligniperda* adults per dose were placed in a Petri dish and treated at 100, 200, 300, 400, or 500 Gy (as described below). Replicates of 30 *A. ferus* were placed individually in 5-mL containers and treated at doses of 50, 100, 150, or 200 Gy. Any eggs

produced during the irradiation treatments were left in treatment vials for monitoring.

Following from the range-finding tests, the main trial of *H. ligniperda* consisted of a single replicate with three pseudo-replicate Petri dishes (with T-shaped inserts) each containing 20 adults, 20 eggs, 20 larvae, and 10 pupae treated at 75, 100, 125, 150, or 175 Gy. The main trial of *A. ferus* consisted of a series of three replicates of *A. ferus* eggs, larvae, and adults with 20 individuals treated per replicate at 20, 40, 60, 80, or 100 Gy.

Gamma-radiation from a Cobalt-60 (^{60}Co) source was applied using a Theratron 80 external beam teletherapy unit (Atomic Energy of Canada Ltd). Radiation doses were delivered simultaneously to all samples in a replicate by placing them at different distances from the ^{60}Co source and utilising the principle of the inverse square law, with minor corrections for the effects of back-scatter off the cell floor, and from the beam collimator system. Dose rate was determined via measurement using a NE2571 ionisation chamber (Nuclear Enterprises, UK) with calibration in terms of air kerma traceable to Australian national standards. Subsequent irradiation times were corrected for ^{60}Co source decay. Treated and control individuals of both species were maintained in a temperature-controlled chamber at a constant 20 ± 1.5 °C after treatment.

Analysis

Observed sterility of treated individuals was corrected for control mortality prior to analysis using the Schneider-Orelli formula that is recommended when assessing percent mortality/sterility of uniform population data (http://www.ehabsoft.com/ldpline/onlinecontrol.htm, accessed 19 April 2016; Schneider-Orelli 1947). Logistic regression was used to model the binomial outcome of irradiation trials and to estimate doses required to achieve sterility at various levels (Venables and Ripley 2002). Where sufficient replicates were available to undertake a formal statistical analysis, a general linear model (GLM) with binomial errors and logit link was applied to analyse the dose response to radiation using R software version 3.2.2 (R Development Core Team 2013). Standardised Pearson residuals versus leverage were examined, and model outliers were identified as points with an extreme Cook's distance (> 2.0). Any potential outliers were individually identified, and due consideration was given to each point to evaluate the validity of the measurement or the potential for a methodological issue (e.g. treatment failure) that would warrant removal. Models were then rerun and examined again for outliers. For each life stage and exposure time, the proportion of sterile organisms served as the response variable which was modelled as a function of temperature. Doses required to achieve 90, 95, or 99% sterility and their associated 95% confidence interval were estimated from the models using the "dose.p" function (R-package MASS (Ripley et al. 2015)).

Results

The IDIDAS (2013) database included information for one species of longhorn beetle and eight species of bark beetles that are from the same family (Cerambycidae) and subfamily (Curculionidae: Scolytinae) as *A. ferus* and *H. ligniperda* respectively (Table 1). A further six studies (four studying Cerambycidae and two Scolytinae) by Chinese researchers were not included by IDIDAS (2013) (Table 1).

Five species of Cerambycidae have been tested previously for their response to irradiation treatments. Lu et al. (2001) tested adult *Anoplophora glabripennis* Motschulsky at a range of doses between 50 and 200 Gy to evaluate the potential use of the sterile-insect technique for control. Sterility of both males and females was observed at doses of 100, 150, and 200 Gy, but not at 50 Gy (Lu et al. 2001). Sterility at 50 and 70 Gy was inconsistent and varied between 71.4 and 100% amongst test replicates. Wang et al. (2006) tested the third to fifth instar larvae of *An. glabripennis* at doses of 40 to 140 Gy. Fifth instar larvae were most resistant to irradiation, and pupation was prevented at 55 and 60 Gy. Wang et al. (2006) tested the larvae, pupae, and adult life stages of *Monochamus alternatus* Hope at various doses between 20 and 140 Gy. Irradiation increased the larval life span, but adult longevity was reduced (Zhan et al. 2011b). Larvae were prevented from pupating by 50 Gy (61.4 Gy $LD_{99.9968}$), pupae did not eclose at 120 Gy, and adults were sterile at 140 Gy (Zhan et al. 2011b). Zhan et al. (2011a) tested the fourth and fifth instar larvae and adults of *Monochamus sutor* (L.) at a range of doses from 25 to 140 Gy. Like *M. alternatus*, irradiation increased larval development time, with larvae not pupating when exposed to 45 to 60 Gy and pupae not emerging at 35 to 40 Gy (Zhan et al. 2011a). Wang Y. et al. (2011) exposed *Xylotrechus rusticus* (L.) larvae and pupae to doses of 20 to 100 Gy. Larval development to the pupal stage was arrested by exposure to 60 Gy, whereas pupae failed to eclose after exposure to 50 Gy. The estimated $LD_{99.9968}$ for larval mortality was 72.3 Gy (64.4–89.1 95% CI) (Wang Y. et al. 2011).

Successful sterilisation (or the prevention of development from juvenile life stages to reproductively capable adults) was found for seven of the nine species of bark beetles tested previously using doses of less than 100 Gy (Table 1). There is conflicting evidence for the required dose to sterilise *Ips confusus* (LeConte). Although Stark (1963) reported that sterilisation occurred at a dose as low as 10 Gy, Wood and Stark (1966) subsequently reported that doses of 75 Gy for males and 100 Gy for females were necessary to obtain sterility (Table 1). The most radiation-tolerant scolytid species tested to date, *Cryphalus fulvus* Niijima, was reported by Yoshida et al. (1974) who noted that tolerance to radiation increased with the progression of life stages and that a sterilising

Table 1 Dose applied and reported treatment effect expressed as sterility (% where specified) or the prevention of development to the adult life stage

Genus/species	Life stage	Dose (Gy)	Response	Reference
Cerambycidae				
Anoplophora glabripennis	Larvae	70–90	Prevented emergence	Wang et al. (2006)[a]
Anoplophora glabripennis	Adult	100	Sterility	Lu et al. (2001)
Monochamus alternatus	Larvae	61.4	$LD_{99.9968}$ prevented emergence	Zhan et al. (2011b)[a]
Monochamus alternatus	Pupae	120	Prevented emergence	Zhan et al. (2011b)[a]
Monochamus alternatus	Adult	140	Sterility	Zhan et al. (2011b)[a]
Monochamus sutor	Larvae	35–40	Prevented emergence	Zhan et al. (2011a)[a]
Monochamus sutor	Adult	140♀	Prevented emergence	Zhan et al. (2011a)[a]
Prionoplus reticularis	Larvae	2476	Mortality	Lester et al. (2000)
Xylotrechus rusticus	Larvae	72.3	$LD_{99.9968}$ prevented emergence	Wang Y. et al. (2011)[a]
Curculionidae: Scolytinae				
Cryphalus fulvus	Egg	50	Not sterile	Yoshida et al. (1974)
Cryphalus fulvus	Larvae	70	Not sterile	Yoshida et al. (1974)
Cryphalus fulvus	Pupae	> 120	Not sterile	Yoshida et al. (1974)
Cryphalus fulvus	Adult	150	Not sterile	Yoshida et al. (1974)
Ips confusus	Unspecified	10	Apparently sterile	Stark (1963)
Ips confusus	Adult	♂75/♀100	♂98/♀100% sterility	Wood and Stark (1966)
Ips sexdentatus	Adult	80 (winter), 140 (summer)	Sterility	Wang X. et al. (2011)[a]
Ips subelongatus	Larvae	80	Prevented emergence	Zhan et al. (2011c)[a]
Ips subelongatus	Pupae	120	Prevented emergence	Zhan et al. (2011c)[a]
Ips subelongatus	Adult	140	Sterility	Zhan et al. (2011c)[a]
Ips typographus	Adult	30	49% sterility	Turčáni and Vakula (2007)
Xyleborus atratus	Adult	40	Sterility	Yoshida et al. (1977)
Xyleborus perforans	Adult	40♀	Sterility	Yoshida et al. (1977)
Xyleborus perforans	Pupae	30–50♂	Sterility	Yoshida et al. (1977)
Xylosandrus compactus[b]	Adult	20–40	Sterility	Yoshida et al. (1975)
Xylosandrus compactus[b]	Larva	50–70	Prevented emergence	Yoshida et al. (1975)
Xylosandrus compactus[b]	Pupa	> 100	Prevented emergence	Yoshida et al. (1975)
Xylosandrus compactus[b]	Egg	30	Prevented emergence	Yoshida et al. (1975)
Xylosandrus crassiusculus[c]	Adult	20♀	Sterility	Yoshida et al. (1977)
Xylosandrus crassiusculus[cb]	Adult	20–40	Sterility	Yoshida et al. (1975)
Xylosandrus crassiusculus[cb]	Larva	50–70	Prevented emergence	Yoshida et al. (1975)
Xylosandrus crassiusculus[cb]	Pupa	> 100	Prevented emergence	Yoshida et al. (1975)
Xylosandrus crassiusculus[cb]	Egg	30	Prevented emergence	Yoshida et al. (1975)
Xylosandrus germanus[b]	Adult	20–40	Sterility	Yoshida et al. (1975)
Xylosandrus germanus[b]	Larva	50–70	Prevented emergence	Yoshida et al. (1975)
Xylosandrus germanus[b]	Pupa	> 100	Prevented emergence	Yoshida et al. (1975)
Xylosandrus germanus[b]	Egg	30	Prevented emergence	Yoshida et al. (1975)

[a]Publications were in Chinese, and only the English abstracts and some tables were consulted; any mistakes in interpretation are our own
[b]Yoshida et al. (1975) reported that all species of *Xylosandrus* tested were rendered sterile with doses between 20 and 40 Gy
[c]Yoshida et al. (1975) and Yoshida et al. (1977) refer to *Xyleborus semiopacus* that is a synonym of *Xylosandrus crassiusculus*

dose for adults was at most 150 Gy. Similar doses (140 Gy) were required to sterilise adult *Ips sexdentatus* (Boerner) and *Ips subelongatus* (Motschulsky) (Wang X. et al. 2011; Zhan et al. 2011c). Additional seasonal effects were noted for *I. sexdentatus* whereby summer-emerging adults required 140 Gy for sterility,

while overwintering adults were sterile at 80 Gy (Wang X. et al. 2011). A dose of 150 Gy represents the upper limit currently reported in IDIDAS for other Scolytinae.

A single range-finding replicate in the current study showed that 94.8% of *H. ligniperda* female adults were sterile after treatment at 100 Gy and 99.8% at doses of 200 to 500 Gy, after adjusting for control mortality (Fig. 1). The lack of any observed non-sterile individuals at 200 Gy or above indicates that the effective dose for sterilisation is likely to be < 200 Gy. Treatment at 75 and 175 Gy resulted in sterility of 99.5 and 97.9% of *H. ligniperda* adults, respectively, whereas 97.8% sterility was achieved at 100, 125, or 150 Gy, after adjusting for control mortality. A single adult individual was observed to produce viable eggs post-treatment at each treatment dose of 100, 125, and 150 Gy (Fig. 2). All *H. ligniperda* eggs and larvae were observed to be sterile at doses between 75 and 175 Gy; however, adjusted sterility (for control mortality) was 93.5 and 99.4% for eggs and larvae respectively (Fig. 2). Although some treated pupae at all doses eclosed (adjusted sterility of 66–74% depending on dose, where emerging adults are considered non-sterile), subsequent mating with untreated "wild-caught" males or laboratory-reared virgin females did not result in egg hatch, irrespective of dose rate (Fig. 2).

Sterility of adult female *A. ferus* from a single replicate treated at 50 to 200 Gy was 99.9% at all treatment levels after correcting for control mortality of 11.7% (Fig. 1). Subsequent tests of three replicates with adult *A. ferus* found that sterility was influenced by dose (GLM, $Z = 4.96$, $P < 0.0001$) (Fig. 3a). Mortality of untreated (control) *A. ferus* adults ranged from 25 to 50%; although high, this was lower than the sterility of treated individuals (Fig. 3a). After correcting for control mortality, the sterility of *A. ferus* adults ranged from 90.5 to 99.1% at 20 Gy and between 99.0 and 99.6% between the three replicates at treatment doses of 40 to 100 Gy. Although the effect of the treatment dose was highly significant, the LD estimates (LD_{99}, 44.1 ± 15.3 Gy, 95% CI) should be treated with caution until this is confirmed by further tests with lower control mortality.

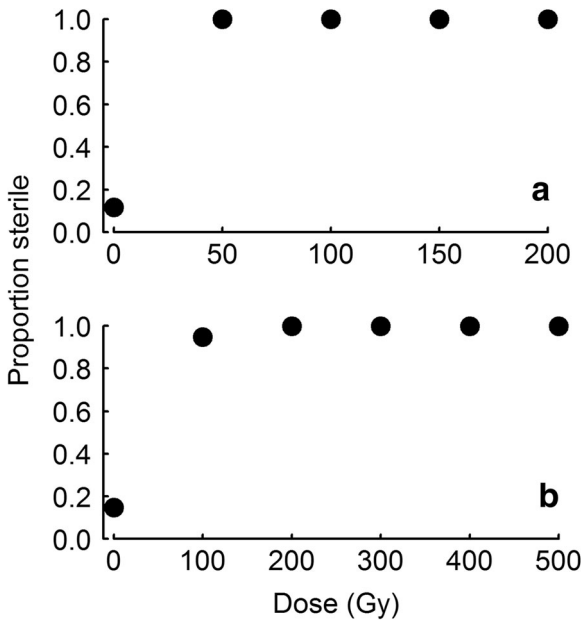

Fig. 1 Results of range-finding dose experiments showing the proportion of **a** *A. ferus* and **b** *H. ligniperda* adults sterilised after exposure to increasing doses of radiation

Control mortality in the three replicates of *A. ferus* eggs ranged from 47.5 to 52.5%. Treatment sterility of *A. ferus* eggs ranged from 96.8 to 99.1% at 20 Gy after correction for control mortality in all replicates and between 99.0 and 99.1% sterility at all doses between 40 and 100 Gy for two of the three replicates (Fig. 3b). However, in the third replicate, sterility varied from 23.8 to 44.8% for treatments between 40 and 100 Gy (Fig. 3b). A two-round procedure identified these four measurements (40-, 60-, 80-, and 100-Gy treatment) from the third replicate as outliers, and they were removed from the model. The resulting model showed a highly significant effect of radiation dose (GLM, $Z = 7.21$, $P < 0.0001$). The estimated LD_{99} to achieve sterility of *A. ferus* eggs was 40.4 Gy (± 9.8, 95% CI). Further replicates are required to provide greater confidence of the estimated LD_{99} for *A. ferus* eggs.

Discussion

Effectiveness of ionising radiation as a sterility treatment

Our results suggest that an effective sterilising dose for all life stages of *H. ligniperda* may exceed 150 Gy. Eggs and larvae were the least tolerant of radiation, which is consistent with the results of other studies that have reported increasing tolerance to radiation as life-stage development progresses (Follett 2008; Tilton et al. 1966; Wang et al. 2006; Yoshida et al. 1974; Zhan et al. 2011b). Larval and pupal development was arrested following treatment at all doses tested (75 to 175 Gy). However, mortality of control eggs does not permit us to assess the impact of radiation on *H. ligniperda* eggs. Pupae tolerated all doses tested, which was demonstrated by successful adult eclosion following all treatments. However, all adults eclosing from the treated pupae were reproductively sterile. All adults were sterilised by doses of either 75 or 175 Gy, but a non-sterile *H. ligniperda* adult was observed after treatment with 100, 125, or 150 Gy. Adult sterility following exposure to 75 Gy is consistent with the results for many other Scolytinae (Table 1). However, several adult scolytines (*C. fulvus* (Yoshida et al. 1974), *I. sexdentatus* (Wang X. et al. 2011), and *I. subelongatus* (Zhan et al. 2011c)) have shown tolerance to 140 to 150 Gy. This higher tolerance

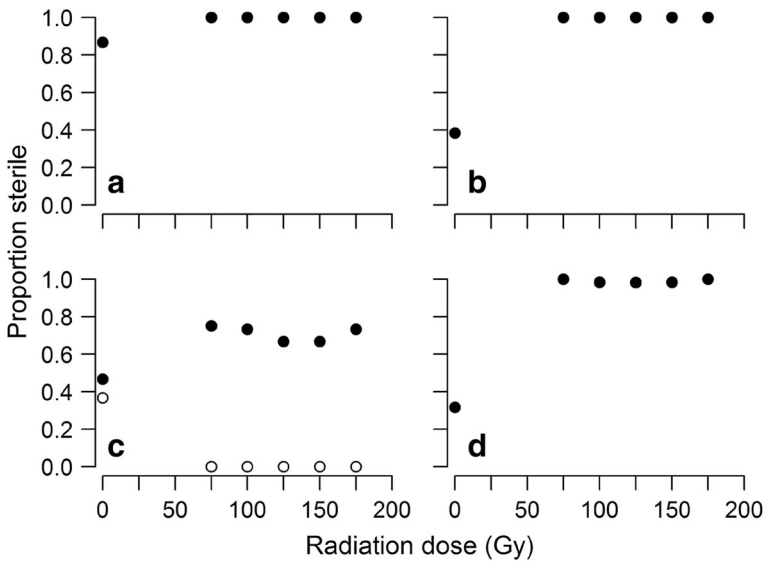

Fig. 2 Proportion of sterile *Hylurgus ligniperda* as a function of irradiation dose (Gy) per life stage. **a** Proportion of eggs that did not eclose. **b** Proportion of larvae that did not successfully emerge as adults. **c** Closed symbols represent the proportion of pupae that did not emerge as adults and open symbols represent the proportion of eclosing adults that laid viable eggs. **d** Proportion of adults that were reproductively sterile and did not produce viable eggs

is consistent with the incomplete sterility we observed following radiation at doses between 100 and 175 Gy for *H. ligniperda* adults. The seasonal differences in adult tolerance to irradiation observed by Wang X. et al. (2011) may reflect decreasing tolerance to irradiation as individual adults age. This relationship needs to be defined for *H. ligniperda* adults as part of future testing to ensure the most tolerant phase of the life cycle is tested.

The LD_{99} for sterility of *A. ferus* adults was 44.1 Gy (± 15.3, 95% CI) and 40.4 Gy (± 9.8, 95% CI) for eggs. This was lower than the estimated 70 to 90 Gy required to sterilise *An. glabripennis* (Lu et al. 2001) and adult *Monochamus* spp. (Zhan et al. 2011a; Zhan et al. 2011b). Lester et al. (2000) did not assess the dose required to cause sterility in the cerambycid *Prionoplus reticularis* White but found that the dose required to cause mortality was 2476 Gy when assessed after 10 days. This result is to be expected as the dose required to achieve mortality is always much greater than the dose required for sterility.

It is difficult to determine the actual cause of the observed partial treatment failure in one replicate of *A. ferus* eggs (at 40 to 100 Gy) post hoc. The level of sterility recorded with the 20-Gy dose and control subjects was similar to that of the other two replicates. Given the outliers present at doses of 40 to 100 Gy in the third replicate, it is necessary to conduct further replicates in the future to provide greater confidence in the LD estimates presented here. On the basis of the current study, the radiation dose required to sterilise *A. ferus* adults and eggs is approximately one order of magnitude lower than the generic radiation dose of 400 Gy currently approved by the USDA for all pests (except for lepidopteran pupae and adults) on horticultural commodities (Follett 2009). No results were obtained on the larval stage as experiments were compromised during post-treatment care.

Conclusions

We recognise the limitations of the data presented here; however, our results provide an indication of an upper limit to an effective dose, taking account of the widely reported increase in tolerance to ionising radiation within a species as a function of increasing development stage (Follett 2008; Tilton et al. 1966; Wang et al. 2006; Yoshida et al. 1974; Zhan et al. 2011b). All adult *H. ligniperda* were sterile following irradiation at 175 Gy. Lower doses cannot be considered as acceptable on the basis of our trials as single non-sterile individuals were observed at 100, 125, and 150 Gy even though all adult *H. ligniperda* tested at 75 Gy were sterile. *H. ligniperda* adults were more tolerant to irradiation than *A. ferus* adults that had a LD_{99} of 44.1 Gy (± 15.3, 95% CI). However, the effective limit to sterilise *H. ligniperda* and *A. ferus* is likely to be far less than the current generic acceptable dose of 400 Gy for horticultural commodities. A dose of 400 Gy, and a series of doses as low as 75 Gy, should be used in an economic assessment to evaluate the potential cost-effectiveness of irradiation as an alternative phytosanitary treatment for bulk export wood

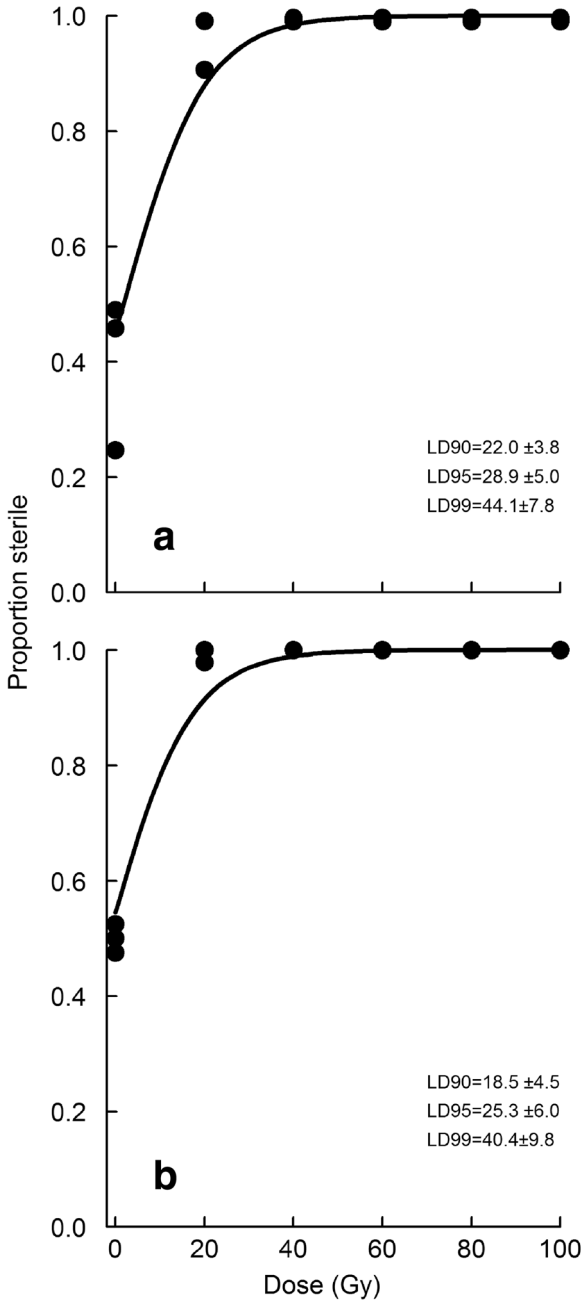

Fig. 3 a Proportion of *A. ferus* adults sterilised after exposure to increasing doses of radiation. **b** Proportion of *A. ferus* eggs sterilised after exposure to increasing doses of radiation. Lines represent the predicted values from the fitted model. LD estimates are provided ± the standard error

commodities. If irradiation is a viable alternative phytosanitary treatment at such doses (as this analysis suggests), then additional research should be conducted to define an International Standards for Phytosanitary Measures (ISPM) 18 and 28 (IPPC 2003, 2011)-compliant irradiation dose for these two species.

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Authors' contributions
AV wrote the first draft as his BSc (Hons) thesis; AV, SP, SW, and TM contributed to the design of the trials; AV, JK, XM, BO, and CR contributed to the experimental work; JL applied the irradiation treatments; and all authors contributed to the revised manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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