Postharvest phytosanitary disinfestation of *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) in citrus fruit: Tolerance of eggs and larvae to ionizing radiation

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Abstract

Historical and current pre- and postharvest strategies are reviewed to identify problems inherent to the control of *Thaumatotibia leucotreta (*Meyrick) (Lepidoptera: Tortricidae). The development of resistance to registered insecticides and the potential risk of damage to export fruit using cold disinfestation protocols necessitated investigating an alternate strategy such as ionizing radiation for the postharvest suppression of the insect. The acute effect of 40–100 Gy of ionizing radiation was assessed on 24–96 h old eggs. It was established that the eggs increased in radiotolerance as they developed and at 96 h the natural and induced egg mortality of non-irradiated and irradiated eggs did not differ greatly. The flight ability and oviposition of moths developing from irradiated 96 h old eggs were totally prevented at 70 and 50 Gy, respectively. A dose of 50 Gy was assessed on 1st to 5th instars. Radiotolerance increased with instar, making the 5th instar the most radiotolerant and the one for which a phytosanitary treatment should be efficacious. Doses of ionizing radiation from 40–200 Gy were used to estimate the lowest effective dose able to prevent further development of 5th instars, or alternatively, totally suppress reproduction and/or flight ability of moths developing from treated larvae. The lowest doses for arrested development of larvae and sterility of moths were 150 and 60 Gy, respectively. Flight ability could not be totally suppressed with 70 Gy.

Key Words: false codling moth; radiation biology; radiosensitivity; immature stages; flight ability; mortality; sterility

Resumen

Estrategias históricas y actuales de pre- y pos-cosecha son revisados para identificar los problemas inherentes al control de *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae). El desarrollo de la resistencia a los insecticidas registrados y el riesgo potencial de daño a la exportación de frutas a través de protocolos de desinfección fríos hizo necesario la investigación de una estrategia alternativa, como la radiación ionizante para la supresión de este insecto en la poscosecha. Se evaluó el efecto agudo de 40-100 Gy de radiación ionizante en huevos de 24 a 96 horas de edad. Se estableció que los huevos incrementaban en radiotolerancia mientras que se desarrollaban y en 96 horas la mortalidad natural e inducida de huevos no irradiados y irradiados no fue muy diferente. La capacidad de vuelo y la oviposición de las polillas en desarrollo de huevos de 96 horas de edad irradiadas fueron totalmente impedídos a 70 y 50 Gy, respectivamente. Se evaluó una dosis de 50 Gy en los estadios 1 al 5. La radiotolerancia aumentó con los estadios, haciendo que el quinto estadio sea el más radiotolerante y el cual un tratamiento fitosanitario debe ser eficaz. Se utilizaron las dosis de radiación ionizante de 40-200 Gy para estimar la dosis efectiva más baja que es capaz para evitar un mayor desarrollo del 5º estadio, o alternativamente, que pueda suprimir totalmente la reproducción y/o la capacidad de vuelo de polillas desarrolladas de larvas tratadas. La dosis más baja para detener el desarrollo de las larvas y la esterilidad de las polillas fue 150 y 60 Gy, respectivamente. La capacidad de vuelo no podía ser totalmente suprimida con 70 Gy.

Palabras Clave: falso gusano de la manzana; biología de la radiación; radiosensibilidad; estados inmaduros; capacidad de vuelo; mortalidad; esterilidad

Thaumatotibia leucotreta (Meyrick) (Lepidoptera: Tortricidae) is considered indigenous to sub-Saharan Africa, the Ethiopian Region, and many islands off the African continent. The insect has a wide range of host plants and has adapted to cultivated crops from its original indigenous host plants. It infests citrus (Sapindales: Rutaceae), among other crops, in South Africa and is a pest of cotton (Gossypium spp.; Malvales: Malvaceae) and maize (*Zea mays* L.; Poales: Poaceae) in tropical Africa (Bloem et al. 2003).

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In the last 40 years *T. leucotreta* had been suppressed with a succession of chemical insecticides: in the late 1970s with larvicidal pyrethroids (Hofmeyr 1977, 1983) followed in the mid-1980s by ovicidal growth inhibitors in the benzoyl urea group (Hofmeyr 1984). The development of resistance by *T. leucotreta* to these insecticides (Hofmeyr & Pringle 1998) necessitated the use of flexible multi-tactic suppression programs. The tactics included strict orchard sanitation as a prerequisite (picking infested fruit and removing fallen fruit at least once a wk), in combination with mating disruption, egg parasitoids, or sterile insect releases (Hofmeyr et al. 2005; Carpenter et al. 2006; Moore & Hattingh 2012). These measures were supplemented when necessary with up to 4 granulosis virus cover sprays per season (Moore et al. 2004). Nonetheless, strict export regulations imposed by a number of countries importing fresh citrus from South Africa necessitated the development of postharvest procedures such as a cold disinfestation treatment (Hofmeyr et al. 1998; Hofmeyr & Hofmeyr 2005). Although effective, the cold protocol resulted in unpredictable and variable degrees of damage to fruit. Ionizing radiation was consequently considered to be a potential alternative treatment to ensure the maintenance of fruit quality and compliance with phytosanitary requirements.

Conventional postharvest control methods such as cold disinfestation and methyl bromide fumigation rely on achieving complete mortality of all treated immature and mature life stages of the target insect (USDA 2014). In contrast a radiation treatment's acute effects are often insufficient to ensure mortality of the treated stage. Instead, initial non-apparent physical damage is manifested later on, in the case of a treated immature usually in one or more of the subsequent life stages, or in the progeny when an adult is treated. This extended effect can result in malformed, debilitated and/or sterile individuals that would be eliminated as a commercial threat.

When the first studies commenced in 2002, the acute effect of 200–400 Gy was assessed on *T. leucotreta*. These doses prevented final (5th) instars to subsequently develop into moths (Hofmeyr, unpublished). Barry et al. (2007) examined the relative tolerance of various citrus fruit cultivars to radiation. A commercial 500 kCi cobalt-60 irradiator was used and dose uniformity ratios of 3.5:1 to 4:1 were encountered in pallets fully stacked with boxes of citrus fruit. It was concluded that 300 Gy would be the maximum dose that citrus fruit in general could absorb with safety. The highest dose absorbed by fruit in the centre of a palletized stack of boxes could therefore not exceed approximately 75 Gy. Complete suppression of *T. leucotreta* in oranges would thus have to occur at this dose or lower. Although 300 Gy was reported as viable for citrus fruit in general, there were subsequent concerns that the dose could be too high for certain cultivars such as lemon, white grapefruit and some mandarin types. A maximum dose of 160 Gy was consequently introduced for experimental purposes, reducing the lowest effective target dose to 40 Gy.

The research on *T. leucotreta* was conducted in 3 successive phases reported in different articles, namely (i) identification of the most radiotolerant life stage and age group of that particular life stage, (ii) establishing the validity of using artificially reared *T. leucotreta* for assessment purposes as an alternative to feral insects occurring in fruit, and (iii) treatment validation for phytosanitary compliance.

Oviposition occurs on the fruit surface and the eggs hatch within 4–5 d. Newly-hatched larvae penetrate into the pulp of the fruit and molt 4 times. The 5th instar invariably leaves infested fruit to pupate in the soil. At harvest only eggs and larvae occur on citrus fruit. Feral populations are poorly synchronized in oranges and eggs and larvae of all age groups can occur coincidently. It was consequently important to examine the effects of radiation on eggs and larvae of different ages to ensure that future research would be conducted using the most radiotolerant age. Here we report studies conducted with these 2 stages.

Materials and Methods

REARING *T. LEUCOTRETA*

Thaumatotibia leucotreta were reared at 26 °C on an artificial diet consisting predominantly of corn flour (Moore 2002). Wide-necked glass jars (500 mL volume; Consul Glass BN8175701, Stellenbosch, South Africa), each filled with 280 g of the medium, were used as rearing containers. Each jar was closed with a screw-on metal lid provided with a 40 mm diam aperture. A paper membrane (112 g/m^2, High Yield Fluting; Sappi Kraft, Milnerton, South Africa) was fitted into the lid to close the aperture, regulate moisture loss, and prevent putrefaction of the diet. Approximately 1,000 eggs, 24 h-old, on wax paper were inoculated into each jar and up to 800 5th instars were obtained per jar 12–14 d later. The larvae pupated in rolled-up strips of corrugated cardboard, 30 mm wide, which replaced the metal lids. The cardboard strips were pulled apart to collect the pupae or were put into emergence containers for moth eclosion and oviposition.

DOSIMETRY

A cobalt-60 point source, panoramic irradiator at the *T. leucotreta* rearing facility of Xsit (Pty) Ltd in Citrusdal, Western Cape, South Africa, was used. The targets were placed on 8 secondary turntables, each 0.3 m in diam, which were located on a primary turntable, 1.37 m in diam. The secondary and primary turntables rotated at 15 and 2 rpm, respectively.

The following irradiation containers were used:

Eggs. Oviposition occurs on the fruit surface only and the eggs were additionally considered to be radiolucent. An egg container manufactured from Nylon-6 was consequently used to provide sufficient dose build-up and ensure charged particle equilibrium. The container consisted of a solid, cylindrical inner core, or egg holder, measuring 120 mm high × 80 mm diam. A section, 105 mm wide × 1.5 mm deep, was removed around the core, creating space for a single wax paper sheet (295 mm × 100 mm) with eggs to be wrapped around. The egg holder was inserted into a tight fitting outer sleeve (135 mm × 100 mm OD × 81 mm ID, 9.5 mm wall thickness).

Larvae. Larvae were treated in the artificial diet in the glass rearing jars as the container sides provided sufficient dose build-up. The set-up for dosimetry and treatment applications consisted of a stack of 3 jars with larvae placed on as many of the secondary turntables as required.

The initial output factor for dose absorbed from the irradiator was conducted using Fricke dosimeters, applying the standard G-value for cobalt-60 of Fe^{3+} = 15.5/100 eV. Optical density readings were performed using a Hewlett Packard diode array spectrophotometer. For this instrument a molar extinction coefficient of 2,081 L/Mole/cm at 304 nm was determined using spectrosol grade Fe^{3+} solutions. This instrument specific constant relates optical density readings in a quartz glass flow cell with an optical path of 10 mm to [Fe^{3+}] concentrations, and is used to calculate dose absorbed values in Gy (J/kg). Different Fricke solutions read within 1% when irradiated in a cobalt-60 reference field. The dosimeter response was checked in a cobalt-60 reference radiation field of 300 mm × 300 mm with 2 mL Fricke solutions placed in a Perspex container producing 6 mm build-up and 50 mm backscatter. The output factor for the reference field was determined using a Farmer tissue equivalent ionization chamber calibrated in a standard field of the South African National Metrology Laboratory.

Mapping was conducted at the top and bottom of the irradiation containers, and the dose uniformity ratio was 1.03 for the egg container and 1.17 for the rearing jars. No reference position was used. The dosimetry was conducted for various positions in the irradiation set-up and the mean was calculated. Actual doses applied in the various experiments on eggs therefore have to be regarded as means ranging from 1.5% higher to 1.5% lower than the specified target dose. The applied doses for rearing jars varied 8.5% above and below the specified target dose. Dose rates were updated before each irradiation treatment using the decay half-life of cobalt-60 at 63.26 months.

Repeated readings taken on the same day at each position showed differences of less than 1% and less than 0.5%, respectively, for the egg container and rearing jars. Each radiation set-up was calibrated using a range of doses that included the radiation levels to be used. Uncertainties in the dose rate from repeated dose readings were less than 1% and 4% for the egg container and rearing jars, respectively. The 95% confidence interval of a reading at any position was 2% and 3%, respectively, for the egg container and rearing jars. All radiation treatments for calibration and experiments were done at 15 °C.

EXPOSURE OF EGGS TO IONIZING RADIATION

Radiotolerance of Progressively Older Eggs

Thaumatotibia leucotreta eggs were obtained from Xsit (Pty) Ltd. A4-sized wax paper sheets were exposed to not less than 1,500 mixed sex moths for oviposition. Eggs from the same oviposition batch were collected after 24 h and incubated at 26 °C for 48–96 h. The eggs were treated as they reached the required age. The wax paper sheets with eggs were divided in half lengthwise (hereafter "egg sheets") to fit into the radiation container. Two egg sheets were used per treatment, each containing approximately 4,500 eggs. Doses ranged between 40–100 Gy. Non-irradiated and irradiated eggs were incubated for 14 d to ensure complete hatch of all viable eggs before examination. A template with 5 randomly distributed sectors, each 20 mm × 20 mm, demarcated areas for counting eggs on each sheet. All eggs were counted in each sector and totalled from 860 to 1,160 between treatments. The eggs were assessed under a stereo-microscope and recorded as hatched or dead.

Delayed Effects of Egg Treatment on Subsequent Stages

Egg sheets with mature 96 h old eggs were treated with 40–100 Gy in 2 consecutive experiments. Immediately after treatment the sheets were cut into pieces containing 500–800 eggs each and inoculated into 10 and 5 rearing jars per treatment per consecutive experiment. The jars were incubated at 26 °C until the larvae had pupated. The larvae were not removed from the rearing jars to avoid injury resulting in unnatural mortality. The number of pupae collected in the control was used to estimate the number of treated larvae. The pupae and subsequent moths were collected and counted per jar. One newly eclosed F_1 female and male each were collected at random from the rearing jars of each experiment and placed into each of ten 100 mL plastic containers for oviposition. Adults developing from non-irradiated eggs were mated to each other, as were adults from irradiated eggs, except in experiment 1 where females from non-irradiated eggs were mated with males from eggs treated with 70 Gy. Oviposition was allowed for 7 d before the moths were removed. Each female was dissected and examined for spermatophores in the bursa copulatrix to confirm successful mating. To ensure complete hatch of all viable eggs the mating containers were incubated for 14 d before examination. An out-of-doors flight test was conducted in the first experiment. Depending on availability, 103–177 moths were used in different treatments. Moths were released individually from a height of 2.5 m above ground level. Those that flew normally compared with non-irradiated control moths were considered to be flight capable. Moths unable to gain height and descending involuntarily, i.e., unable to maintain flight, or falling directly to the ground, were regarded as unable to fly.

EXPOSURE OF LARVAE TO IONIZING RADIATION

Different instars can occur concurrently in pre- and postharvested fruit. The effect of radiation on the various instars consequently had to be examined to ensure that research would be conducted using the most radiotolerant age group.

Radiotolerance of Different Instars

In 3 experiments 4 to 8 jars per instar category were used per treatment, viz., 1,600 to 3,200 larvae per treatment. The rearing jars were allocated at random ensuring comparable numbers of insects in each treatment. Forty larvae were collected randomly from an additional randomly selected rearing jar in each experiment. The instar category was verified with head capsule measurements (Daiber 1979) using an electronic caliper. Rearing jars were selected for 3rd, 4th, and 5th instars and treated with doses ranging between 200–400 Gy in 50 Gy increments (experiments 1 and 2). In the third experiment, 1st, 3rd, and 5th instars were selected and treated with a single dose of 50 Gy. The following factors were assessed:

Larval Mortality. The numbers and natural mortality of the larvae in the diet could not be established in situ or be removed from a jar, counted, and replaced without injury resulting in unnatural mortality. Larvae in the control and treatments were therefore allowed to pupate and the pupae were collected and counted. The rearing jars were allocated at random and the number of pupae developing in the untreated control is therefore comparable to the number of larvae that would have pupated in the irradiated jars in the absence of treatment.

Pupal Mortality. The pupae from different jars were combined into one batch per treatment. From each batch 160 pupae were collected at random and placed individually into glass vials, 50 mm × 15 mm, with foam rubber stoppers. All moths eclosing from the 160 pupae were sexed and dead pupae were recorded.

Reproductive Potential: Ten pairs of moths chosen at random from the 160 pupae were used to assess fecundity and fertility as described above in the egg study. The 4 possible mating combinations were assessed:

- females and males both developing from non-irradiated larvae,
- females and males both from irradiated larvae,
- females from non-irradiated larvae and males from irradiated larvae, and
- females from irradiated larvae and males from non-irradiated larvae.

Flight Test: All moths eclosing from the remainder of the 160 pupae were used for out-of-doors flight tests conducted as described above in the egg study.

Direct and Delayed Effects of Ionizing Radiation on 5th Instars

Five experiments were conducted to confirm results of the previous studies and determine a treatment dose that could be validated with much larger numbers of test insects. Three to 8 rearing jars were used per treatment in the different experiments, viz., 1,200 to 3,200 larvae per treatment. Larvae treated with 40–200 Gy were incubated and the pupae were collected and placed into emergence containers for moth eclosion. Assessments similar to those discussed in the previous section were conducted.

Table 1. Acute effect of ionizing radiation on percent hatching of progressively older *Thaumatotibia leucotreta* eggs.

	Mean ± SE hatched eggs (%)			
	Age of eggs when treated			
Dose (Gy)	24 h	48 h	72 h	96 h
0	76.2 ± 1.2	—	—	—
40	4.1 ± 1.1	50.5 ± 1.0	61.4 ± 2.8	64.9 ± 1.1
60	0.0	45.1 ± 1.1	60.7 ± 2.6	65.1 ± 1.9
70	0.0	44.4 ± 1.4	55.0 ± 0.8	64.3 ± 0.7
85	0.0	33.7 ± 2.0	53.0 ± 1.8	62.5 ± 1.6
100	0.0	21.0 ± 0.8	50.6 ± 1.2	61.7 ± 1.1

Results

EXPOSURE OF EGGS TO IONIZING RADIATION

Radiotolerance of Progressively Older Eggs

Seventy six percent of eggs eclosed in the non-irradiated control (Table 1). In various past studies with non-irradiated eggs, the natural mortality had consistently been higher on egg sheets than on fruit, on average 15–25% on egg sheets compared with 3–8% on fruit (Hofmeyr, unpublished). Eggs are mostly deposited in clusters on wax paper or any other smooth surface. Eggs in these clusters are frequently partially or fully overlapped by other eggs. Many more dead eggs are recorded in the clusters than when they are typically laid discretely in small indentations texturing the rind of oranges.

Dose effects were noticeable and egg hatch decreased as the dose was increased (Table 1). There was a strong age related response to treatment; up to 24 h-old all eggs were killed by all but the lowest dose of 40 Gy (Table 1). The percent eclosion of 48-96 h eggs increased progressively and was somewhat similar for all treatment doses at 96 h; from a practical point of view it was not much different from egg hatch in the untreated control, differing only by 11.1–14.5%. At 96 h larvae with heavily sclerotized head capsules were noticeable in the transparent egg shells and they would eclose in a few h.

ACUTE AND DELAYED EFFECTS OF EGG TREATMENT ON SUBSEQUENT STAGES

Acute Effect

Ninety six h-old eggs treated with 40–70 Gy eclosed, and 5th instars eventually pupated (Table 2, experiment 1). The number of pupae and moths decreased progressively with increased dose. The numbers of malformed pupae with, inter alia, pupal cases resisting normal splitting and preventing moth eclosion increased commensurately. The 60 Gy and 70 Gy treatments in particular resulted in many dead, partially eclosed moths with pupal cases still attached. Malformation was common in the surviving moths displaying wing aberrations and an inability to stay upright or move around. The survivors were predominantly male. Relatively few of the larvae that developed from the eggs treated with 100 Gy were ultimately able to pupate (Table 2, experiment 2). Virtually all these pupae died, and only 10 severely malformed males eclosed. In all treatments the general physical condition of the moths, including many that showed no obvious symptoms of malformation, was detrimentally affected and resulted in impaired flight ability (Table 2, experiment 1). Only 3 moths of 177, 1 moth of 116 and none of 103 moths, respectively, could fly in the 50, 60, and 70 Gy treatments, respectively.

Reproductive Capability

The reproductive potential of the moths from irradiated eggs was severely affected (Table 3). As the dose rate was increased the moths' mating ability decreased and the females that mated were decreasingly fecund and increasingly sterile. A dose of 40 Gy could not prevent the production of fertile eggs. At 50 Gy and above fecundity was completely suppressed. The few females that developed from irradiated eggs in the 70 Gy treatment were too malformed and lethargic to attempt a mating study. Males from this treatment were consequently paired with non-irradiated control females. No mating occurred and no eggs were produced, indicating a total absence of male reproductive potential. The reproductive capability of moths in the 100 Gy treatment could not be assessed due to the acute effects discussed above.

EXPOSURE OF LARVAE TO IONIZING RADIATION

It was accepted from the various mating combinations assessed that matings between moths from irradiated larvae would be the only probable outcome if *T. leucotreta* escaped from consignments of fruit in an environment free from the pest. These data only are presented.

Radiotolerance of Different Instars

In experiments 1 and 2 trends indicated increased radiotolerance as the larvae molted from 3rd to 5th instar. However, the selected doses of 200–400 Gy resulted in larval mortalities too great for differences between instars to be adequately emphasized.

Head capsule measurements in experiment 3 indicated that the larvae selected for the experiment were virtually all correct for their respective instar categories, namely 1st instar (100%), 3rd instar (100%)

Table 2. Delayed effects of ionizing radiation on *Thaumatotibia leucotreta* pupae and moths from 96 h old irradiated eggs.

		Mean ± SE pupae[a]		Mean ± SE moths[a]		
Exp. no.	Dose (Gy)	Produced (%)	Malformed (%)	Produced (%)	Males (%)	Moths able to fly (%)
1	0	[2,014][b]	0.0	98.4 ± 0.1	50.8	90.4
	40	92.6 ± 37.2	1.9 ± 0.7	20.3 ± 2.9	55.8	33.0
	50	72.1 ± 8.7	5.2 ± 0.9	13.7 ± 0.8	65.0	1.7
	60	67.9 ± 16.8	13.9 ± 1.3	9.7 ± 0.8	79.7	0.9
	70	51.5 ± 13.3	23.2 ± 1.3	4.0 ± 1.1	96.8	0.0
2	0	[3,447][b]	—	96.1 ± 0.4	47.6	—
	100	10.3	—	0.3 ± 0.1	100.0	—

[a]Relative to 0 Gy control.
[b]Number of pupae from untreated eggs in [brackets].

Table 3. Delayed effects of ionizing radiation on the reproductive potential of *Thaumatotibia leucotreta* moths from 96 h old irradiated eggs. Moths from non-irradiated and irradiated eggs were assessed separately.

		Reproductive potential		
Exp. no.	Dose (Gy)	Mated ♀♀ (*n* = 10)	Mean ± SE no. of eggs/♀	Mean ± SE egg hatch (%)
1	0	10	281.8 ± 37.9	83.8 ± 2.3
	40	4	44.8 ± 33.0	4.9 ± 1.1
	50	1	0.0	0.0
	60	0	0.0	0.0
	70	0[a]	0.0	0.0
2	0	10	307.7 ± 21.0	85.1
	100	No ♀♀	0.0	0.0

[a]Females and males from non-irradiated and irradiated eggs respectively.

and 5th instar (90%, the remainder being 4th instar). Larval and pupal mortality decreased from 1st to 5th instar and in the latter instar there was little difference in either larval or pupal mortality between irradiated and non-irradiated 5th instar larvae (Table 4). Approximately 3% of the pupae from irradiated larvae, regardless of instar, were malformed compared to less than 0.5% of pupae from non-irradiated larvae. Obviously malformed moths were absent but flight ability was nonetheless detrimentally affected, showing an instar-related response (Table 4). Mating incidence, fecundity, and fertility of moths increased as progressively later instars were treated (Table 5). The relatively large percentage of dead eggs in the controls (Table 5) is attributed to egg clustering.

This study presented sufficient proof that 5th instars were the most radiotolerant stage and justified their use in supplementary studies. This agrees with Hallman et al. (2010) who reviewed the literature and found that radiotolerance increases with development, meaning that the most radiotolerant stage for phytosanitary irradiation would be the most developed stage that could be found in the exported commodity.

Direct and Delayed Effects of Ionizing Radiation on 5th Instars

These experiments were comprised of 76% to 97% of 5th instars; the remainder being 4th instars. The effects of any particular treatment were similar across repetitions and yielded comparable conclusions. The lowest dose (40 Gy) had little acute effect on larvae (Table 6). However, mortality increased commensurate with dose increases and, apart from a few short-lived, malformed individuals intermediate between larva and pupa, all 5th instars were prevented from pupating by a dose of 200 Gy. Pupal mortality increased similarly, and at ≥ 125 Gy no moth eclosed (Table 6). The gender ratio was little affected from 40 Gy to 75 Gy. Higher doses increased the mortality of female pupae compared to male pupae, as reflected in a ratio of 1 female moth to 9 male moths at 100 Gy (Table 6). The flight ability of moths could not be totally eliminated with doses of 40-70 Gy (Table 6).

Table 4. The development of *Thaumatotibia leucotreta* pupae and flight ability of moths from 1st to 5th instar treated with 50 Gy of ionizing radiation.

Instar treated	Larval mortality (Mean ± SE %)[a]	Pupal mortality (%)[a]	Moths able to fly (%)[a]
1st	19.6 ± 5.6	16.0	12.6
3rd	16.8 ± 8.4	9.9	29.8
5th	1.0 ± 2.8	0.7	65.9

[a]Relative to respective controls.

Table 5. The fecundity and fertility of *Thaumatotibia leucotreta* moths from 1st to 5th instars treated with 50 Gy of ionizing radiation.

Dose (Gy)	Instar treated	No. of mated ♀♀(max. 10)	Mean ± SE no. eggs per mated ♀	Hatched eggs (%)
0	1st	10	446.1 ± 30.9	86.3
	3rd	10	443.3 ± 20.3	87.9
	5th	10	468.7 ± 54.1	86.2
50	1st	3	0.0	—
	3rd	8	1.6 ± 0.9	0.0
	5th	9	19.4 ± 13.1	90.3

Malformation of pupae and moths was recorded in 3 experiments. Up to 70 Gy, pupal malformation was not observed, but increased to 60% at 100 Gy. At the same dose 94% of moths were malformed, females more so than males; this result was also reported by Bloem et al. (2003). The abnormalities included bloated bodies, warped wings, and an inability to move around. Moths were often unable to shed their pupal cases completely resulting in partial eclosion.

Successful mating was slightly suppressed by 40 Gy and 50 Gy treatments. Doses ≥ 60 Gy sharply decreased mating occurrence (Table 7). Egg eclosion in the controls was normal for oviposition on wax paper sheets. Fecundity was sharply reduced by 40 Gy, but a relatively large percentage of the eggs that were produced eclosed. Both factors were progressively more affected as the dose was increased, and at 60 Gy only a few eggs were deposited and none hatched. At 70 Gy and above fecundity was totally suppressed. A dose level of approximately 60 Gy can therefore provisionally be regarded as the lowest effective radiation dose for 5th instar *T. leucotreta* provided the result can be confirmed when much larger numbers of insects are treated.

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Table 6. The development of *Thaumatotibia leucotreta* pupae and moths from 5th instars treated with ionizing radiation.

	Mean ± SE %			
Dose (Gy) applied to 5th instars	Larval mortality	Pupal mortality	♂ Moths (%)	Moths able to fly (%)
0	[413.9][a]	11.8 ± 1.7	51.6	90.8
40	0.8 ± 4.4	16.6 ± 4.9	—	52.6
50	9.8 ± 2.1	—	52.1	34.6
60	12.8	—	—	4.2
70	14.4	52.7	—	5.9
75	29.9	96.5	59.8	—
100	47.5 ± 7.9	82.2 ± 4.4	90.2	—
125	63.1	98.6	100.0	—
150	80.4	100.0	No moths	—
200	100.0	No pupae	No moths	—

[a]Mean number of untreated larvae per rearing jar in [brackets].

Table 7. The reproductive potential of *Thaumatotibia leucotreta* moths from 5th instars that were treated with ionizing radiation. Ten pairs of moths chosen at random from 160 pupae were used to assess fecundity and fertility.

Dose (Gy) applied to 5th instars	No. of mated ♀♀ (*n* = 10)	Mean ± SE no. of eggs per ♀	Mean ± SE egg hatch (%)
0	10	442.7 ± 18.1	83.2 ± 2.7
40	8	89.3 ± 19.8	39.5 ± 9.9
50	8	11.1 ± 2.2	15.3 ± 4.5
60	1	3.4	0.0
70	1	0.0	0.0
75	2	0.0	0.0
100	0	0.0	0.0

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