

Effects of X-rays on *Tuta absoluta* for use in inherited sterility programmes

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Abstract *Tuta absoluta* is a key pest of tomato crops originating from South America. The consequences of X-radiation on this species were studied under laboratory controlled conditions. The effect of radiation on adult emergence was evaluated exposing male and female pupae to increasing X-rays. Adult emergence decreased as doses of X-radiation increased, with the appearance of deformities such as malformed wings and bent legs at doses ≥ 350 Gy. Besides, males and females obtained from irradiated pupae were out crossed with untreated counterparts to explore the effects of X-radiation on inherited sterility. (a) Irradiated male \times untreated female crosses. Both fecundity and fertility of the untreated females were reduced by radiation, and the effect was stronger as the doses increased. Neither the longevity of parental males and F1 adults nor the sex ratios of the F1 and F2 generations were affected by X-radiation (F1 and F2: first and second generation of descendants of irradiated adults). Inherited sterility effects were manifested by a significant reduction in the F1 fecundity, F1 fertility, and the amount of larvae and pupae produced. Doses of 200–250 Gy could be used to induce inherited sterility in *T. absoluta* males. (b) Untreated male \times irradiated female crosses. The minimum dose at which irradiated

females were completely sterile was 200 Gy. The present study is the first study in *T. absoluta* that provides the starting point for implementing the inherited sterility in this species.

Keywords South American tomato pinworm · X-radiation · Inherited sterility · Sublethal effects

Introduction

Tuta absoluta (Meyrick 1917) (Lepidoptera: Gelechiidae) is an important tomato pest native to the Neotropics (Desneux et al. 2010). This oligophagous species attacks eggplants, potatoes, and especially tomatoes, feeding on their leaves, stems, apices, flowers, and fruits throughout the entire growing cycle (Botto et al. 2000). This moth was only present in South American countries until 2006 when it was detected in Spain. The present study is important given the exceptional speed and extent of *T. absoluta* invasion. It rapidly spread over several European countries causing enormous yield losses of up to 100 % and represent a serious threat to the Afro-Eurasian countries (Desneux et al. 2010, 2011).

The main method of control of *T. absoluta* relies on the application of chemical insecticides (Desneux et al. 2010; Haddi et al. 2012). Their intensive use has led to the development of resistant populations to some of the recommended insecticides in Brazil (de Siqueira et al. 2000), Chile (Salazar and Araya 1997), and Argentina (Lietti et al. 2005).

One of the pest control options that does not develop resistance and has also no significant negative impact on the environment is the Sterile Insect Technique (SIT). SIT involves the use of radiation to sterilize insects and then release them into a wild population of the same species so

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that they are able to mate and prevent the reproduction of wild females (Knippling 1955). Historically, sterilization has used γ radiation with a high radioactive material dependency. Nowadays, this type of irradiators have several difficulties to be purchased because the Nordion INC company (formerly MDS INC) stopped the production of self-shielded cobalt-60 irradiators and by the difficulties related to the transportation of Co sources (Mastrangelo et al. 2010; Mehta and Parker 2011). On the contrary, the X-irradiators do not depend on a radioactive source and they are easier and safer to obtain than the cobalt-60 ones.

SIT programs have been successful against a number of Diptera pests (Bloem and Carpenter 2001). However, Lepidoptera species are more resistant to radiation compared to most other insects (LaChance 1967). The radioresistance in Lepidoptera can be attributed to their holokinetic chromosomes and to the large kinetochore plate to which the spindle microtubules attach during cell division (LaChance 1967; Wolf et al. 1997; Tothová and Marec 2001). It covers a significant portion of the chromosome length (Wolf 1996), insuring that most radiation-induced breaks will not lead to the loss of chromosome fragments. The fragments are stable during both mitosis and meiosis, and can even be transmitted through germ cells to the next generation (Marec et al. 2001; Carpenter et al. 2005). On the contrary, dipterous have a typical monocentric chromosome with the sister chromatids joined by the centromere where the kinetochore is localized. The radiation-induced chromosome fragments are unstable leading to the loss of chromosomal parts. Due to the high radioresistance in Lepidoptera species, higher doses for full sterilization are required. However, these high doses of radiation not only sterilize the moths but also reduce their ability to compete with wild moths. This last undesirable effect can be avoided using sub sterilizing doses that do not affect the quality and competitiveness of irradiated males (North 1975) while they produce completely sterile females because they are generally more sensitive to radiation than males (Proverbs 1962; LaChance 1985; Carpenter et al. 2001). When partially sterile males mate with wild fertile females their deleterious effects induced by radiation are inherited and expressed for several generations, mainly in the F1 generation (the first generation of descendants of irradiated adults) (Carpenter et al. 2005). In the F1, egg hatching is reduced and the resulting offspring is both highly sterile (more than the irradiated parent moths) and predominantly male (Bloem and Carpenter 2001; Seth and Sharma 2001).

The use of inherited sterility (IS) or F1 sterility is considered a key strategy in management programs for Lepidoptera pests and it should be based on the area-wide concept of integrated pest management (AW-IPM) (Carpenter et al. 2005). Inherited sterility is compatible with the use of other area-wide control tactics such as mating disruption, biological control, cultural control methods, and

the use of bio-rational pesticides (Carpenter et al. 1987; Bloem and Carpenter 2001; Carpenter et al. 2005). Field releases of partially sterile insects have demonstrated that some Lepidoptera species like the cabbage looper *Trichoplusia ni* (Hübner), the corn earworm *Helicoverpa zea* (Boddie), the gypsy moth *Lymantria dispar* (L.), and the codling moth *Cydia pomonella* (L.) could be controlled with this technique (North and Holt 1969; Proverbs et al. 1978; Carpenter et al. 1987; Carpenter and Gross 1993; Mastro 1993; Bloem et al. 1999, 2001).

Little is known about the effect of radiation on *T. absoluta*. The only study that analysed the effect of gamma rays on its different stages was made by Arthur (2004), to develop a quarantine treatment for packaged tomatoes. Thus, and due to the economic relevance of *T. absoluta*, it is important to assess the viability of IS as a control technique. Our goal was to study the effects of different doses of X-radiation on the emergence, fecundity, and fertility of *T. absoluta* with the purpose of determining the dose at which females were sterile and males were partially sterile.

Materials and methods

Laboratory colonies

The research was carried out at the Insectario de Investigaciones para Lucha Biológica (IILB), IMYZA, INTA (Castelar, Buenos Aires, Argentina). Eggs and larvae of *T. absoluta* were collected on tomato plants from local greenhouses to establish a colony. Insects were reared on tomato plants for about 30 generations and they were periodically refreshed with new insects from local greenhouses. The colony was maintained in a controlled environment room at 22–30 °C and 60–85 % relative humidity. The voucher specimens were preserved in the IILB collections.

In the following studies, the insects were allowed to develop in a room with controlled environmental conditions: maximum temperature: 25.5 ± 0.2 °C, minimum temperature: 20.8 ± 0.2 °C, maximum relative humidity: 78.9 ± 1.0 %, minimum relative humidity: 56.2 ± 3.1 %, and photoperiod: 14L:10D. These environmental conditions were measured with a TFA digital thermo-hygrometer model 30.5003 (Dostmann GmbH & Co.KG, Germany).

First study: effect of X-rays on adult emergence

Pupae obtained from the rearing of *T. absoluta* were separated by sex and randomly assigned to the treatments. The position of the genital opening (in the eighth abdominal segment for females and in the ninth for males) was the

diagnostic method for sex assessment in *T. absoluta* pupae (de Coelho and Franca 1987).

Eight pupae ($n = 8$) of each sex (48–72 h before adult emergence) were placed in a Petri dish (1.5 cm high and 5.5 cm in diameter) and irradiated using a Constant Potential X-Ray System Mg 160 Philips at a dose rate of 0.679 Grays/Second (Gy/s). The characteristics of the irradiator were anode voltage 120 kVp, current 15 mA, beam quality Cx120 approx. 0.07 mm aluminum (at 160 kV). Dosimeter PTW model UNIDOS® E (calibration was made with secondary standards dosimetry (CRRD) by regional reference center located in Ezeiza Atomic Center). It was calibrated by the method of substitution in terms of air kerma by the supplier with the expanded 1.2 % uncertainty in the calibration factor. Traceability to last calibration is 14 June 2011. The ion chamber was Farmer-Type Ionization chamber volume 0.6 cm³ (TN30010-2339 PTW-Freiburg) with the following characteristics: Measuring Quantity: Air Kerma (Kair), Calibration Factor: $N_k = 4.938 \times 10^7$ Gy/C.

The treatments were the radiation doses applied (minimum–maximum dose/treatment) = 0 (control), 49.68–50, 99.1–100, 149.05–150, 198.73–200, 248.42–250, 298–300 and 347.78–350 Gy. From now on, doses would be referred as 0, 50, 100, 150, 200, 250, 300, and 350 Gy. Four to five Petri dishes (replicates) were used for each sex-dose combination. They were exposed sequentially and the arrangement of the dishes was centered in relation to the radiation field. The effect of X-radiation on both male and female emergence was evaluated considering the following variables: (1) complete adult emergence estimated as the proportion of adults that emerged completely/total irradiated pupae, and (2) proportion of deformed individuals estimated as the deformed individuals/total adults that emerged completely. Complete adult emergence refers to adults that emerged from the pupae without any problem; it includes both normal and deformed adults but exclude those that remained stuck to their pupal skin.

Since the complete adult emergence is a binary response variable (success or failure), the proportion of complete adult emergence was analysed by a logistic regression with dose and sex as independent variables. The significance of model parameters was checked by Wald statistics and model fit by likelihood-ratio tests using the generalized linear model procedure in Statistica for Windows (Agresti 1996; StatSoft 2000).

Second study: effect of X-rays on irradiated males and inherited sterility

Completely emerged males obtained from pupae exposed to increasing doses of X-radiation (48–72 h before emergence), were crossed with untreated females, establishing

the parental generation (F0). For each dose level (0, 100, 150, 200, 250, and 300 Gy) four couples (replicates) were individually placed in a cylindrical acetate cage (24.5 cm high and 7.5 cm in diameter) containing a tomato plant. The plant was renewed twice at 5-day intervals. The first two plants used were carefully inspected for the eggs laid by each female during the first 10 days of its lifespan. This procedure guaranteed that about 90 % of the fecundity is recorded, since after females are 10 days-old the number of eggs/female/day decays to zero (Pereyra and Sánchez 2006). The third plant was kept until both adults were dead. Adult longevity was then recorded.

Tomato plants with eggs were individually placed on humid tissue paper in rectangular plastic containers (26 cm long, 18 cm wide and 5 cm high). After 7 days, F1 larvae (first and second instars) were counted and placed on a new tomato plant in a cage of acetate (39 cm high and 22 cm in diameter, with a plastic floor and fine mesh net in the upper side) until pupation. The pupae were counted and sexed.

Fecundity (total eggs laid/female), longevity of adults, fertility, number of F1 larvae/female, number of F1 pupae/female, and F1 sex ratio (number of males/total individuals) were recorded for all doses. The effect of radiation dose on the fecundity, longevity and number of F1 larvae and pupae were analysed by a Negative Binomial regression for count data using the generalized linear model procedure in STATGRAPHICS Centurion XVI v16.1.17 (StatPoint Technologies, Inc. 1982–2011). The significance of model parameters was checked by Wald statistics and model fit by likelihood-ratio tests.

Fertility was estimated as the proportion of emerged larvae from the recorded eggs (number of larvae/number of eggs). This resulted in an underestimation as the chorion of *T. absoluta* egg is very small and they broke and lost among the hairs of the leaves when larvae emerged. Since fertility and sex are binary response variables, the proportion of emerged larvae from the eggs and sex ratio were analysed by a logistic regression with dose as the independent variable. The significance of model parameters was checked by Wald statistics and model fit by likelihood-ratio tests (StatSoft 2000).

In order to analyze the inherited effects, emerging F1 adults were used for each dose level in two different types of crosses: $M_{F1} \times F_U$ and $M_U \times F_{F1}$ (where M_{F1} = male descendent from an irradiated male, M_U = unirradiated male, F_U = unirradiated female, and F_{F1} = female descendent from an irradiated male). Four replicates were carried out for each cross. F1 adult couples were held on a tomato plant in a cage as described above. F1 fecundity, longevity of adults, fertility, number of F2 larvae/female, number of F2 pupae/female, and F2 sex ratio (number of males/total individuals) were recorded for all crosses. The effect of dose radiation and cross type on the F1 fecundity and longevity and number of F2 larvae and pupae were analysed by a

Table 1 Effects of X radiation on the parental generation (Irradiated female × Untreated male) and on their F1 progeny

Dose (Gy) ^a	Crosses (F0 Male × F0 female)	Number of F1 larvae/female (Mean ± SE)	Number of F1 pupae/female (Mean ± SE)
0 (8)	U × U	57.63 ± 4.39	47.63 ± 4.78
150 (8)	U × I	0.75 ± 0.49	0
200 (10)	U × I	0	0
250 (3)	U × I	0	0

Gy Gamma, I irradiated, U unirradiated, F0 parental, F1 first generation of descendants of irradiated adults

^a Radiation was administered with a Constant Potential X-Ray System Mg 160 Philips at a dose rate of 0.679 Gy/s

Negative Binomial regression for count data using the generalized linear model procedure in STATGRAPHICS Centurion XVI v16.1.17 (StatPoint Technologies, Inc. 2011). Fertility and sex ratio were analysed by a logistic regression with dose and cross type as independent variables. The significance of model parameters was checked by Wald statistics and model fit by likelihood-ratio tests (StatSoft 2000).

Third study: effect of X-rays on irradiated females

Completely emerged adult females, obtained from pupae exposed to increasing doses of X-radiation 48–72 h before emergence, were crossed with untreated males, establishing the parental generation (F0). Three to ten replicates were carried out for the doses applied to the pupae (Table 1). Adult couples were held on a tomato plant in a cage as described above.

F0 fecundity, longevity of adults, F0 fertility, number of F1 larvae/female, and number of F1 pupae/female were recorded for all crosses. F0 Fecundity, longevity of adults, F0 Fertility, number of F1 larvae/female, and number of F1 pupae/female were recorded for all crosses. Data of F0 fecundity and longevity were analysed by a Negative Binomial regression for count data (StatPoint Technologies, Inc. 1982–2011). Fertility was analysed by a logistic regression with dose as independent variable. The significance of model parameters was checked by Wald statistics and model fit by likelihood-ratio tests using the generalized linear model procedure in Statistica for Windows (StatSoft 2000).

Results

First study: effect of X-rays on adult emergence

The proportion of complete adult emergence decreased significantly with the increase of the X-radiation (Wald =

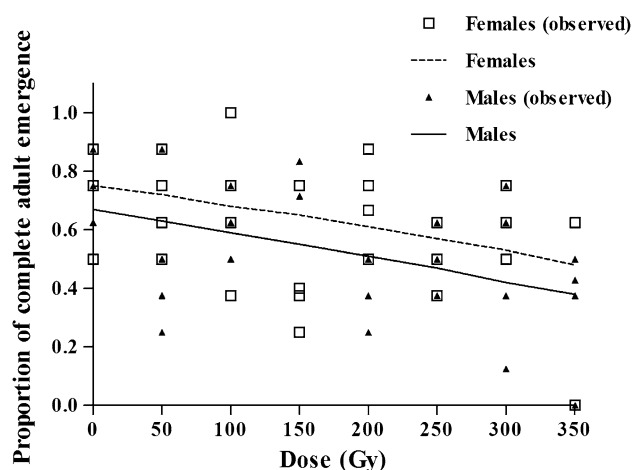


Fig. 1 Proportion of complete adult emergence (number of adults that completely emerged/total number of individuals) in the emergence study

19.81; $P < 0.05$; $\chi^2_{(1)} = 20.64$; $P < 0.05$) (Fig. 1). For the same dose of radiation, the effect depended on the sex of the individual, being the female emergence higher than that of males (Wald = 5.52; $P < 0.05$; $\chi^2_{(1)} = 5.54$; $P < 0.05$). It was also observed that with 350 Gy some adults presented deformities such as malformed wings and bent legs.

Second study: effect of X-rays on irradiated males and inherited sterility

Fecundity of *T. absoluta* females mated with irradiated males decreased significantly with increasing doses of radiation (Wald = 1,590.17; $P < 0.05$; $\chi^2_{(1)} = 42.88$; $P < 0.05$). However, when the highest tested dose (300 Gy) was excluded of the analyses, there was no significant relationship between fecundity and dose (Wald = 1.90; $P > 0.05$; $\chi^2_{(1)} = -1.95$; $P > 0.05$). That means that females mated with males exposed to the highest dose failed to lay a normal number of eggs (8.25 ± 2.56). Due to 300 Gy affected the mating ability of treated males, this dose was not considered useful for IS and was not taken into account in the following analyses (Fig. 2a).

Longevity of treated males (Wald = 2.26; $P > 0.05$; $\chi^2_{(1)} = 0.51$; $P > 0.05$) was not affected by the application of radiation. The mean longevity was 17 ± 1 days. On the other hand, the dose affected significantly the fertility in the F0 generation (Wald = 101.84; $P < 0.05$; $\chi^2_{(1)} = 112.79$; $P < 0.05$) (Fig. 3). A significant reduction in the number of F1 larvae and pupae was observed when pupae of parental males were irradiated with increasing doses of X-rays (Wald = 388.31; $P < 0.05$; $\chi^2_{(1)} = 24.02$; $P < 0.05$ and Wald = 225.75; $P < 0.05$; $\chi^2_{(1)} = 20.54$; $P < 0.05$, respectively) (Fig. 2b, c). There were no significant differences in the F1 sex ratio among doses

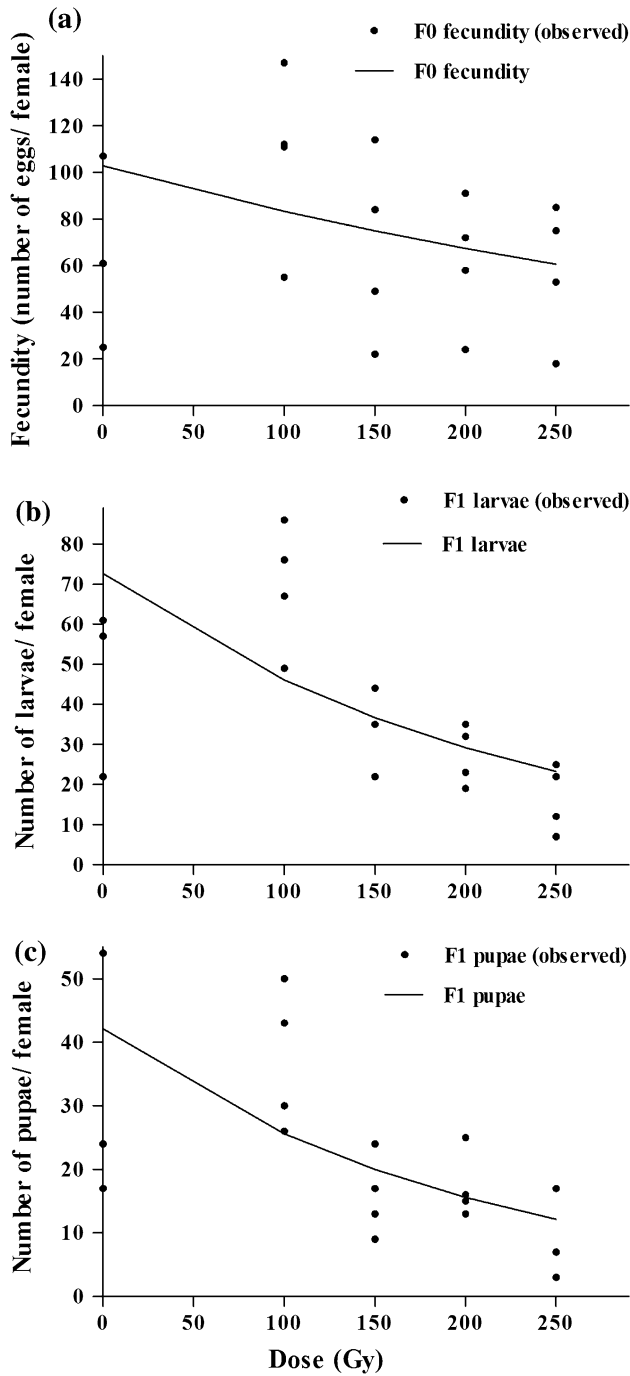


Fig. 2 **a** Parental fecundity (number of eggs/female), **b** number of F1 larvae and **c** number of F1 pupae when *T. absoluta* males were irradiated as pupae and mated with untreated females. *F0* parental generation, *F1* first generation of descendants of irradiated males

(Wald = 2.92; $P > 0.05$; $\chi^2_{(1)} = 2.96$; $P > 0.05$). The proportion of males was 0.66 ± 0.036 .

Since the number of pupae obtained from males irradiated with 250 Gy was very low (8.5 ± 2.99), and females

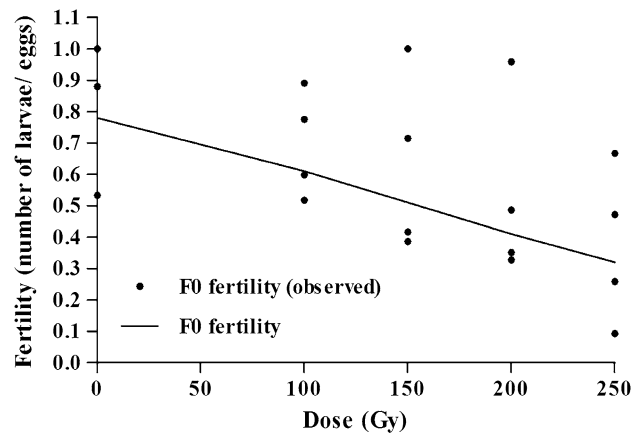


Fig. 3 Parental fertility (number of larvae/number of eggs) when *T. absoluta* males were irradiated as pupae and mated with untreated females

did not emerge from the pupae, this dose was discarded to analyze the F2 progeny. Fecundity of F1 adults mated with untreated counterparts was affected by the dose of X-radiation applied to the F0 male (Wald = 911.80; $P < 0.05$; $\chi^2_{(1)} = 107.31$; $P < 0.05$) and the type of F1 mating ($M_{F1} \times F_U$ or $M_U \times F_{F1}$) (Wald = 141.10; $P < 0.05$; $\chi^2_{(1)} = 26.01$; $P < 0.05$) (Fig. 4a). F1 Fecundity decreased with the dose but daughters of irradiated males laid more eggs than females mated with the sons of irradiated males regardless of the dose applied.

The dose was the only factor that affected significantly the fertility in the F1 generation (Wald = 217.70; $P < 0.05$; $\chi^2_{(1)} = 268.35$; $P < 0.05$), while the type of crossing did not have significant effects and was removed from the analysis (Wald = 1.19; $P = 0.27$; $\chi^2_{(1)} = 1.14$; $P > 0.05$) (Fig. 5). There were no significant differences in F1 adult longevity among treatments (Wald = 2.46; $P > 0.05$; $\chi^2_{(1)} = 1.40$; $P > 0.05$ for dose and Wald = 3.53; $P > 0.05$; $\chi^2_{(1)} = 2.01$; $P > 0.05$ for type of crossing). The mean longevity was 14 ± 1 days. The total number of F2 larvae decreased significantly when doses applied to F0 males increased (Wald = 930.30; $P < 0.05$; $\chi^2_{(1)} = 210.29$; $P < 0.05$) and, according to F1 fecundity results, it was affected by the type of F1 crossing ($M_{F1} \times F_U$ or $M_U \times F_{F1}$) (Wald = 29.79; $P < 0.05$; $\chi^2_{(1)} = 4.12$; $P < 0.05$) (Fig. 4b). Moreover, a similar result was obtained for the total number of F2 pupae (Wald = 673.18; $P < 0.05$; $\chi^2_{(1)} = 116.77$; $P < 0.05$ for dose and Wald = 61.26; $P < 0.05$; $\chi^2_{(1)} = 5.55$; $P < 0.05$ for type of crossing) (Fig. 4c). Doses did not affect the F2 sex ratio (Wald = 1.08; $P > 0.05$; $\chi^2_{(1)} = 1.71$; $P > 0.05$) and there were no significant differences in this variable among types of crossing (Wald = 0.53; $P > 0.05$; $\chi^2_{(1)} = 0.62$; $P > 0.05$). The proportion of males was 0.49 ± 0.042 .

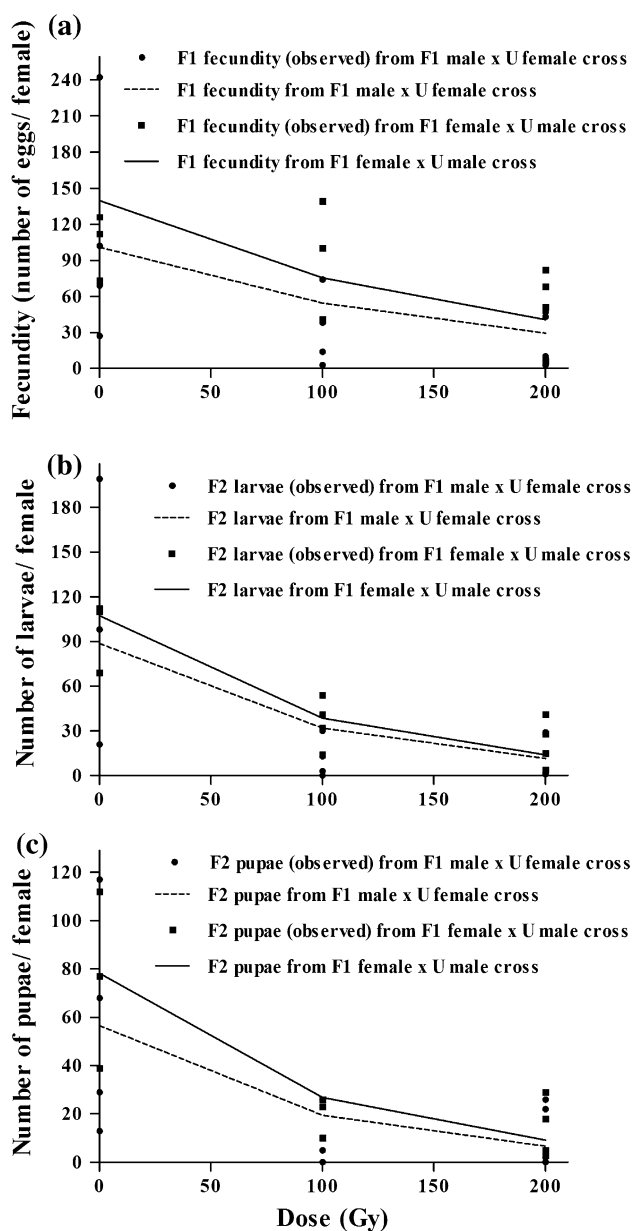


Fig. 4 For both types of F1 crosses (F1 male \times untreated female and untreated male \times F1 female), **a** Fecundity of the F1 progeny (number of eggs), **b** number of F2 larvae and **c** number of F2 pupae. U unirradiated, F1, F2 first and second generation of descendants of irradiated adults

Third study: effect of X-rays on irradiated females

The fecundity of irradiated females was significantly affected by the X radiation doses applied (Wald = 591.86; $P < 0.05$; $\chi^2_{(1)} = 465.49$; $P < 0.05$) (Fig. 6). Besides, the X-radiation affected significantly the fertility of the irradiated females (Wald = 73.28; $P < 0.05$; $\chi^2_{(1)} = 79.91$; $P < 0.05$) (Fig. 7). The eggs laid by females that were irradiated as pupae with 200 and 250 Gy did not hatch. The

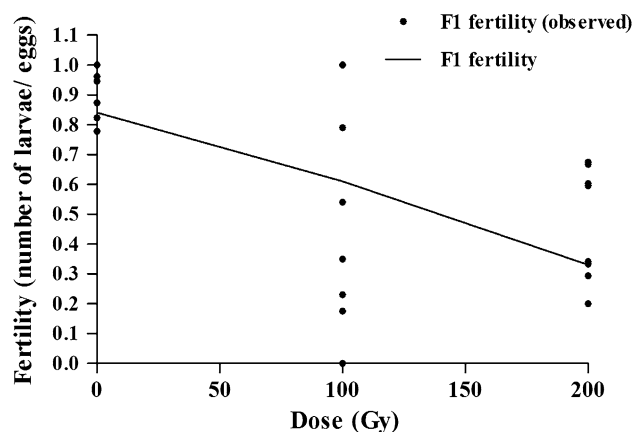


Fig. 5 Fertility of the F1 progeny (number of larvae/number of eggs) when *T. absoluta* parental males were irradiated as pupae and mated with untreated females

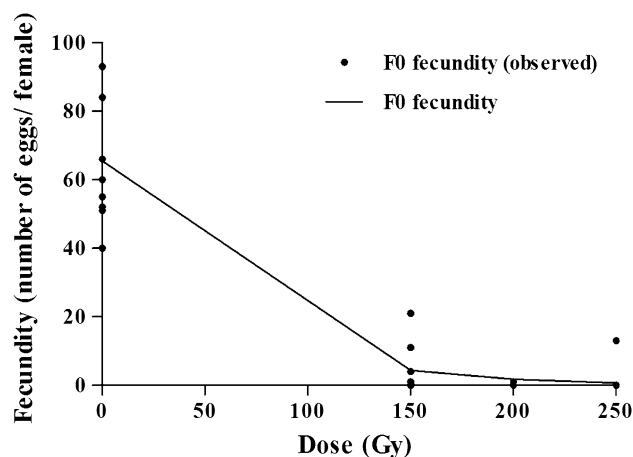


Fig. 6 Parental fecundity (mean number of eggs) when *T. absoluta* females were irradiated as pupae and mated with untreated males

only F1 larvae that developed to pupae, and then to adults were the descendants from untreated females (Table 1).

On the other hand, longevity of treated females (Wald = 2.23; $P > 0.05$; $\chi^2_{(1)} = 2.52$; $P > 0.05$) was not affected by the doses of radiation applied to the pupae. The mean longevity was 12 ± 1 days.

Discussion

First study: effect of X-rays on adult emergence

Selection of the most appropriate sub sterilizing dose of radiation to induce IS in moths is a critical point. Thus, it should be high enough to avoid the release of fertile females but not extremely high, to maintain the mating competitiveness of the males (Bloem et al. 2003). Moreover, this dose should not affect normal adult emergence.

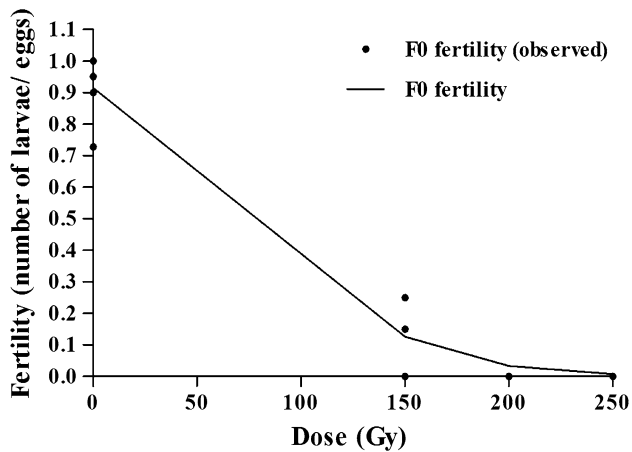


Fig. 7 Parental fertility (number of larvae/number of eggs) when *T. absoluta* females were irradiated as pupae and mated with untreated males

The proportion of complete adult emergence of *T. absoluta* decreased with the increase of the X-radiation applied to parental pupa. Several authors have mentioned that Lepidoptera females are generally more sensitive to radiation than males (LaChance 1985; Carpenter et al. 2005). However, we observed that for a same dose of radiation, the effect depended on the sex of the individual, and the emergence of female was higher than that of males. The results also suggest that a dose ≥ 350 Gy would affect not only the emergence of adults but also their normal morphology. Doses between 50 and 300 Gy reduced adult emergence compared to the control but none of the moths showed deformities. This is consistent with other reports in other Lepidoptera species (Nguyen Thi and Nguyen Thanh 2001; Dhouibi and Abderahmane 2001). On the other hand, our results do not agree with those of Arthur (2004) who studied the effect of gamma radiation applied on *T. absoluta* pupae and found that 300 Gy was the lethal dose. The type of radiation and the way this affects the insect development can be a significant factor to explain the differences between these studies (Bakri et al. 2005).

Second study: effect of X-rays on irradiated males and inherited sterility

Once the dose range that does not affect adult emergence was established, another key point for IS is that the sub sterilizing dose of radiation should maintain mating ability of treated males (North and Holt 1971; Carpenter et al. 2001; Bloem et al. 2003; Ayvaz et al. 2007). The results of fecundity indicated that mating ability of treated males have been affected by the highest tested dose (300 Gy). North and Holt (1971) showed that when *Trichoplusia ni* (Hübner) wild-type females mated with an irradiated male, they often failed to

lay a normal number of eggs. They suggested that this might be caused by an inadequate sperm or accessory gland fluid transference to the female during mating. On the other hand, radiation did not affect male mating ability in other moth species such as *Cryptophlebia leucotreta* (Meyrick) in the dose range of 100–350 Gy (Bloem et al. 2003), and *Plutella xylostella* (L.) and *Cactoblastis cactorum* Berg in the dose range of 100–500 Gy (Sutrisno et al. 1993; Carpenter et al. 2001).

The fertility in the F0 generation was affected by the application of radiation. A decrease in the F0 fertility due to increasing doses of radiation was observed for untreated females that mated with males irradiated as mature pupae in different species of Lepidoptera (Carpenter et al. 1986; Henneberry and Clayton 1988; Carpenter et al. 2001; Nguyen Thi and Nguyen Thanh 2001; Bloem et al. 2003; Ayvaz et al. 2007; Boshra 2007). For example, Henneberry and Clayton (1988) showed a decrease in fertility in *Pectinophora gossypiella* (Saunders) when pupae were treated with 150 Gy (gamma rays) (43 %) compared to the control dose (81 %). This species is a gelechiid like *T. absoluta*. However, F0 fertility in *T. absoluta* was higher than that in *P. gossypiella* at 150 Gy. This could indicate that *T. absoluta* is more resistant to radiation than this species. Differences in the sensibility to radiation could be related to quantitative differences in total DNA content and to the developmental stage of the individuals when they are irradiated (Tobías 1952; Ostergen et al. 1958; Sparrow et al. 1965; Mansour 2010). On the other hand, the differences between *T. absoluta* and *P. gossypiella* could also be due to the type of radiation applied (X-rays or gamma rays) since both have different energy levels and, as a consequence, affect the chromatin differently (Bakri et al. 2005).

The number of F1 larvae and pupae was affected by increasing X-radiation doses. This result suggested that a smaller number of F1 larvae and adults from irradiated males was obtained with high doses of radiation.

One of the advantages of IS in Lepidoptera males is the distortion of the F1 sex ratio in favor of males (Proverbs 1962; LaChance 1985; Makee and Saour 1997). Lepidoptera species show a typical ♀WZ/♂ZZ sex determination system (Suomalainen 1969; Traut and Marec 1997; Makee et al. 2008). Marec et al. (1999) suggested that the sex ratio distortion in the F1 generation in Lepidoptera is a result of recessive lethal mutations induced in the Z sex chromosomes of treated parents that are responsible for the death of F1 females. However, in our study it was not observed this bias against females. We notice that Carpenter et al. (2001) (and references therein) also found a lack of F1 sex ratio distortion in *C. cactorum*.

One of the most important attributes of the IS in Lepidoptera is that F1 male and female offspring are more

sterile than the irradiated F0 parents (Proverbs 1962; North 1975; LaChance 1985; Carpenter et al. 2005). The results of the present study showed that the F1 fertility was smaller than the F0 fertility. This result and the decrease in the number of larvae and pupae in the F1 and F2 generations with increasing X radiation doses, suggest that the deleterious effects induced by X radiation in F0 male were still present in F1 and F2 generations. Taking into account all the studied variables, we suggest that 200–250 Gy doses could be used to induce IS in *T. absoluta* males.

Third study: effect of X-rays on irradiated females

The results of this experiment suggested that full sterility of *T. absoluta* females might be reached at 200 Gy when they are irradiated as pupae. Besides, the larvae that descended from females irradiated with 150 Gy failed to reach pupae stage. This is consistent with other Lepidoptera species studied such as *C. cactorum* and *E. kuehniella* whose females became completely sterile at a dose of 200 Gy (Carpenter et al. 2001; Ayvaz et al. 2007). Concerning other gelechiid species, full female sterility in *P. gossypiella* and *Phthorimaea operculella* (Zeller) was achieved at 150 and 200 Gy, respectively (Henneberry and Clayton 1988; Makee and Saour 2003).

Conclusion

In summary, the most appropriate strategy to control *T. absoluta* populations would be the release of completely sterile females and partially sterile males with an optimum dose of radiation of 200 Gy. To conclude, our study is the first study analyzing the effects of radiation on *T. absoluta*, providing first step to address the viability of implementing IS as a control technique on this species.

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