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Full Length Research Paper

Gamma radiation sterilization of *Bactrocera invadens* (Diptera: Tephritidae) from southern Ghana

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The African invader fly, *Bactrocera invadens*, an invasive pest in Africa since 2003, causes damage and poses a threat to the mango and horticultural industry. Its control is therefore needed. Sterilization of males using gamma radiation doses (25, 50 and 75 Gy) as a means of population control was investigated. Irradiation at the pupal stage (about 6 days after pupation) was found to be suitable. It was observed that a gamma radiation dose of 75 Gy rendered males of *B. invadens* completely sterile, while doses of 25 and 50 Gy induced partial sterility in the males. Females were made completely sterile by all doses of radiation tested.

Key words: *Bactrocera invadens*, mango, gamma radiation doses, Sterile Insect Technique, fertility.

INTRODUCTION

In 2003, the African invader fly, *Bactrocera invadens* (originating from Asia) was detected in Kenya and was reported to be spreading across tropical Africa (Lux et al., 2003a). This pest has since been detected in several countries including Tanzania (Mwatawala et al., 2004), Benin (Vayssieres et al., 2005) and Ghana (Billah et al., 2006). Since its detection in Ghana, *B. invadens* has spread through all ten regions of the country (PPRSD, 2006; Utomi, 2006). *B. invadens* attacks mango and other fruits, and has been described in detail by Drew et al. (2005). Prior to the invasion of sub-Saharan Africa by *B. invadens*, the major mango pest was the marula fly, *Ceratitis cosyra,* whose average damage was given at about 20 to 30% of mango production by Lux et al. (2003b). An assessment of the damage of *B. invadens* on mango in Benin shows production losses varying from 10 to 57% between April and June (Vayssieres et al., 2005), and losses higher than this may be recorded elsewhere. Furthermore, the presence of *B. invadens* in sub-Sahara Africa hampers trade between this region and other regions of the world (Guichard, 2008, 2009).

Control of this invasive pest of high economic importance is therefore very important to safeguard the mango industry and the horticultural industry in general, and to facilitate free trade in fruits. The Sterile Insect Technique (SIT) is a non-disruptive and species-specific pest control strategy that is effectively used against fruit flies (Hendrichs et al., 2002). It involves the release of large numbers of sterile males into the field where they mate with wild females of the same species and cause decline in wild population as reproduction is blocked (Knipling, 1955). Benedict and Robinson (2003) pointed out that sterilization by irradiation is presently the most practical way to sterilize insects. Reproductive sterility is induced by exposing the insects to X-rays, electron beams, or most commonly gamma rays from a Cobalt-60 or Caesium-137 source (LaChance, 1975; Bakri et al., 2005; Robinson, 2005). Helinski et al. (2006) noted that though irradiation is intended to target the germ cells, the process is non-specific and somatic cells may also be damaged.

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Figure 1. Incubation of eggs and larvae inside fruits in plastic racks: A. Wooden cage with mangoes in racks and trays of sand; B. Close view of rack and tray.

According to Calkins and Parker (2005), sterility increases as the radiation dose increases, but insect quality will also decrease. Quality is defined by parameters like flight ability, longevity, startle activity and mating competitiveness. However, irradiating insects in a reduced-oxygen atmosphere, near or after completion of adult development and using minimum sterilizing doses are strategies that are used to minimize somatic damage and preserve insect quality (Lance and McInnis, 2005). Bakri and Hendrichs (2002) investigated radiation doses to prevent fruit flies from the *Anastrepha, Bactrocera, Ceratitis, Dacus* and *Rhagoletis* genera from reproducing and their study showed that the sterilizing doses varied for different species within the same genus. Since SIT has proved successful for many pests in various countries, and considering the fact that no SIT study has been carried out on *B. invadens* in Ghana, it is worthwhile to investigate its potential in the control of *B. invadens*.

MATERIALS AND METHODS

Fruit collection and study locations

Fallen mango fruits were collected from the Botanical gardens (W 00°11.14″ and N 05° 39.63″) and the University farm of the University of Ghana, Legon (W 00° 11.59″ and N 05° 39.59″) in the Greater Accra region, and also from the Agricultural Research Station (ARS) Kpong (E 00° 04.29″ and N 06° 07.89″) in the Eastern region of Ghana. The three collection sites are in the southern part of Ghana.

Incubation of eggs and larvae in fruits

Incubation was done according to the method used by Utomi (2006). Fruits were put in plastic racks of 0.45 x 0.29 x 0.09 m dimension (length x width x height) with openings in the bottom (Figures 1A and B). A tray of moist sand was put under each rack to collect any larva dropping from the fruits in the rack through the openings. The set-up (rack and tray) was put into a wooden cage measuring 0.5 x 0.5 x 0.8 m dimension (length x width x height) covered with wire gauze to prevent other insects from contaminating or infesting the fruits (Figures 1A and B). The fruits were discarded when no more larvae dropped from them, which normally took 2 to 3 weeks.

Collection of pupae

Trays of moist sand were removed every night and the sand was poured onto a wire mesh. The sand in each tray was sieved to collect any pupa that had pupated in it. Pupae collected from incubated fruits were transferred to a glass tube and the tube plugged with cotton wool (Figure 2A).

Irradiation and handling of irradiated pupae

Irradiation of pupae was done at the Radiation Technology Centre (RTC) of the Ghana Atomic Energy Commission (GAEC). Pupae were transported in glass tubes insulated with dry cotton wool and put inside a plastic container. The glass tubes, plugged with cotton wool, were placed in the metal holding cage in the irradiation chamber of the irradiation facility and exposed to gamma radiation from a Cobalt 60 source. Exposure was done at different doses of 25, 50, 75 and 100 Gy at a dose rate of between 0.5208 to 0.5155 Grays per second (Gy/s). Irradiation was carried out with pupae of different ages (2, 3, 4, 5, 6 and 7 days) to find out the suitable age at which subsequent irradiation treatments should be carried out. Pupae older than 7 days were not used because adults of *B. invadens* usually start to enclose from pupae about 8 days after pupation. Fifteen pupae were taken from each age group and exposed to the different doses of radiation. The same number of pupae from each age group were not irradiated and kept as control. Subsequent to the determination of a suitable age for pupae

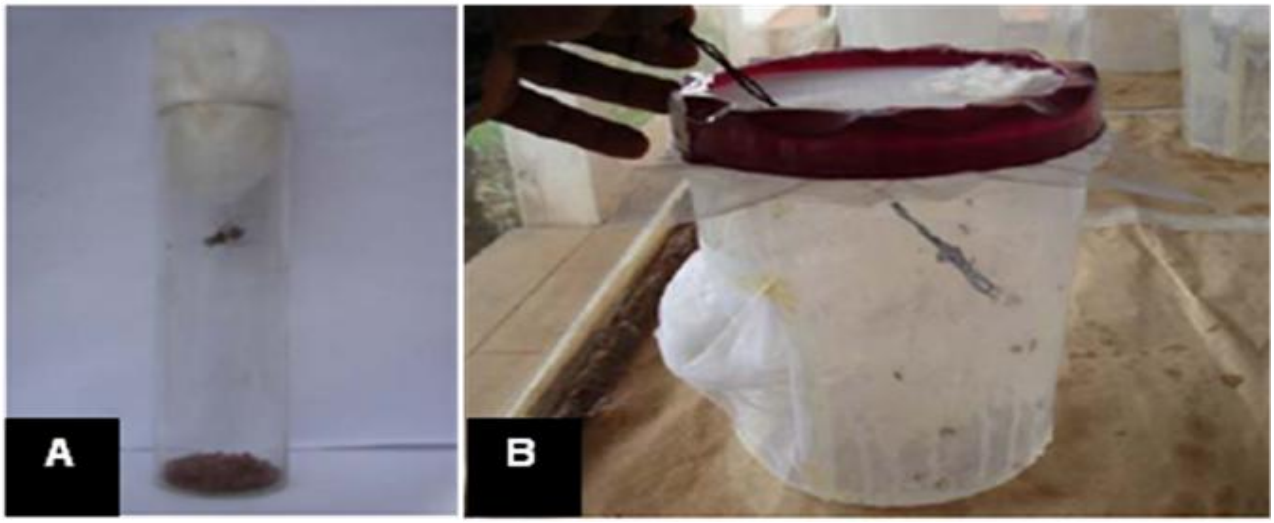

Figure 2. Handling and rearing of flies: A. An emerged adult fly held in a glass tube; B. Feeding of flies contained in a plastic cage.

treatment, irradiation of pupae of the suitable age(s) was carried out at the different doses mentioned earlier. Fifty pupae were irradiated at the different doses. Irradiation for each dose was done in 3 replicates. After irradiation, the glass tubes containing irradiated pupae were kept in plastic cages, which were labelled appropriately and kept for the adults to emerge.

Handling and rearing of adults

Plastic cages (plastic buckets with wire gauze on the screw tops) of dimension 0.24 × 0.22 m (height x top diameter) were used to keep the flies (Figure 2B). The flies were fed on a solution of sugar and yeast (3:1). Flies were fed twice daily (morning and late afternoon). Feeding was done by putting a piece of cotton wool attached to the tip of a metal stick into the food solution and dabbing the wet cotton wool on the side of the cage to create droplets on it (Figure 2B). Water was provided by placing a piece of moist cotton wool on top of the wire gauze of the cage. Flies were kept at ambient conditions between 28 and 34°C and 61 and 93% relative humidity. Adult females emerging from pupae in the same batch of non-irradiated pupae were put in one cage and kept as non-irradiated virgin females. The cages were labelled appropriately. Adults emerging from the irradiated pupae were kept in cages according to their ages and doses of treatment. Some irradiated adult females were also kept in a different cage as virgin females.

Fecundity and fertility of irradiated and non-irradiated adults

Ten days after adult emergence, irradiated and non-irradiated adults were paired. Transfer of flies to different cages was done using an aspirator. Irradiated males were paired with non-irradiated virgin females, while irradiated females were paired with non-irradiated males. Irradiated males were also paired with irradiated females. Control cages contained non-irradiated males paired with non-irradiated females. Each dose treatment was replicated three times. Eggs were collected from females using perforated fruit domes as described by Nishida et al. (1963) and kept in a cavity block with some water. About 4 days after pairing (to give time for mating and egg laying), the fecundity of the females in the different cages was assessed. The percentage hatch of the eggs collected from the different categories was assessed and the fertility of the males in the different treatment cages was determined by the percentage hatch from eggs laid by the respective females in the mating pair. Eggs and larvae were counted under a binocular light microscope.

Statistical analysis

Column chart showing adult emergence after gamma radiation treatment was drawn in Microsoft office Excel (2007 version), while Analysis of Variance (ANOVA) to compare the fertility of males in different gamma radiation treatment groups was done using SPSS 10.0 for Windows.

RESULTS

Establishment of suitable pupa irradiation age

Adult emergence for the non-irradiated pupae (0 Gy) from all the age groups (day 2, 3, 4, 5, 6 and 7) was above 50%, while when pupae were treated at ages below 4 days the adults either failed to emerge or had below 50% emergence (Figure 3). A few population of pupae irradiated at the age of 4 days emerged (adult emergence values below 50%), except for those treated with 25 Gy who showed an adult emergence of 67%. Pupae irradiated at the ages of 5, 6 and 7 days had adult emergence values above 50% (Figure 3).

Post irradiation fertility of adult flies

Male fertility (determined, in this study, by the ability of female pair's eggs to hatch) generally decreased as the

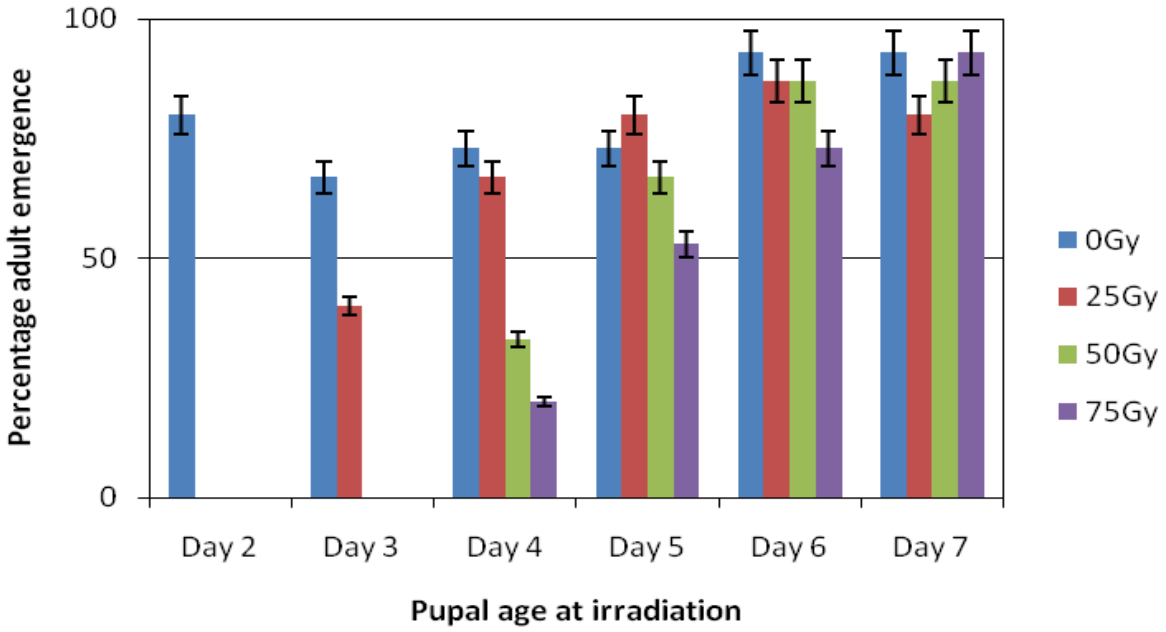

Figure 3. Adult emergence from pupae of different ages irradiated with gamma radiation. Adults successfully emerge from more than half the number of treated pupae when treatment (25, 50 or 75 Gy) was done between the 5th and 7th day after pupation. Error bars for the chart series are with 5% values.

Table 1. Effect of gamma radiation on fertility of male flies.

Dose (Gy)	Mean fertility (%)
0	35.0[a]
25	23.3[a]
50	22.7[a]
75	0.0[b]

[a] Has a statistically significant difference from [b]($p < 0.05$).

dose of radiation increased (Table 1). ANOVA analysis (Analysis of Variance) showed that no statistically significant difference existed between the percentage mean fertility of non-irradiated males and those irradiated at 25 and 50 Gy, but a statistically significant difference existed between the percentage mean fertility of non-irradiated, 25 and 50 Gy irradiated males and that of 75 Gy irradiated males ($p < 0.05$). Eggs laid by the non-irradiated virgin females paired with irradiated males hatched except for those of females paired with 75 Gy irradiated males. The larvae that hatched out from eggs laid by non-irradiated females paired with non-irradiated males had defined epidermal layers that were normal and cylindrical in shape (Figure 4A), while larvae that hatched out from eggs laid by non-irradiated females paired with males irradiated at 25 and 50 Gy were generally gel-like, amorphous, immobile, and had no defined epidermal layer (Figure 4B, C and D). Females were rendered sterile by all doses applied (25, 50 and 75 Gy) and none of them was able to lay eggs irrespective of pairing with irradiated or non-irradiated males.

DISCUSSION

The study carried out to establish the suitable age at which pupae irradiation should be performed showed that the ages of 5, 6 and 7 days (mean of 6 days) were suitable for irradiation of pupae of *B. invadens* between the doses of 25 and 75 Gy. This followed the use of 50% as a selection limit, since the non-irradiated pupae of different ages always had adult emergence values above 50% and this value also implied that at least half the number treated could be used for SIT. The percentage of adults that successfully emerges from irradiated pupae determines the number that can be released. This can be influenced by radiation dose (Calkins and Parker, 2005) and as seen in this study, the age of irradiated pupae. Irradiation of adults was not considered, since transport of adults is somewhat cumbersome.

The results obtained also show that gamma radiation treatment reduces the fertility of males in *B. invadens* and though the fertility of males irradiated at 25 and 50 Gy was lower than that for non-irradiated males, the

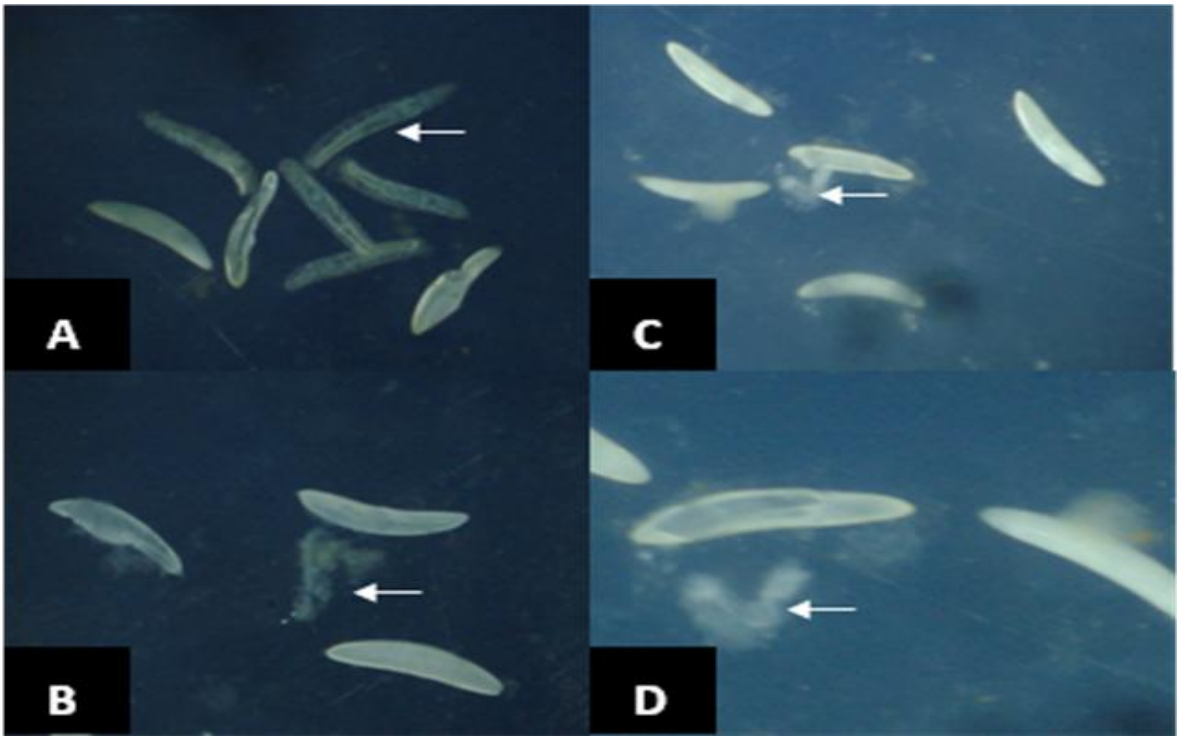

Figure 4. Effect of irradiation on larval hatchlings: A. Representative larvae that hatched out of eggs laid by non-irradiated adults; B, C and D show representative larvae that hatched from eggs laid by non-irradiated females mated with males irradiated at 25 or 50 Gy. White arrows in the pictures point at larvae that had hatched out of eggs.

differences were not significant. However, a statistically significant difference existed between the mean fertility of males irradiated at 75 Gy and other males (non-irradiated, 25 and 50 Gy). Generally, sterility increased as the dose of radiation increased, with flies irradiated at 75 Gy being completely sterile. The results obtained also agree with that from Calkins and Parker (2005), who showed that females of many insects become 100% sterile at lower doses than the males. The observed immobility and lack of shape of larvae hatching from eggs laid by females paired with 25 and 50 Gy males showed that embryonic development was impaired and the larvae died consequently.

This goes further to suggest that lower radiation doses could be used to induce partial sterility leading to non-viable hatch in *B. invadens*. However, a radiation dose of 75 Gy can be used to prevent production of offspring. This result agrees with the results of a related study by Bakri and Hendrichs (2002), who achieved sterilization in *B. zonata* and *B. dorsalis* using radiation doses of 60 and 90 Gy respectively. Both *B. zonata* and *B. dorsalis* are related to *B. invadens* and the relationship between these three *Bactrocera* species may explain why the doses required are similar. As gamma radiation treatment renders *B. invadens* males sterile, it can possibly be used for SIT against this fly. However, other parameters such as male flight ability, longevity and competitiveness will have to be assessed and optimized in line with suitable sterilization doses.

Conclusion

In conclusion, gamma radiation treatment is promising for the control of *B. invadens* and can be applied to 6 days old pupae at the dose of 75 Gy to cause total sterility in adult males, while a dose between 25 and 50 Gy might be used to induce partial sterility.

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