Irradiation as a quarantine treatment against *Bactrocera invadens*, in *Mangifera indica*, L. in Ghana

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The detection of the African invader fly, *Bactrocera invadens*, in Ghana has led to limitations in the mango export industry. The limitations ranging from increased control costs to rejection of exports has necessitated studies in the area of quarantine treatment. A study was conducted to ascertain the effective dose of gamma irradiation for the control of *B. invadens* in fruits intended for export. Pupae were obtained after incubation of mango fruits collected from various locations. Adults were reared out and 5 – 20 females were placed on fruits in cages and allowed to oviposit. Infested mangoes were then examined to determine larval infestation levels. Late instar (14-21 day old) larvae in fruits were irradiated at 15–75 Gy to determine an effective dose for the control of *B. invadens*. The mortality of the fly was determined at the various doses to obtain a probit 9 figure of 70 Gy as the effective dose. Confirmatory tests using 3,050 larvae endorsed the effective dose as the probit 9 dose.

Key words: Gamma irradiation, *Bactrocera invadens*, mango fruits, mango industry.

INTRODUCTION

*Bactrocera invadens* was not known in Africa until its recent detection by Lux et al. (2003) in Kenya and Tanzania in 2003. This pest is known to have originated from Sri Lanka from where it got into Africa (Drew et al., 2005). In a space of one year, it had spread from the East African Countries to West and Central Africa, extending from DR Congo to Senegal (Hanna, 2005). This was detected in Ghana through a survey conducted by Billah et al. (2006). Since then, it has spread throughout the country (Wilson and Cobblah, 2007).

The damage caused by fruit flies stem from the puncturing activity of the female when it is about to lay its eggs. Rot causing bacteria are introduced into the fruit from the intestinal flora of the fly, thereby causing rot of the tissues surrounding the eggs laid. Larvae from the hatched eggs feed on the fleshy portion of the fruit, leading to the creation of galleries which serve as entry points for pathogens. This increases the decay of the fruit, thereby making it unsuitable for human consumption (Billah and Ekesi, 2006). The damage caused is seen as
direct loss of yield, increased control costs, as well as the loss of export markets, and the cost of the maintenance of treatment and eradication facilities and trade barriers (Bech, 2008).

In Ghana, farmers experience 3-85% losses, exporters 10-15%, processors 2-10% and the total national loss of mango to fruit flies is 65% (Billah, 2008). Irradiation has been used as a quarantine measure as an alternative to methyl bromide when it was banned in the United States of America and other countries (IAEA, 2004). This method of quarantine control has become common for arthropods which are of economic importance. Irradiation has been used as a quarantine measure for sweet potato weevil (Cylas formicarius elegantulus [Summers]), oriental fruit moth (Grapholita molesta [Busck]), sugarcane borer (Diatraea saccharalis [F.]), southwestern corn borer (D. grandiosella [Dyar]), Mexican rice borer (Eoreuma loftini [Dyar]) and Indian meal moth (Plodia interpunctella [Hubner]). Thus, irradiation encouraged trade among nations since countries would not want to have the introduction of pests of economic importance into their country. The USA demands that mangoes from affected countries in West Africa, such as Ghana are irradiated as a guarantee that will prevent the introduction of the African invader fly into that country (Bech, 2008).

In Australia, it is a necessity that mangoes from India are irradiated in order to prevent the introduction of the fruit fly. Fruits from the Philippines are to be irradiated before they are sent to the USA (Ignacio, 2008). There is a generic dose of 150 Gy for fruit flies which was proposed for the tephritids in 1986, based on irradiation data for many tephritid fruit fly species and a limited number of other insect pests, and 300 Gy for other insects (ICGFI, 1999; Heather, 2004). It is therefore imperative that doses for specific species of tephritids are established in order to reduce cost of irradiation since the higher the dose the more finance is committed to it and vice versa (Torres and Hallman, 2007). This will lead to increased capacity for treatment facilities by decreasing the required time for treatment (Follet and Armstrong, 2004). Doses below the generic dose have been observed in some fruit flies, some of these include: 100 Gy for Ceratitis capitata (Hallman and Torres, 2004); 125 Gy for Bactrocera dorsalis and 150 Gy for B. cucurbitae (Follet and Armstrong, 2004); 70 Gy for Anastrepha spp.; 101 Gy for Bactrocera jarvisi and B. tryoni, (Hallman and Loaharanu, 2002).

MATERIALS AND METHODS

Fruit collection

Infested Keitt mango fruits were collected from the University farm and the Botanical Garden (W 00° 11.14’ and N 05° 39.63’) of the University of Ghana, Legon, in the Greater Accra Region of Ghana. They were transported to the Zoology Department of the University of Ghana and the Ghana Atomic Energy Commission (GAEC, 00° 13.15’ and -05° 40.33’) where experimental studies took place.

Incubation of eggs and larvae in the fruits

Infested fruits were picked from the University of Ghana Farms. These were incubated based on the method used by Utomi (2006). The fruits were kept in screened racks to prevent the access of other insects. The racks measured 0.45 x 0.29 x 0.09 m (length x width x height). Trays were fitted under each rack to collect ready- to- pupate larvae which had bored their way through the fruits. The trays contained moist sterilised soil for the larvae to pupate in them.

Pupae collection

The moist soil containing the pupae was sifted to collect the pupae. The soil was put back into the trays for further collection of subsequent pupae. The collected pupae were kept in jam jars (which had dimensions of bottom and top diameters as 0.195 m and 0.230 m respectively and height of 0.095 m) and kept for sex ratio, percentage emergence and mortality.

Rearing of adults

Emerged adults were counted, and sex ratio determined. They were kept in infestation cages (which had top and bottom diameters of 0.66 and 0.81 m respectively and 0.24 m high). The temperature was between 28 and 30°C. They were fed with a solution of three parts of sugar and one part of yeast. Clean water was provided (soaked in cotton wool) for them to drink. Mortality was recorded on a daily basis.

Infestation level studies

The weight of non-infested fruits was recorded. 5, 10 and 20 males and females of B. invadens were put in each cage (in triplicates). A fruit was put in each cage for infestation (in triplicates) for five days. The number of ovipunctures on each fruit per cage was counted. The infested fruits were incubated in plastic containers, which had sterilised soil in them for pupariation. Pupae from each container were counted, incubated in glass containers for adult emergence. Percentage emergence was calculated for each container.

Gamma irradiation of infested fruits

Two hundred and forty insects (males and females in the ratio 1:1) were put into eight infestation cages for infestation (in 3 replicates). Each infestation cage contained a fruit. The fruits were removed after 24 h based on the method used by Follet and Armstrong, (2004) and kept in plastic containers with sterilised soil on racks in the laboratory. The number of larvae in each fruit was estimated based on the infestation levels done as above. Larvae in the fruits were allowed to reach the third instar stage which is the most radiation resistant stage (Ballock et al., 1963).

Infested fruits were irradiated at the Radiation Technology Centre (RTC). The larvae in the fruits were irradiated at 15, 25, 35, 45, 50, 60 75 Gy at a dose rate of 193.52 Gy hr⁻¹ at a distance of 0.50 m by 0.70 m from a Co⁶⁰ source (type SLL-02) at the Ghana Atomic Energy Commission. Three hundred and forty-one larvae (in the fruits) were irradiated at each dose in three replicates with a control. Frick dosimeter was used to calibrate the irradiation area and the dose distribution. Pupae collected were kept in Kilner jars with a mesh top for good ventilation for the flies when they emerged. They
Table 1. Development pattern at various infestation levels of *Bactrocera invadens*.

<table>
<thead>
<tr>
<th>No. Flies</th>
<th>No. of holes/fruit</th>
<th>No. of eggs/ hole</th>
<th>No. of larvae/fruit</th>
<th>Emergence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.5 ± 0.1</td>
<td>92.6 ± 11.4</td>
<td>234.7 ± 26.7</td>
<td>83.2± 0.7</td>
</tr>
<tr>
<td>10</td>
<td>3.6 ± 0.3</td>
<td>164.5 ± 15.2</td>
<td>569.6 ± 43.5</td>
<td>81.5±1.6</td>
</tr>
<tr>
<td>20</td>
<td>6.3 ± 0.3</td>
<td>176.0 ± 16.6</td>
<td>1068.7 ± 84.1</td>
<td>78.0±1.6</td>
</tr>
</tbody>
</table>

Mean separation (Tukey’s test): a, b and c are significantly different from each other (p<0.05), in the same column.

were kept in the insectary at the Animal Science Department of the Biotechnology and Nuclear Agriculture Research Institute of Ghana Atomic Energy Commission. Natural mortalities were corrected using Abbott’s formula. SPPS 16 was used to analyse the data.

Confirmatory test

A large population of 3,050 late third instar larvae (in infested fruits) were irradiated at 70 Gy at a dose rate of 191.88 Gy h^{-1}. Fricke dosimeter was used to calibrate the irradiation area as well as the dose distribution area. A control group of 345 (larvae in fruit) were not irradiated based on the method used by Follet and Armstrong (2004).

RESULTS AND DISCUSSION

Oviposition behaviour

The adult females (from 10 days old) responded immediately and showed oviposition behaviour when the fruits were put into the infestation cages. The females climbed onto the fruits in the various cages apparently searching for holes before creating one as reported by Ogaugwu (2007). They then spent about five minutes on the fruits until oviposition is completed.

Signs of Infestation

After oviposition, exudates from the holes were visible. This gave the exact position where infestation had taken place. Points of infestation darkened until the eggs hatch after two days. The feeding habits of the larvae caused more exudates to come from the point of infestation and the ovipunctures made the fruits unattractive. As the larvae tunnel throughout, the fruits decayed at a faster rate. The skin of the fruits sunk when pressed, with some larvae occasionally coming out of the fruits. It was observed that the number of larvae increased considerably as the population of the flies was increased. There were significant differences (p<0.05) in the number of larvae per colony of flies shown in Table 1.

Pupariation

Larvae came out of the fruits when they reached the last instar stage. They entered into the soil in the containers to pupate. They pupated a day after entering the soil. The pupae were barrel shaped and had a creamy colour. Pupae had segmented lines as well as two dark projections at one end and the other end smooth.

Adult Emergence

Adults emerged from the collected pupae at a mean age of 8±0.6 (SD) days after pupation with a mean percentage of 80.89 ±5.46 (SD). The development at various infestation levels is shown in Table 1. The number of larvae per fruit was dependent on the number of female flies which have started ovipositing and visited the fruit (Table 1). After the eggs hatched into larvae, the early instars aggregated at the point where they were laid before spreading throughout the fruit as they grow, which confirms the general feeding habit of fruit flies as was reported by Pena et al. (2008). The number of larvae per infestation were significantly different (p<0.05) from each other. Ogaugwu (2007) observed that a female could lay 70 eggs per day on the average. Hence, the significant differences (p<0.05) was due to the number of females that laid eggs in each cage. Percentage emergence was significantly different (p<0.05) from each other among the population. Larger number of larvae in the fruits led to lower percentage of emergence. This was due to larger population feeding on the same fruit. This led to competition among the larvae for food.

Irradiation of infested fruits

After irradiation, fruits were opened (dissected) with a knife to allow the late irradiated instars to get out of the fruits after 24 h in order to pupate in the soil provided in each of 24 containers. All the larvae pupated. There were significant differences (p<0.05) in the number of non-emergence of adults for the various doses applied to the larvae. Irradiation increased the mortalities of the pupae seen in Figure 1, as the doses increased (Ogaugwu, 2007). A higher dose applied led to a high disruption of the cells of the flies and a higher effect of the life process taking place in the larvae. This led to the higher mortalities in the irradiated as compared with the control.
Figure 1. Mortality and the doses of irradiation treatment.

Confirmatory test

The probit 9 figure gave a dose of 68.506 Gy, rounded off to the nearest whole number as 70 Gy. This dose will lead to a mortality of 99.99% mortality of the pupae that where irradiated. All the larvae (3050) that were irradiated at the effective dose of 70 Gy at a dose rate of 191.878 Gyr⁻¹ did not emerge. This confirms the dose as the effective dose for B. invadens.

Conclusion

The findings of this study showed that irradiating late larval instars at a dose of 70 Gy will lead to 99.99% of mortality (non-emergence) in B. invadens. The results with the large larval population of 3,050 at probit 9 level (99.99% mortality) confirmed that 70 Gy can be used as a quarantine treatment dose against B. invadens in Mangifera indica.

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REFERENCES


Lux SA, Ekesi S, Dimbi S, Mohammed S, Billah M (2003). Mango-


