Effect of different radiation doses on Medfly quality

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Radiation is used to sterilize Medflies, Ceratitis capitata (Wied.), for SIT programmes. Mass-reared flies in the Madeira-Med sterile insect technique (SIT) programme are sterilized at the pupal stage about 24 hours before adult eclosion with 100 Gy using a Gamma Cell 220 loaded with Cobalt 60. The pupae are in anoxia/hypoxia when treated. It is known that the higher the radiation dose given to the Medfly pupae, the more the overall quality of the adults is reduced. A series of tests was conducted to determine if we could reduce the radiation dose so as to measurably improve fly quality while maintaining the sterility required for a successful Medfly control (as compared to eradication) programme using SIT. Our target sterility in the irradiated males was 97.5% as measured in mating tests with untreated fertile females. The flies were subjected to standard quality control tests, including field-cage tests. Two series of irradiations were carried out. The first used 0 Gy, 50 Gy, 75 Gy, 100 Gy, 125 Gy and 145 Gy, and the second 0 Gy, 50 Gy, 60 Gy, 70 Gy, 80 Gy, 90 Gy and 100 Gy. In both tests we measured egg hatch, larval production, pupal production, emerged adults and flying adults. In the second test any F1 males that were produced were mated with unirradiated females. Field-cage tests were run with both series of doses to estimate competitiveness of mass-reared flies treated with the various radiation dosages. Results indicated that Medfly control programmes using could use a radiation dosage of 80 Gy.

INTRODUCTION

Madeira-Med is a programme to control the Medfly on the island of Madeira using the sterile insect technique (SIT). It is the first planned use of SIT for Medfly control (Pereira et al. 2000), as eradication has been the objective of all other Medfly SIT programmes. The programme takes advantage of the recently developed genetic sexing strains (GSS) that provide for the release of sterile males only. This eliminates the sterile sting problem and greatly increases efficacy of SIT (Hendrichs et al. 1995). The GSS used was V7 (mix 2000).

A Medfly mass-production factory was constructed with a planned production of 50 million sterile flies per week (Pereira et al. 1997).

For successful control with SIT it is essential to produce sterile males that will compete successfully with wild males for mating with wild females (Orozco & Lopez 1993). The compatibility (ability of sterile male flies to mate with wild females) and competitiveness (mating performance in competition with wild males) are first tested in laboratory (Fried, 1971). However, laboratory tests cannot assess the full behavioural repertoire of mass-produced insects, and field-cage tests are therefore essential (IAEA/USDA/FAO 1998).

The objective of the field-cage tests was to determine the mating competitiveness of males irradiated with different doses. In addition, the use of male-only strains and the development of control programmes, such as Madeira-Med, open the possibility to decrease the irradiation dose (Pereira 2001) and delay the time of pupal irradiation. The male-only strain can be irradiated 24 hours before emergence, instead of the 48 hours required when also irradiating female pupae in bi-sexual releases (Hendrichs et al. 1995).

MATERIALS AND METHODS

Irradiation

We set up two tests with different radiation doses: 0 Gy, 50 Gy, 75 Gy, 100 Gy, 125 Gy and 145 Gy, and 0 Gy, 50 Gy, 60 Gy, 70 Gy, 80 Gy, 90 Gy and 100 Gy. Pupae were irradiated in anoxia/hypoxia one day before adult emergence, in a Nordion 60Co gamma cell irradiator. For each experiment 20 irradiated males were mass-mated with 20 unirradiated females (two cages, 10 pairs per cage).

Quality parameters

In both tests an egg sterility test was run according the International Quality Control Manual (IAEA/USDA/FAO 1998). The eggs obtained were seeded on larval diet, the pupae obtained were allowed to emerge and the number of flying males counted. Quality parameters calculated were: egg hatch, pupal survival, adult emergence

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and flight ability. Eggs from the mated females from the different treatments were collected for 10 days after the females started to oviposit. Egg hatch was assessed five days after egg laying, and survival was determined at the larval stage. In the first test, the quality of pupae, adults and flying males of the F1 generation were determined. In the second test F1 males were back-crossed to unirradiated females and the same quality parameters of the resulting offspring were tested.

Field-cage tests
For the field-cage tests, male Medflies were irradiated at the above-mentioned doses. Wild Madeira female Medflies were collected as pupae from larval survey samples (mixed hosts). Pupae from both sterile and wild strains were placed in a standard quality-control perspex cage (30 × 30 × 40 cm) until emergence. After emergence the insects were sexed and females and males kept in separate rooms to avoid contact with the male pheromone before the tests. All flies were maintained under low stress conditions (24°C, 65% RH and daylight conditions) in plastic 2-litre containers with water and food (1:3 protein hydrolysate:sugar). Healthy flies of both strains were selected for the tests and marked with a dot of water-based paint (Deka) on the notum (IAEA/USDA/FAO 1998).

The field cages were cylindrical, with flat floor and ceiling (2.9 m diameter, 2.0 m high) (Chambers et al. 1983). Each cage was supported by a PVC frame (Calkins & Webb 1983). Each field cage was set up over a small citrus or mango tree. Pruning was sometimes necessary to facilitate observation in the cage.

The test period covered the time of maximum sexual activity of both wild and mass-produced flies (sunrise to mid-afternoon). Male flies were released 30 minutes before the females so that they could start forming leks (Prokopy & Hendrichs, 1979). The mating performance of GSS sterile males when competing with wild males for wild female was measured. Temperature, relative humidity and light intensity in the field cage were recorded every hour. The mating pairs were monitored continually, and 5 minutes after initiation of mating, each pair was collected in a separate 20 ml vial. Because the wild and sterile mass-reared males might not have occupied the same part of the tree, the initial location of each mating pair on the tree or on the cage screen was recorded according its vertical position (bottom, middle and top).

In the field-cage tests we measured the proportion of flies mating (PM). The PM measures the suitability of the flies and the environment for mating. It represents the overall mating activity of the flies, both wild and sterile, and it is defined as follows (Cayol et al. 1999):

\[
PM = \frac{\text{Number of pairs collected}}{\text{Number of females released}}
\]

As mass-reared males were used we also measured the mating frequency. Ten males per treatment were used in a field cage. In total, 60 males were used in the first test and 70 males in the second. A sex ratio of two males per one female was maintained. The mating frequency is described by the formula below (IAEA/USDA/FAO, 1998)

\[
\text{Mating frequency} = \frac{\text{No. of matings per treatment}}{\text{Total of matings}}
\]

F1 sterility
In the second test we also studied the induced sterility. The males produced in the first generation were crossed with unirradiated females. Males were not irradiated only to see if some sterility was induced from the first generation.

Table 1. Percentage egg hatch, pupal recovery, adult emergence and fliers obtained when female Medflies were mated with males subjected to radiation doses up to 145 Gy.

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>No. of eggs used</th>
<th>% Egg hatch</th>
<th>% Pupal recovery</th>
<th>% Adult emergence</th>
<th>% Adult fliers</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6149</td>
<td>91.98</td>
<td>33.29</td>
<td>20.20</td>
<td>15.42</td>
</tr>
<tr>
<td>50</td>
<td>5929</td>
<td>17.74</td>
<td>5.43</td>
<td>3.00</td>
<td>2.21</td>
</tr>
<tr>
<td>75</td>
<td>2982</td>
<td>5.37</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>100</td>
<td>5679</td>
<td>1.99</td>
<td>0.49</td>
<td>0.23</td>
<td>0.19</td>
</tr>
<tr>
<td>125</td>
<td>4893</td>
<td>1.10</td>
<td>0.26</td>
<td>0.22</td>
<td>0.18</td>
</tr>
<tr>
<td>145</td>
<td>5394</td>
<td>0.50</td>
<td>0.13</td>
<td>0.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>

1Percentages obtained from the number of eggs tested.
2Treatment invalid as the flies escaped from the cage.
methodology was the same as was followed for the previous generation. In this test we only measured egg hatch.

RESULTS AND DISCUSSION

The effect of the two series of radiation doses on Medfly quality is presented in Tables 1–5. Results from the field-cage tests are presented in Table 2.

Radiation doses from 50 to 145 Gy

Table 1 shows the effect on Medfly quality after males were irradiated at doses up to 145 Gy. All quality parameters decreased as the radiation dose for sterile males increased.

Radiation doses from 50 to 100 Gy

In the second series, radiation doses between 50 to 100 Gy were used. The data presented in Table 3 show similar results to those presented in Table 1; all the quality parameters decreased with increasing radiation dose. The same result was found by Franz (2000).

Table 3. Percentage egg hatch, pupal recovery, adult emergence and fliers obtained when female Medflies were mated with males subjected to radiation doses up to 100 Gy.

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>No. of eggs used</th>
<th>% Egg hatch</th>
<th>% Pupal recovery</th>
<th>% Adult emergence</th>
<th>% Adult fliers</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5930</td>
<td>90.03</td>
<td>28.35</td>
<td>19.02</td>
<td>17.55</td>
</tr>
<tr>
<td>50</td>
<td>4273</td>
<td>16.99</td>
<td>7.54</td>
<td>5.13</td>
<td>4.56</td>
</tr>
<tr>
<td>60</td>
<td>4865</td>
<td>11.06</td>
<td>4.05</td>
<td>2.71</td>
<td>2.24</td>
</tr>
<tr>
<td>70</td>
<td>5956</td>
<td>9.23</td>
<td>2.30</td>
<td>1.76</td>
<td>1.54</td>
</tr>
<tr>
<td>80</td>
<td>5097</td>
<td>5.38</td>
<td>1.84</td>
<td>1.16</td>
<td>0.94</td>
</tr>
<tr>
<td>90</td>
<td>5995</td>
<td>3.40</td>
<td>0.80</td>
<td>0.45</td>
<td>0.32</td>
</tr>
<tr>
<td>100</td>
<td>5053</td>
<td>3.15</td>
<td>0.83</td>
<td>0.47</td>
<td>0.36</td>
</tr>
</tbody>
</table>

1Percentages obtained from the number of eggs tested.

If PM <0.2, the data cannot be used (IAEA/USDA/FAO, 1998). In these tests, the overall PM was >0.6. Tables 2 and 4 indicate that more than 60% of the total number of possible matings took place. Mating competitiveness decreased as radiation dose increased.

In the second test we monitored the males obtained to determine whether they had induced F1 sterility. As seen in Table 5, the percentage F1 egg hatch decreased with an increase in radiation dose. All values obtained were less than the egg hatch in the control (0 Gy). We therefore conclude that there is evidence of F1 sterility.

CONCLUSIONS

The 100 Gy radiation dose that is now used at the Madeira-Med Medfly factory results in approximately 2% egg hatch. With 80 Gy, egg hatch is approximately 5%, with 1% fliers. This result is acceptable for a control programme.

The mating competitiveness increases with the decrease in radiation dose.

Table 2. Number and frequency of Medfly matings in field-cage tests with males subjected to radiation doses up to 145 Gy.

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>Matings</th>
<th>Total number</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>48</td>
<td></td>
<td>0.216</td>
</tr>
<tr>
<td>50</td>
<td>36</td>
<td></td>
<td>0.162</td>
</tr>
<tr>
<td>75</td>
<td>36</td>
<td></td>
<td>0.162</td>
</tr>
<tr>
<td>100</td>
<td>27</td>
<td></td>
<td>0.122</td>
</tr>
<tr>
<td>125</td>
<td>39</td>
<td></td>
<td>0.176</td>
</tr>
<tr>
<td>145</td>
<td>36</td>
<td></td>
<td>0.162</td>
</tr>
<tr>
<td>Total</td>
<td>222</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM</td>
<td>0.617</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Number and frequency of Medfly matings in field-cage tests with males subjected to radiation doses up to 145 Gy.

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>Matings</th>
<th>Total</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>54</td>
<td>0.179</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>51</td>
<td>0.169</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>53</td>
<td>0.174</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>45</td>
<td>0.149</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>37</td>
<td>0.123</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>30</td>
<td>0.099</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>32</td>
<td>0.106</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>302</td>
<td></td>
<td>0.719</td>
</tr>
<tr>
<td>PM</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
It appears that sterility was induced in the F1 generation, but we need to repeat these tests to confirm this.

REFERENCES


