SEX PHEROMONE PRODUCTION AND REPRODUCTIVE BEHAVIOUR IN GAMMA-IRRADIATED TENEBRIO MOLITOR

MAYA MENON and K. K. NAIR

Pestology Centre, Department of Biological Sciences, Simon Fraser University, Burnaby 2, British Columbia

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Abstract—Studies to determine the effects of gamma-radiation on sex pheromone production in female Tenebrio molitor showed that irradiation of females either as newly emerged or as pharate adults with 3.5 or 7 krad had no significant effect on their sex pheromone activity when compared to that of normals. However, treatment of irradiated insects with a juvenile hormone analogue increased their pheromone activity, whereas it decreased the pheromone activity in the normals.

Ovary maturation was inhibited in irradiated insects, and it could not be alleviated by treatment with juvenile hormone analogue. Since ovarian transplants from normal to irradiated insects showed yolk deposition, whereas reciprocal transplants did not, it is concluded that the inhibition in yolk deposition in the ovaries of irradiated insects was due to radiation damage to the ovary itself rather than to the neuroendocrine system.

Irradiation of the males with 7 krad did not affect their ability to respond to the female sex pheromone or their sexual vigour. At higher doses their response was slow and it decreased with increase in radiation dose, and also as a function of time after irradiation.

INTRODUCTION

In recent years evidence has accumulated in favour of the existence, in most groups of insects, of a sex pheromone which plays a significant role in bringing the two sexes together for reproduction (Berzoa, 1970; Wood et al., 1970). Therefore, sterilizing insect pests for control can be successful only if sterilizing techniques do not interfere with (1) the production of their sex pheromone, (2) their ability to perceive the pheromone, and (3) their normal behaviour. The only studies to date on the above aspects are on the American cockroach, Periplaneta americana (Wharton and Wharton, 1957), the gypsy moth, Porthetria dispar (Statler, 1970), and the codling moth, Laspeyresia pomonella (White and Hutt, 1971).

We have investigated in the beetle Tenebrio molitor: (1) the effects of gamma-radiation on sex pheromone production and reproductive physiology of the females, (2) the ability of irradiated males to perceive the female sex pheromone, and (3) the sexual vigour of irradiated males. We chose this insect because its pheromone history (Valentine, 1931; Tschinkel et al., 1967; Tschinkel, 1970; Happ, 1969,
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1970; HAP and WHEELER, 1969; MENON, 1970; AUGUST, 1971) and reproductive physiology (MORDUE, 1965, 1967; LAVERDURE, 1967; MENON, 1970) are well documented. Since implantation of active larval corpora allata into allatectomized female T. molitor promoted yolk deposition (LAVERDURE, 1967) and since topical application of a juvenile hormone (J.H.) analogue induced sex pheromone production in a decapitated female T. molitor (MENON, 1970), we also investigated the influence of J.H. on the above aspects in gamma-irradiated T. molitor.

MATERIALS AND METHODS

A stock colony was maintained as described by MENON (1970). Pupae were collected, sexed, and held separately in two incubators at 25°C and 12 hr photoperiod. The insects were used to study: (1) bioassay of pheromone of females exposed to gamma-radiation and J.H. treatment, (2) ovarian development in females exposed to gamma-radiation and J.H. treatment, (3) ovarian transplants from normal into irradiated females and vice versa, (4) sensitivity of irradiated males to pheromone extracts of normal females, and (5) assessment of sexual vigour of irradiated males.

Irradiation of females

The insects were irradiated in a 60Co gamma source (Gamma Cell 200, A.E.C.L.) at a dose rate of 1.2 krad/min, measured in air with a Victoreen dosimeter. Newly emerged females were exposed to 3.5 and 7 krad and pharate adults, 1 day before ecdysis, to 7 krad of gamma-radiation. Irradiated and control insects were kept in groups of 5 to 10 in plastic Petri dishes lined with filter paper and provided with bran and water.

Hormone treatment

The J.H. analogue used was trans-trans-N,N-diethyl-3,7,11-trimethyl-10,11-epoxydodeca-2,6-dienamide (Syntex), made up in acetone. One to 2 days after adult emergence about 50 per cent of the insects irradiated with 7 krad as pharate adults and 50 per cent of the unirradiated controls were treated with 2 μg of J.H. in acetone by topical application on the mesothorax with disposable micropipettes. The other 50 per cent of the two groups of insects received 2 μl of acetone only and these served as controls.

Pheromone assay

Ten days after adult emergence the control and the irradiated insects, with or without the J.H. treatment, were extracted individually in tetrahydrofuran (1 insect/ml) as described by MENON (1970). Each extract was then tested for biological activity using 20 males by the assay method of TSHINKEL et al. (1967).

Estimation of ovary maturation

After extraction of the pheromone the insects were preserved in 70% ethanol. They were dissected and the number of insects with mature ovaries, the number
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of eggs per insect, and the number of eggs per insect with mature ovaries were determined.

**Ovary transplantation**

Ovaries of 1- to 2-day-old adult females were transplanted into females of the same age group. Transplantations were made from (1) normal to normal, (2) normal to irradiated (7 krad), and (3) irradiated to normal. Only females irradiated as pharate adults were used. Ovaries with ducts were excised from donor insects and placed in *Tenebrio* saline (BELTON and GRUNDFEST, 1962). The recipient insects were anaesthetized for 1 hr in water through which carbon dioxide bubbled continuously. Then they were affixed on their dorsum in a Petri dish by a strip of Plasticine. Under a binocular microscope a small rectangular incision was made on the ventral side of the eighth abdominal segment, a piece of fat body was removed, and a single ovary from the saline was inserted into the body cavity. The cuticular flap was replaced, surface sterilized with chloremphenicol, dried with cotton wool, and sealed with melted paraffin wax. The recipient insects were returned to the incubators and provided with bran and water 1 day after the operation. After 10 days they were sacrificed and the terminal oocytes of the transplants were examined for yolk deposition.

**Irradiation of males**

Insects were collected at random from a month-old population of males and were divided into four groups of 60 each. One group was kept as control and the other three groups were exposed to 7, 16, or 32 krad of gamma-radiation. The sensitivity of these males to pheromone extracts of normal females was tested on days 1, 3, and 6 after irradiation. Furthermore, the sexual vigour of these irradiated males was assessed on day 7 by using combinations involving normal females, normal males, and irradiated males. Three marked males (one normal, one exposed to krad, and the other to 16 krad) per female were confined under a Petri dish for 10 min and their order of mating was observed. Thirty tests were made using 90 males and 30 females.

**Statistical analyses**

One way analysis of variance and the *t*-test were used to test the significance of differences in pheromone activity in the various groups after different treatments. The data on ovary maturation and on the males' sensitivity to pheromone extracts of females were analysed by the exact hypergeometric one-tail test using LIEBERMAN and OWEN'S (1961) tables. The data on males' sexual vigour was analysed by the exact Wilcoxon one-tail test using the above tables. In all tests the minimum significance level was set at \( P < 0.05 \).

**RESULTS**

**Mortality**

Adult mortality was about 15 per cent in the irradiated females and 5 per cent in the controls, up to day 10. Seventy per cent of the normals with ovary transplants
and 58 per cent of the irradiated females with ovary transplants survived during the experimental period of 10 days. Exposure of mature males to 16 and 32 krad increased the mortality to 10 and 90 per cent respectively within 8 days of irradiation.

**Pheromone activity**

Exposure of newly emerged females to 3·5 and 7 krad and pharate adults to 7 krad did not significantly alter their pheromone activity up to 10 days (Table 1). However, application of J.H. to females irradiated with 7 krad increased the pheromone activity significantly above that of controls. On the other hand, treatment of unirradiated females with J.H. decreased their pheromone activity significantly below that of control insects.

**Table 1—Pheromone activity of extracts of female T. molitor 10 days after emergence and various treatments**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of female extracts tested</th>
<th>Percentage of males responded (mean ± S.E.)</th>
<th>Comparison of means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newly emerged adults</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Unirradiated (controls)</td>
<td>8</td>
<td>52·50 ± 3·85</td>
<td>1 vs. 2, N.S.*</td>
</tr>
<tr>
<td>2. Irradiated (3·5 krad)</td>
<td>5</td>
<td>48·00 ± 6·26</td>
<td>2 vs. 3, N.S.</td>
</tr>
<tr>
<td>3. Irradiated (7 krad)</td>
<td>7</td>
<td>55·70 ± 4·87</td>
<td></td>
</tr>
<tr>
<td>Pharate adults</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Unirradiated, treated with 2 µl of acetone (controls)</td>
<td>35</td>
<td>51·70 ± 2·68</td>
<td>4 vs. 5, P &lt; 0·025</td>
</tr>
<tr>
<td>5. Unirradiated, treated with 2 µg of J.H.</td>
<td>25</td>
<td>39·20 ± 4·08</td>
<td>5 vs. 6, P &lt; 0·025</td>
</tr>
<tr>
<td>6. Irradiated (7 krad), treated with 2 µl of acetone</td>
<td>21</td>
<td>57·20 ± 3·50</td>
<td>6 vs. 7, N.S.</td>
</tr>
<tr>
<td>7. Irradiated (7 krad), treated with 2 µg of J.H.</td>
<td>27</td>
<td>62·77 ± 2·97</td>
<td></td>
</tr>
</tbody>
</table>

* Not significant (P > 0·05).

**Ovary maturation**

Irradiation of newly emerged females with 3·5 krad significantly reduced the number of insects with mature ovaries. However, the number of eggs per insect
with mature ovaries was not significantly different from that of the controls. A dose of 7 kR significantly reduced not only the number of insects with mature ovaries but also the number of eggs per insect with mature ovaries, irrespective of whether the insects were irradiated as newly emerged or as pharate adults. Treatment of irradiated insects with J.H. did not repair the inhibition in yolk deposition, whereas J.H. treated controls showed a slight, but not significant (P = 0.07), increase in the number of eggs per insect (Table 2).

Table 2—Effect of gamma-radiation and J.H. application on ovary maturation in T. molitor

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of insects examined</th>
<th>Percentage of insects with mature ovaries</th>
<th>Total No. of eggs</th>
<th>Mean No. of eggs/insect with mature ovaries</th>
<th>Mean No. of eggs/insect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Newly emerged adults</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Controls (normal)</td>
<td>68</td>
<td>96a</td>
<td>700</td>
<td>10.9a</td>
<td>10.2a</td>
</tr>
<tr>
<td>2. Controls, treated with 2 μg of J.H.</td>
<td>22</td>
<td>95a</td>
<td>281</td>
<td>13.4a</td>
<td>12.8a</td>
</tr>
<tr>
<td>3. Irradiated (3.5 krad)</td>
<td>17</td>
<td>71b</td>
<td>81</td>
<td>6.8a</td>
<td>4.8b</td>
</tr>
<tr>
<td>4. Irradiated (7 krad)</td>
<td>27</td>
<td>26c</td>
<td>33</td>
<td>4.7c</td>
<td>1.2c</td>
</tr>
<tr>
<td><strong>Pharate adults</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Irradiated (7 krad)</td>
<td>34</td>
<td>15c</td>
<td>11</td>
<td>2.2c</td>
<td>0.3c</td>
</tr>
<tr>
<td>6. Irradiated (7 krad), treated with 2 μg of J.H.</td>
<td>23</td>
<td>15c</td>
<td>10</td>
<td>3.3c</td>
<td>0.4c</td>
</tr>
</tbody>
</table>

Means in the same column followed by the same italic letter are not significantly different at the 5 per cent level of probability.

**Ovary transplants**

Young ovarian transplants from normal to normal and from normal to irradiated females showed conspicuous yolk deposition 10 days after transplantation, whereas transplants from irradiated to normal females did not (Table 3).

Table 3—Results of transplants of normal and irradiated (7 krad) ovaries into normal and irradiated (7 krad) female T. molitor

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of insects</th>
<th>Survived (%)</th>
<th>Percentage with yolk laden transplants</th>
<th>Comparison of means</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normal to normal</td>
<td>11</td>
<td>77</td>
<td>90</td>
<td>1 vs. 2, N.S.*</td>
</tr>
<tr>
<td>2. Normal to irradiated</td>
<td>32</td>
<td>58</td>
<td>55</td>
<td>2 vs. 3, P&lt;0.005</td>
</tr>
<tr>
<td>3. Irradiated to normal</td>
<td>25</td>
<td>70</td>
<td>0</td>
<td>1 vs. 3, P&lt;0.005</td>
</tr>
</tbody>
</table>

* Not significant (P>0.05).
Sensitivity of irradiated males to female pheromone extract

The sensitivity of males exposed to 7 krad was not significantly different from that of control insects on all the 3 days tested. Males irradiated with 16 krad showed a slight decrease in their sensitivity to female extracts 6 days after irradiation, and those exposed to 32 krad did significantly less than those of the other three groups on all the three days tested (Table 4).

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Radiation dose (krad)</th>
<th>No. of insects tested</th>
<th>Percentage of males responded</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>1</td>
<td>0 (controls)</td>
<td>40</td>
<td>67.5</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>40</td>
<td>85.5</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>40</td>
<td>85.0</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>40</td>
<td>55.0</td>
</tr>
</tbody>
</table>

Group 1 vs. 2, not significant; 1 vs. 3, not significant; 1 vs. 4, \( P < 0.05 \) on all days tested; 2 vs. 3, not significant; 2 vs. 4, \( P < 0.05 \); 3 vs. 4, \( P < 0.05 \).

Sexual vigour of irradiated males

There was no significant difference in the mating ability of normal males or of those exposed to 7 krad of gamma-radiation. However, the mating ability of males exposed to 16 krad was significantly inhibited (Table 5).

<table>
<thead>
<tr>
<th>Rank</th>
<th>Normal</th>
<th>7 krad</th>
<th>16 krad</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of males mated first</td>
<td>33.3</td>
<td>46.0</td>
<td>6.6</td>
</tr>
<tr>
<td>Percentage of males mated second</td>
<td>45.0</td>
<td>18.8</td>
<td>10.7</td>
</tr>
<tr>
<td>Percentage of males mated third</td>
<td>0</td>
<td>15.4</td>
<td>16.7</td>
</tr>
<tr>
<td>Percentage of males mated from each group</td>
<td>78.3</td>
<td>80.2</td>
<td>34.0</td>
</tr>
</tbody>
</table>

Normal vs. 7 krad, N.S.
Normal vs. 16 krad, \( P < 0.05 \).
Seven krad vs. 16 krad, \( P < 0.05 \).

DISCUSSION

Our data show that doses of gamma-radiation that inhibit yolk deposition do not interfere with sex pheromone production in female *T. molitor*. This is in conformity with Statler's (1970) observations from field studies that radiation sterilization of female gypsy moth, *P. dispar*, does not affect its attractiveness to...
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males. WHARTON and WHARTON (1957) showed that in P. americana low doses of 2 MeV electrons damaged oothecal production permanently and pheromone production temporarily, but on recovery the pheromone production overshot the normal level. Since there is an inverse relationship between oothecal production and pheromone production, WHARTON and WHARTON concluded that the increase in pheromone production was due to the destruction by irradiation of the mechanism associated with oothecal production. Whether this is due to radiation damage to the colleterial glands, ovaries, or via damage to the neuroendocrine system is not evident from their studies. Although the role of J.H. in yolk deposition in insects is established (ENGELMANN, 1970), the inhibition of yolk deposition observed in the irradiated T. molitor was not alleviated by external application of J.H. It is also known that J.H. is involved in the production of sex pheromone in the cockroach, Byrsotria fumigata (EMMPRICH and BARTH, 1968), the bark beetle, Ips paraconfusus (confusus) (BORDEN et al., 1969), and in T. molitor (MENON, 1970). Therefore, if the pheromone synthesizing mechanism is not damaged by irradiation, the excess of J.H. applied to the insect may be utilized by it to increase pheromone production. This perhaps accounts for the higher level of pheromone activity in the irradiated T. molitor treated with J.H. On the contrary, normal females treated with J.H. showed a decrease in pheromone level on day 10. It is likely that the excess of J.H. triggers the mechanism of yolk deposition, which in some manner inhibits the mechanism of pheromone production. BELL and BARTH (1970) have demonstrated this inverse relationship between yolk deposition and pheromone production in B. fumigata that have been treated with excess of J.H.

The ovaries of irradiated T. molitor failed to mature either in the presence of J.H. or after their transplantation into normal females, whereas irradiated beetles deposited yolk in ovarian transplants from normal insects. Hence, there is strong inference that the inhibition in yolk deposition in the ovaries of irradiated insects is due to radiation damage to the ovary itself rather than to the neuroendocrine system. BAILIE and SHIPP (1970) came to similar conclusions using irradiated Dacus cucumis. According to LA CHANCE and BURNS (1963) the inhibition in yolk deposition in irradiated Cochliomyia hominivorax was due to damage to the nurse cells. In the case of the T. mohtor, however, it is not certain whether the radiation damage is to the nurse cells and/or to the follicle cells.

Radiation sensitivity of the various developmental stages of T. molitor have been reported (NICHOLAS and WIANT, 1959; MENVINICK and CROSSLEY, 1968) but little is known of the effect of radiation on the induction of sterility in males or their ability to perceive sex pheromone after exposure to radiation. Some preliminary observations indicated that 7 krad is a substerilizing dose, whereas 16 krad induces 99-9 per cent sterility in the males of T. molitor. Although irradiation of males with 7 and 16 krad did not impair their ability to perceive the pheromone from female extracts, their mating competitiveness (sexual vigour) was severely affected at 16 krad. It is pertinent to mention here that WHITE and HUTT (1971) found that when males of L. pomonella were irradiated with various doses of gamma-radiation and released in a field, the number of irradiated males caught in traps baited with
females decreased with increase in radiation dose. This has been attributed to loss in vigour in irradiated insects. In our studies we observed that most of the males irradiated with 16 kR were lethargic within 7 days of irradiation. Whether this was due to structural damage to the sarcoplasmic reticulum and mitochondria of the skeletal muscles similar to that observed in the flight muscles of gamma-irradiated housefly Musca domestica (NAIR and BHAKTHAN, 1969) is currently being investigated.

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