CHAPTER 7. SEXUAL COMMUNICATION OF BACTROCERA PAPAYAE

7.1 INTRODUCTION

The definition of communication includes any stimulus produced by one animal that induces a response in other. From ethologists point of view communication behaviour allows the exchange of information between interaction descriptive (Arakaki et al., 1984; Foote et al., 1993; and Briceno et al., 1996)

7.1.1 Significance of the study:

Due to the public awareness of the excessive use of chemical insecticides entomologists concerned with insect pest control are showing greater interests toward integrated pest management based on solid biological and ecological knowledge (Clark et al., 1967; Guthrie and Sheets, 1970; Moreno et al., 1991; and Shelly, et al., 1996). An important step is to reduce the pest population to levels below the economic threshold. The economic threshold is the density at which control measures should to applied to prevent increasing pest population from reaching the density that will cause economic damage (Stern et al., 1959). However for the fruit flies the economic threshold is often near zero due to the acceptance of only perfect fruits by consumers and by those concerned with importing fruits. As a result, research entomologists currently place great emphasis on the Sterile Insect Release Method (SIRM) for eradication. The SIRM involves the release of laboratory reared insects which have been sterilized with gamma radiation and allowed to compete for mates with pest males. Eradication can be achieved on relative low cost compared to insecticide on long term basis without ecological problems associated with insecticide use. However the effectiveness of the SIRM relies to a great extent on the ability of the laboratory reared males to mate successfully with wild females, Thus proper mating behaviour and efficient function of sterilized mass reared males in the field are of major concern.
Two important aspects of behavioural quality are: (1) searching ability for mating sites or sexual partners and (2) mating behaviour are affected in mass reared insects (Boller, 1972). Examples include the alteration of female responsiveness to male pheromone in screw worm flies Cochliomyia hominivorax (Fletcher et al., 1968) the production of a less attractive sex pheromone in boll weevils Anthonomus grandis male and the unsynchronized mating period of laboratory and wild population in codling moths Carpocapsa pomonella (Boller, 1972). However tephritid species-specific example of laboratory effects are reported in two species. Laboratory males of C. capitata have a lower insemination rate than recently established laboratory male (Rossler, 1975; and Susan et al., 1986). Moreover both sexes of the laboratory strain reach sexual maturity much earlier than the wild (Wong, and Nakahara, 1978). From the laboratory rearing sterilization with gamma radiation there were some problems related to mating A. suspensa pupa decrease the frequency of signaling behaviour of males (Sharp and Webb, 1977). In C. capitata the ability of sterilized males to compete successfully with non sterilized males in mating is reduced by 50 % and the peak of mating activity of both sexes is delayed as a result of sterilisation causing unsynchronised sexual activity (Holbrook, and Fujimoto, 1970; and Robert et al., 1992). Therefore it is not surprising that deviation in sexual ability sterilised laboratory reared flies from that of ones has suggested as a primary cause for the failure of several SIRM programs (Ito and Kawamoto, 1979). Successful eradication of B. dorsli using effective SIRM program requires a complete knowledge of its complex sexual behaviour in order to develop laboratory strain which can either mate in the field or prevent normal mating of wild male. Basic research on the sexual behaviour of pest had recently been recognized as an important component of insect control. Presently, sexual communication of this species poorly is understood (Arakaki et al., 1984; Keiser et al.,
1973; Kobayshi et al., 1978; Roan et al., 1954, and Headrick et al., 1995). Therefore detailed experimental data regarding the sexual behaviour of B. dorsalis will certainly be of value in the effort to control this pest.

The objective of study.

Studies on the sexual behaviour of B. papayae was confined to examining the significance of acoustic, olfactory or visual signals produced by the wing fanning of males and how these signals are used in sexual communication. This is divided into two main phases.

**Attraction phase** which brings males and females together from far distance (average 50 cm) to the same place and at the same time for mating.

**Courtship behaviour** at close range (average 5 cm) which is displayed by males and use by the females to either accept or reject the courting individual. The data would provide additional information endurable or likely to be useful in developing effective control strategies. For example if sound and pheromones are as important in attracting and mating with females, a control method utilising these data can possibly be developed. Synthetic sources of odour and sound may potentially be used to confuse wild females which are attempting to locate male and as a result mating will be prevented.

Knowledge obtained from this research on B. papayae will have potential for both direct and indirect application to developing programs for this pest species as well as other related species.

**7.2 MATERIALS AND METHODS**

**7.2.1 Description of sexual behaviour:**

Three pairs of sexually mature virgin male and females were kept in a small cage \((30 \times 30 \times 30 \text{ cm})\) during 1030 to 1130 h to adapt them until the afternoon experiment. Observation of sexual behaviour were made at 25 - 27 °C, relative humidity 79 - 85 % RH and under natural lighting conditions from 1630 to 1930 h. Detailed activities performed including movement and interactions performed
by both sexes were recorded. Observations ended when all activities stop or ceased at about 30 min after sunset.

7.2.2 Sexual activities with respect to age: Some specific sexual activities was recorded in both sexes from day 1 to 28. These activities included male wing fanning, female response (visiting wing fanning male) and mating were examined separately in addition to the relationships between them.

A. Male wing fanning.

Fifty marked males were kept together in a cage from the first day after emergence. Each day male observed to fan his wings were removed from the cage during the observation period (1630 to 1930 h) and replaced with a new virgin unmarked male of the same age. This way a constant density of flies in the cage was maintained to minimize any possible influence of density dependent factors. The total number of marked original males of each age engaging in wing fanning behaviour was recorded. The experiment was continued until about 95 % of males seen to fan their wings.

B. Female responsiveness.

Fifty one day old females were marked and kept into screened large cage (100 × 100 × 50 cm) containing a chilli plant. Sexually mature virgin male inserted in mini screen cages were hung on the plant. These mini cages (4 cm in diameter and 5 cm in height) (plate 10) were screened with white nylon covering contained one or two males per cage. Each day any female which was seen to be visited by male, were removed
Plate 10: Laboratory mini cage
from the cage during the observation period (1630 h to 1930 h) and replaced with a
new virgin unmarked females of the same age. The total number of the marked original
females at each age visiting males was recorded. The experiment was terminated until
about 90 % of the 50 females had visited males.

C. Matings.

Fifty 1-day old males and 50, 1-day old females were marked and kept together
in a screened large cage of containing chilli plant. Each day any flies found copulating
were removed at about 15 - 30 min after darkness had fallen and substituted with new
virgin unmarked flies of the same age. The total number of marked males of each age,
which copulated, was recorded. The location where matings took placed was recorded.
The experiment was continued until about 90% of the 50 male and 50 females had
mated.

7.2.3 Responsiveness and receptivity of mated females:

Ten virgin sexually mature (ages between 25 -30 days) males and 10 females
from the same age group were introduce together in each of 20 cage to allow matings
for three days. Every day at near 15 - 30 min after sundown, pairs observed copulating
were carefully removed from the cage using small glass vials and held until they
separated. The mated males and new virgin females were placed back into the same
cage, but mated females were held for further experiments. The experiment was
repeated three times, each time was terminated when all females mated. By this way a
sufficient supply of mated females was obtained for further tests.
The following experiment were carried out on mated and virgin females of the same
ages to determine the effects of having mated once on their sexual responsiveness and
receptivity to more matings. The experiments were not extended to 18 days because the
effect of aged of flies may influence the results.
A. Responsiveness of mated females.

Female responsiveness is defined as female approaching and showing response towards signaling males.

The experiment on female responsiveness to males were done by releasing 20 virgin and 20 marked mated females from the same age into a large screen cage of. One to two sexually mature males were placed in each of several mini screened cages hung on a plant (chilli) inside the larger cage. During the observation period (1630 to 1930 h). The experiment was repeated every two days (2, 4, 6, 8, 10, 12, 14, 16, and 18 days) after the first mating of mated females. The number of virgin and marked females visiting males was recorded.

B. Receptivity of mated females.

Female receptivity is defined as a female readiness for mating. Two experiments were conducted to determine if a first matings would lead to further matings of females.

Experiment 1. Mated and virgin females together.

Ten virgin females and 10 marked mated females were placed with 10 males of the same age to test for female receptivity to matings. Each day pairs in copula were removed from the cages and the number of matings was recorded. Mated females were discarded and replaced. Mated males were placed back into the cage to maintain the constant proportion of sexes. The experiment continues every 3 consecutive days. New flies were set up in the same method for successive three day periods (1-3, 4-6, 7-9, 10-12, 13-15, and 16-18 days) after the first mating of mated female. A total of three replicates were done at each time period.

Experiment 2. Mated and virgin females tested separately.

Virgin and mated females were tested separately for their mating receptivity.

Ten males and 10 virgin or mated females were placed together and allowed to mate for
3 consecutive days. Every day, pairs in copula were removed from the cages and the number of matings was recorded. Mated females were discarded and replaced but mated males were replaced into the cages. By this way, a constant proportion of sexes and a high number of experienced mating males that were potentially able to copulate were maintained. The experiments were carried out as 1-3, 4-6, 7-9, 10-12, 13-15, and 16-18 days of mated females.

7.3 Results

7.3.1 Description of sexual behavior.

Sexual behavior of *B. papayae* can be divided into the following:

**A. Signal emission.** As light intensity decreased during the one hour before dusk sexually mature male increased activities including rapid walking and short distance flight (about 5 - 8 cm). As a result, encounters between individuals became frequent. Females were mostly stationary but showed some movements such as walking or aggression with encountered individuals. Then males assumed signaling activities of rapid wing fanning and rhythmic abdominal stroking with the rear legs. During this period an odour, hypothesized to be a sex pheromone, would be emitted. The male fanned their wings up and down rapidly while holding them over their backs. This wing fanning produced audible buzzing sound emitted in a series of pulses for varying duration from 5sec. to 2 min. At the same time the males frequently stroked the posterior region of the abdomen by using both of hind legs alternately. This abdomen stroking rhythmically coincided with a sound that could be heard intervening the buzzing sound produced by the wing fanning. Based on my observation of males signaling activities such sound that was produced by touching the wings with their legs when males were stroking their abdomen. The odour mentioned above was also easily detectable during the active signaling by males. During signaling, a male responded
quickly to movement near by. Between bursts of wing fanning a male frequently changed direction and then resumed wing fanning. The signaling male usually remained in one place or walked toward other individuals. Various activities usually occurred several times prior to the first mating attempt by males. The signaling by males appeared to excite females, which became active walking about and quivering their abdomen vertically. After 3 hours observation i.e. the onset of dusk alone did not seem to excite the sexual activity females. Females confined in a cage without males were inactive displaying no walking or quivering movements.

**B. Courtship behaviour.** Females approached signaling males rather than vice versa. A female either walked towards male or flew about until she landed near him. The male then turns toward the female usually in face to face position and walk close to while still fanning his wings rapidly in a long burst. During a brief frontal orientation after the female approached to about 1 cm from the male wing fanning continued. When the female stopped the male flew (while in the under side the leaf) or jumped (while on the vertical surface the leaf) from the frontal position over the head of female and onto her back. The male usually flapped his wings during mounting attempts and later settle on her back. However this wing flapping seemed to function merely as way to balance himself while attempting to achieve intromission rather than serving as an acoustic signal like the rapid wing fanning performed previously.

**C. Copulation.** The female in non-receptive state repeatedly resisted males attempting to mount. She would bend her abdomen when the male attempts to mount. The bending of her abdomen and ovipositor downwards preventing the male from successfully mounting her. Simultaneously the female tried to dislodge the male from her back using her hind legs and wings. Both of them struggled and eventually the female dislodged the male or the attempting male would give up. However, when female was receptive she
would turn the distal end of her abdomen upward and extend her ovipositor. The male would bend his abdomen downwards attempting to insert his aedeagus into the females ovipositor and copulation could be achieved within a few seconds. The male held the female by the abdomen with fore legs but held his middle and hind legs on to the substrate. The copulating pair sometimes flew as short distances or walked away from the area of intense activities to settle on upper level of the cage. Pairs remained in copula beyond dusk and into darkness when the observation ceased. Other sexual activities e.g. male wing fanning and female responses subsided suddenly with the onset of darkness.

7.3.2 On sexual Activities with Respect to Age.

Figures 7 exhibiting cumulative frequency (%) of male wing fanning and female approaching respective. Males first exhibited wing-fanning behaviour at an earlier age even before female first become responsive to males signals. The first time that wing fanning in males was observed was at the age 8 days but female first approached males at age 10 days. More than 50% of females were observed responding to male signals at 18 days. At the age 28 days 96% of the males were observed wing fanning and 84% of the female were observed to respond to signaling males.

C.Mating. The first mating pair was observed at the age of 9 days (Fig. 7 b) which was close to the age of first wing fanning male and first approaching female. At the age of 15 days more than 50% of the matings observed. At the age of 26 days, 92% of the mating observed and no further mating was seen observed through days 28.

In this mating experiment the majority of signaling males and matings were seen on the under side of the leaves. Out of 150 mating pairs observed, 55% were on the bottom surface of the leaves, 39.4% were on the cage walls and remaining 5.6% were on the ceiling or bottom of the cage and on the plant pot.
Fig. 7. Cumulative percentages of mating for different ages in (a) males (b) females
During the dark phase with about 1 hour after sundown brief check (using flash light) did not observed any sexual activity or new copulating pairs. Most of the flies were immobile but slight movements or short range walking if the flies were disturbed by strong flashlight for longer than 30 sec.

7.3.3 Responsiveness and Receptivity of Mated Female.

A. Responsiveness of mated females.

In all tests, the period 2 - 18 days after mating, females were significantly less responsive to signaling males than virgin females (Table 13). At 2 days after first mating only 15 % of responding females were mated compared to 75 % virgins of the same age. The approaches by mated females did not increase with increase number of days after first mating.

B. Receptivity of mated females.

In experiment 1 when both virgin and mated females were caged with males, mated females were significantly less receptive to further mating than virgin females (Table 14). This highly significant level decreased receptivity to further mating continued in mated females throughout the period of 1 - 18 days after the first mating. The total mating by both mated females and virgin females did not increase with increasing time (days) (Table 14).

Experiment 2. When virgin or mated females were caged separated with males, mated female were still significantly less receptive to further matings than virgin females (Table 15). This highly significant level of decreased receptivity to further matings was continued in mated female throughout the period of 1 - 18 days after first mating. The table 15. Also showed the matings by females whether mated or virgin did not increase with increasing time (days).
Table 13. The effect of the first mating on the approach by B. papayae* females to males during the period of the 2 - 18 days

<table>
<thead>
<tr>
<th>Days after 1st Mating</th>
<th>Females approached males#</th>
<th>Percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Virgin fem.</td>
<td>mated fem.</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
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<tr>
<td>14</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>18</td>
<td>12</td>
<td>2</td>
</tr>
</tbody>
</table>

* = 20 virgin females and 20 mated females
** = highly significant difference (P = 0.001)
# = means of 3 replicates
M. = mated female
V. = virgin female
fem. = Female
Table 14. The effect of first mating on the subsequent of mating behaviour of *B. papayae* females during the period 3-18 days in the presence of virgin females

<table>
<thead>
<tr>
<th>Days after 1st. mating</th>
<th>Mean number of</th>
<th>Total mean of tested</th>
<th>Percentages</th>
<th>Sig. level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V. F</td>
<td>M. F</td>
<td></td>
<td>V. F</td>
</tr>
<tr>
<td>3</td>
<td>94</td>
<td>26</td>
<td>120</td>
<td>78.33</td>
</tr>
<tr>
<td>6</td>
<td>101</td>
<td>11</td>
<td>112</td>
<td>90.02</td>
</tr>
<tr>
<td>9</td>
<td>102</td>
<td>7</td>
<td>109</td>
<td>93.56</td>
</tr>
<tr>
<td>12</td>
<td>95</td>
<td>10</td>
<td>105</td>
<td>90.48</td>
</tr>
<tr>
<td>15</td>
<td>91</td>
<td>8</td>
<td>99</td>
<td>91.92</td>
</tr>
<tr>
<td>18</td>
<td>50</td>
<td>10</td>
<td>60</td>
<td>83.33</td>
</tr>
</tbody>
</table>

** = highly significant difference (P = 0.001)
Table 15. The effect of first mating on the subsequent mating behaviour of *B. papayae* females during the period of 1 - 18 days

<table>
<thead>
<tr>
<th>Days after mating</th>
<th>Number of female* tested</th>
<th>Mean number of female mated$^6$</th>
<th>Percentages</th>
<th>Sig. level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>virgin fem.</td>
<td>mated fem.</td>
<td>virgin fem.</td>
</tr>
<tr>
<td>1 - 3</td>
<td>100</td>
<td>30</td>
<td>9</td>
<td>60</td>
</tr>
<tr>
<td>4 - 6</td>
<td>100</td>
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<td>7</td>
<td>70</td>
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<td>100</td>
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<td>13 - 15</td>
<td>100</td>
<td>27</td>
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<td>54</td>
</tr>
<tr>
<td>16 - 18</td>
<td>100</td>
<td>44</td>
<td>14</td>
<td>88</td>
</tr>
</tbody>
</table>

* virgin and 50 mated

** highly significant differences (P = 0.001)

Virgin and mated female were tested separately
7.4 Discussion

*B. papayae* carries its mating to about an hour each day during the decrease in light intensity at dusk. This narrow daily period of mating reported in oriental fruit fly by Roan et al., (1954) and was confirmed several times (Arakaki et al., 1984; Keiser et al., 1973; Kobayashi et al., 1978; and Susan, 1986). In cases where the components of sexual behaviour of *B. papayae* were examined, the results again confirmed that this species exhibit its sexual behaviour and mating only at dusk, (Keiser et al., 1973). In addition, several checks made after sundown did not detect any more copulating pairs or any sexual activities suggesting that darkness contribute to decrease in their sexual beh0000aviour and mobility. However copulation of *B. dorsalis* in total darkness reported by Keiser et al., (1973) may be consequence of the limited space in the small cages that were used leading to encounters and matings of small number of flies of other species of sub tropical and tropical tephritids that normally mate only at dusk like, *B. cacuminatus*, *B. tryoni*, *B. oleeae* and Mexican fruit fly *A. ludens* (Back and Pemberton1917; Hiroyuki et al., 1984 and Iwahashi et al., 1986, Causses et al., 1966; Mcphail and Bliss, 1933; Myers,1952; and Myburgh, 1962). The restricted period of sexual activity may result from an interaction between the declining light intensity and an endogenous daily rhythm of sexual responsiveness in both sexes as found in *B. tryoni* (Tychsen, 1975 and Fletcher, 1977).

In *B. papayae* the wing fanning by males and abdominal stroking with wings at dusk appeared to generate sound. The buzzing and chik sounds heard rhythmically intervening with the buzzing sound were generated by wing fanning and touching of the legs with the wing respectively. These sounds serve as auditory stimulus to females. Sound produced in some tephritids species such as *B. cucurbitae* and *B. tryoni* has been reported to result from stridulation of the wing on abdomen cilia (Fletcher, 1968;
Monro, 1953; Suzuki & Koyama, 1980; and San Jose et al., 1996). In addition to production of sounds, wing fanning may be associated with the release of a sex hormone in this species as odour was released during the signaling of males. This is also reported in *B. cucurbite*, and *B. tryoni* (Kuba et al., 1984; and Drew, 1987).

The wing fanning males increased in the number and responding to males signal and mating are closely correlated with the ages of adults (Fig. 1 and Fig. 2). Since the onset of mating assume sexual maturation, the results showed that the onset of all sexual activities of *B. papayae* investigated in this study coincided with the attainment of maturity (Fig.1). Though the onset of sexual responsiveness in *B. tryoni* does not depend on ovarian maturation (Fletcher and Giannakakis, 1973) there was a positive correlation between the percentage of female mating and ovarian development in *B. cucurbiteae* (Suzuki and Koyama, 1980). Furthermore the response of *A. suspensa* females occured as the ovary reaches matured size and decreased in both younger and older females (Nation, 1972). It is reasonable to hypothesize that the females of *B. papayae* response of approaching to signaling males were correlated to female ovarian development.

The female responsiveness and receptivity to males would not be totally lost after single mating as the results of this study indicate tremendous effect of mating on both the responsiveness and receptivity of females (Table 1, 2, and 3). Mated females neither approached signaling males nor accepted subsequent mating as readily as virgin females. The inhibition of responding and remating did not decline even at 18 days after the first mating. It was also noted that even when the males were monopolized by caging with only one type of female the results were not different from when they were caged with both types of females i.e. virgin and mated. Such results agree well with the finding of Kobayashi et al., (1978) that the previously mated females of *B. dorsalis* did
not respond to either live male or rectal glands. However the results disagree with the report of Christensen and Foote (1960) when females of B. dorsalis mate and remate frequently at 4 - 5 day intervals. It is possible that the inhibition of responsiveness and receptivity of mated females over a period of time during this study due to lack of opportunity to utilize the sperm from the first mating. In this experiment study, females were not provided with substrate for oviposition after the first mating. On the other hand, oviposition may not be the actual inhibiting factor because mating occurs repeatedly in both laying and non laying females of another species of tephritids, Euleia fratria (Tauber and Toschi, 1965).

In B. papayae mated females becomes sexually unresponsive and unreceptive for at least a few days. Similarly in C.capitata female were not attracted to male pheromone at least 10 days after mating (Feron, 1962). The male pheromone of B. tryoni did not elicits any response in females that mated two days previously (Fletcher and Giannakakis, 1973). The refractory period in B. papayae mated females lasted for a few days as B. tryoni and B. oleae. Females that mated may abstain several weeks after mating (Fay and Meats, 1983; Tzanakakis et al., 1968).

The reason for the refractory period to remating may be associated with the time energy costs of mating. First copulation may be a disadvantage to females if she have obtained a sufficient sperm supply. This agree with report of Nakagawa et al., (1971) that females of C.capitata do not remate for several weeks after receiving sufficient amount of sperm. Secondly it would be advantageous for the male to develop any mechanism to deter chance of further mating of the same female by different male. Possibly in this species a male substance or chemical stimulus associated with sperm may induce female unreceptivity as has been suggested in B. oleae (Tzanakakis et al., 1968).
Sexual receptivity may be regained when the sperm in the spermatheca have been utilized. For example, the proportion of remated females in *C. capitata* gradually increases to about 30% at four weeks, and at seven weeks after the initial mating (Nakagawa *et al.*, 1971). Such female receptivity to repeated mating is negatively correlated with the sperm content in the spermatheca. However, the return of sexual receptivity of mated *B. papayae* females did not occur during the period of 18 days experiment. The reason might be a lack of opportunity for females to oviposit and delete their sperm supply. Form this study, it indicates a reduction in responsiveness and receptivity in mated *B. papayae* females as compared to virgin ones at least during the 18 days after the first mated. The mechanism reduction female responsiveness and receptivity is unknown. If the inhibition of remating in females can be overcome and sperm can compete successfully with those from a previous mating, there may be selection favoring male that males court mated female (Parker, 1970, 1974). In the other study, SIRM (Sterile Insect Released Method) the reduced receptivity of females to further matings is advantageous to released sterilized males (*A. suspensa*) due to their higher proportion in the wild. If there is different sperm viability a return of receptivity of mated females may affect the use of this method. Wild females may be able to produce viable offspring if wild males whose sperm can compete successfully with those from the previous sterilized males subsequently inseminated them. On the other hand remating of wild females with sterilized males whose sperm are as viable as those the previous wild male is advantageous to SIRM programs (Sharp and Weeb 1977).