

Full Length Research Paper

# Study on wing fanning as a signal of sexual response and courtship behavior of *Bactrocera papayae*

M. Sofian-Azirun, F. A. Muttardi, M. Rosli Hashim and M. Motior Rahman\*

Institute of Biological Sciences, Faculty of Science, University of Malaya 50603 Kuala Lumpur, Malaysia.

Accepted 25 May, 2011

**A series of experiments was carried out at the Institute of Biological Sciences, University of Malaya to identify wing fanning as an attraction or signal of sexual response and courtship behavior of *Bactrocera papayae*. Sexual natures such as signal or attraction and courtship behavior were determined through males and females with modified wings and sensory receptors, respectively. Wing fanning was effective to attract female towards male (42 to 48%) and vice versa (22 to 25%). Sexual attractiveness and signaling by males varied with light intensity. Females were attracted more at 18:20 to 18:55 by frequent wing fanning by males and sharply declined after sunset. Least attraction (10%) was observed by females toward males with modified wings compared to males with non modified wings. Similarly, males were less attracted by females with modified sensory receptors. Significantly, weaker sexual response was found when both the aristae and antennae were removed. Mating rate (12 to 14%) was reduced significantly by males wing removal and clipping (>50%) and wing bases waxed. Modified females mated less than non-modified ones due to the preventive measure of the pheromone receptor. Olfactory signals appear to be more important than acoustic signals in determining whether a female would mate or not. Pheromone along with acoustic receptor modification played a key factor in the success of mating. Acoustic and olfactory signals appear less important when eliminated singly, but combined elimination of olfactory and acoustic signal reduces the mating of females by a greater magnitude.**

**Key words:** Attraction, courtship, response, sexual, signaling.

## INTRODUCTION

Sexual attraction signals are used in sexual communication which is emitted by any individual over a reasonably long range to attract the opposite sex. The attraction signals include visual, acoustic or olfactory that are noticed in some species, for example, in gall forming tephritids where signaling consisted of simple wing fanning (Zwolfer, 1974), while some species do not produce any attraction signals as the case of *Tephritis dilacerate* (Berube, 1978). In *B. papayae*, sexual communication occurs by a combined action of attraction and courtship. Some of the tephritid males, such as: *Rioxa pornia* (Pritchard, 1967; Hee and Tan, 1998), *C. capitata* (Bateman et al., 1976; Feron, 1962) and *B. tryoni* (Bellas and Fletcher, 1979) produce pheromones for attracting females, while *A. suspense* (Nation, 1972;

Perdomo, 1974; Bateman et al., 1976; Webb et al., 1976, 1983a) produces calling song generated by burst of rapid wing fanning along with the release of sex pheromones. The males of *B. cucurbitae* (melon fly) produce sound by vibrating their wings together with pheromones to attract female (Rolli, 1976). The shiny gold body, distinctive patterned color markings on the body, or wings and elaborate movements or postures possesses a significant visual signal of sexual approach in *A. suspense* (Dodson, 1978).

Acoustic signals are used as an important tool during courtship in several tephritid species. A particular song serves to persuade a female of *Toxotrypana curicuda* (fruit fly) to remain in place for the courtship (Sivinski and Webb, 1985a), while in *A. suspense*, sound produce by the wings of the male or female stimulates sexual arousal in both sexes (Sivinski and Webb, 1985a; Webb et al., 1984). *B. tryoni* and *C. capitata* produced specific acoustic signals during courtship (Monro, 1953; Webb et al., 1983b). In addition, *C. capitata* male produced three

\*Corresponding author. E-mail: [mmotiorrahman@um.edu.my](mailto:mmotiorrahman@um.edu.my)/[mmotiorrahman@gmail.com](mailto:mmotiorrahman@gmail.com).

different types of acoustic signals comprising sound, calling courtship and copula sound prior to a successful mating (Feron, 1962; Webb et al., 1983b). However, the actual roles of these signals are not clear yet. Pheromones (sex hormone) are produced by males in numerous tephritids, including *A. suspensa*, *C. capitata*, *B. cucurbitae*, *B. tryoni*, *Rhagoletis pomonella* and *R. pomonia* (Fletcher, 1977; Kobayashi et al., 1978; Nation, 1972; Pritchard, 1967; Prokopy, 1975). The pheromones stimulate the copulatory responses at close range of *B. cucurbitae* and *B. tryoni* (Baker et al., 1982; Bellas and Fletcher, 1979; Fitt, 1981). Wing fanning is a common phenomenon of sexual activity and acoustic and olfactory stimuli have been produced frequently by males of *B. papayae*. Wing fanning generates a buzzing sound (Roan et al., 1954) and release of pheromones that localize in gland complex of rectal sac, which is highly attractive to virgin females (Schnelez and Boush, 1971; Kobayashi et al., 1978). Even though the behavior and responses suggested that stimuli played an important role in the success of mating, there is no experimental proof to pinpoint which stimuli elicited sexual arousal in the females. However, the visual signals such as variety of markings and colors or movement position have not been reported in any of the tephritids.

Sexual signal produce by a species causes a specific reaction in the opposite sex for its reception. Several types of sensory receptors are known to utilize for signal reception by individuals of various species. In Dipteran species of *Drosophila melanogaster*, the feather arista is the acoustic receptor situated on the 3rd antennal segment of the female. When the arista is stimulated by the sound wave, it stimulates the Johnston's organ located in the 2nd antenna segment (Clark, 1971; Manning, 1967). However, different species of Diptera possess a similar type of sound receptor, but no such information is available on the location of acoustic receptors in tephritids. In addition to the sound receptor, the 3rd antennal segment of *D. melanogaster* also bears olfactory receptors (Kellogg et al., 1962). Among tephritids, *B. tryoni* and *B. oleae*, which have the same third antennal segment, are covered with large numbers of chemosensilla (Giannakakis and Fletcher, 1985; Hallberg et al., 1984). Studies on *B. oleae* indicate that sensilla on the antennae can respond to sex pheromone and other volatiles (Bateman and Morton, 1981; Van der et al., 1984). Many tephritid species, including *A. ludens* (Mexican fruit fly), *A. suspensa*, *C. capitata*, *B. susurbitae* and *B. dorsalis* possess sensilla on the 3rd antennal segment (Dickens et al., 1988; Robacker and Hart, 1987). The sensilla are believed to function as olfactory receptors because of their similarity in morphological characteristics with other reported chemoreceptor in insects. The sensilla found on the antennae of both male and female of *A. ludens* and *A. suspensa* gave similar response to the tested chemicals (Robacker and Hart, 1987; Robacker et al., 1986).

Although, much of the studies have been conducted on

different species of tephritid, only few reports are available on *B. papaya*. In depth studies on attraction and courtship behavior are limited in this species. Therefore, wing fanning as an attraction of sexual signal studies were conducted to get better understanding on the sexual communication mechanism and courtship behavior in this economically important species in Malaysia. Based on the these specific studies of sensory receptors and behavioral evidence, it is expected that the interference in the functioning of sensory receptors in receiving signals would provide some information regarding sensory modalities used by the species under this study. Therefore, modification of various sensory receptors in females was the primary approach for this study. In addition, male wing fanning has never been experimentally demonstrated to be a part of sexual communication. However, experiments were designed to determine the significance of wing fanning in order to evaluate sexual attractiveness and courtship behavior over time.

## MATERIALS AND METHODS

In this study, a series of experiments were conducted under both attraction and courtship phases. Assemblies of flies were an intermediate range for attraction experiments. In the experimental cage, distance was maintained by an intermediate range (70 x 110 x 70 cm), which was much less than the distance available in the wild. During the experiment, average distance was maintained for about 60 cm (varied range was from 20 to 100 cm) depending on the location of an individual. Individuals (1 or 2) were released into the cage at morning hours in order for them to be acclimatized with it before the afternoon experiment, and were tested for their attractiveness in several small screen mini cages, made with white nylon (5 cm in diameter and 6 cm in height). Two males were placed together in a mini cage, and it was noticed that the fan wing of one was more frequent than the other. These mini cages were hung on potted plants (chili plant) and were kept inside a large cage. Flies that landed on the mini cages were kept one minute and recorded as being attracted by the tested flies. Mini cages were placed on the plant about two hours before sunset. Observation started about one hour later and continued until darkness fell when all activities stopped. The experiments were conducted during different time of the year to minimize the day length difference in the experiment. Generally, the experiment was conducted during three hours period around dusk. The number of times that flies landed on the mini cages was recorded in each experiment.

### Attraction phase

#### Experiment 1: Study of attraction between male and female

Two experiments were conducted to determine the attractiveness of both male and female to the same sex. Prescreened selected wing fanning mature virgin males and mature virgin females were confined in each of the several mini cages (1 or 2 flies per cage) and were hung on chili and tomato plants. Both were placed on either side of a large screened cage. Each day, the confined flies were alternated between the two plants to minimize any effect of plant preference that could occur. Fifty (50) flies of each sex were tested. Any individual that landed on each mini cage, placed on the plants was recorded at 2-min intervals. Flies used in the study were

replaced for each observation period. A total of 5 groups of males and females were tested.

#### **Experiment 2: Study on the distribution of attractiveness over time**

An experiment was carried out to examine the attractiveness during a two-hour period around dusk (17:30 to 19:30 h). Prescreened mature (1 to 2) male flies were placed in each of the several mini cages and males were hung on plant and placed in a large screen cage. Hundred (100) female individuals were tested and 20 females were released into this cage to be attracted by wing fanning males. Individuals landed frequency on the mini cages for 5 min was recorded.

#### **Experiment 3: Effect of wing modification on the attractiveness of female**

A study was conducted to determine the role of male modified wing fanning to attract females. Pre-screened males were anaesthetized by chilling in the freezer of a refrigerator for 1 to 2 min. Modification of wing was done by a dissecting microscope which was used to clip more than 50% of the wing and by putting a drop of wax at the base of each wing. The purpose of clipping was to remove the portion of the wing that may rub against the abdominal cilia, but to allow males to still fan their wings normally. On the contrary, waxing was intended to inhibit the male wing fanning completely. The males without any modification of wing were used as control treatment. In this experiment, 1 to 2 modified and non-modified mature males were placed separately in each of the several mini cages. These cages were then hung on chili and tomato plants, one containing modified males, and the other containing non-modified males. Both plants were placed in a large screened cage, where 40 mature virgin females were released. Visiting time (approximately 2 min.) of females to males during 1-h period around dusk was recorded and all new virgin individuals were replaced for each test per day. The plants with modification males were alternated each day to minimize any effect of plant preference that could occur. Out of the 100 tested males, 50 males had modified wings, while the rest 50 males had non-modified wings (control). Consequently, a new batch of virgin females was replaced for each test per day.

#### **Experiment 4: Study on female sensory receptors in response to male**

This study was conducted to determine the significance of male wing fanning over intermediate range sensory receptors, such as acoustic, as well as olfactory that females may use for receiving male signals. Mature virgin females were anaesthetized by chilling in freezer of a refrigerator for about 1 to 2 min. Dissecting microscope was used for three types of modifications of female sensory receptors. Each method of modification was intended to prevent the female reception of a specific type of signal from males. These were removal of the arista (acoustic receptor), entire antennae (both acoustic and olfactory receptors), by using micro-scissors and painting the antennae (olfactory receptor) with black enamel leaving the arista free to receive acoustic signals. The modified females were tested to determine whether they were able to approach males or not as that of non-modified females. In attraction test, 1 to 2 mature males were placed in mini cages by maintaining distance of an intermediate range. Each of the modified and non modified 20 females was released into the large cage. Females landing on male cages were captured during test and identified to obtain the number of each type of female attracted to males. New virgin flies were replaced for each day of observation.

#### **Courtship phase**

Several experiments were conducted to examine the significance of sexual signal utilized at a close range (average closeness of 5 cm, and range from 2 to 10 cm) courtship. Mating measured by actual copulation of males was used as a criterion indicating the significance of signals produced. Pre-screened selected mature males exhibited wing-fanning behavior in all experiments. Before any modification, flies were anaesthetized by chilling in freezer of a refrigerator for 1 to 2 min. Mating was conducted by placing tested individuals in a small cage (5 cm in diameter and 6 cm in height) and recorded each day until sunset. The test, in the cage, was terminated when mating was observed or after 7 days, although, it was always observed earlier.

#### **Experiment 1: Study on male wing fanning in mating**

Wing fanning performed by modified and non-modified males at close range was examined in two separate experiments to understand its importance in a successful mating. Modification procedure of the wing was the same as employed in the attraction phase. In the 1<sup>st</sup> experiment, mating was conducted by placing two males, both non-modified and modified wing males together with one female. In each type of modification, 10 to 50 groups of males were tested. In the 2<sup>nd</sup> experiment, mating was conducted by both modified and non-modified males, or two males modified in different ways, together with one female. A total of 10 to 50 groups of males from each type of modification were tested. The normal wings of immobilized males were altered under a dissecting microscope in three different ways. Less than 50% or more than 50% of wings were clipped off, or a drop of wax was put at the base of each wing.

#### **Experiment 2: Mechanism of female response**

Modified and non-modified females sensory receptor was tested to evaluate the sensory mechanism on mating by which females accepted or rejected copulation with the courting male. Three major sensory receptors, that is, visual, acoustic and olfactory were modified in females. Normal sensory receptors of anaesthetized females were altered under a dissecting microscope in four different ways in order to prevent reception of certain type of signal from males. They were either a removal of arista (acoustic receptor) or the entire antennae (both arista and olfactory receptors) by clipping with micro scissors and painting the eyes (visual receptor) or antennae (olfactory receptors) with black enamel paint. The painting of the antennae left the arista to receive acoustic signals only. The non-modified females were used as control. These females were tested in two separate experiments. In the 1<sup>st</sup> experiment, mating was conducted by placing two females, one each with non-modified (control) and modified sensory receptors, together with one male. A total of 40 to 50 groups of females from each type of modification were tested. In the 2<sup>nd</sup> experiment, mating was conducted by placing two of both types of females, that is, non-modified (control) and modified females in two different ways together with one male. A total of 10 to 40 groups of males from each type of modification were tested.

## **RESULTS**

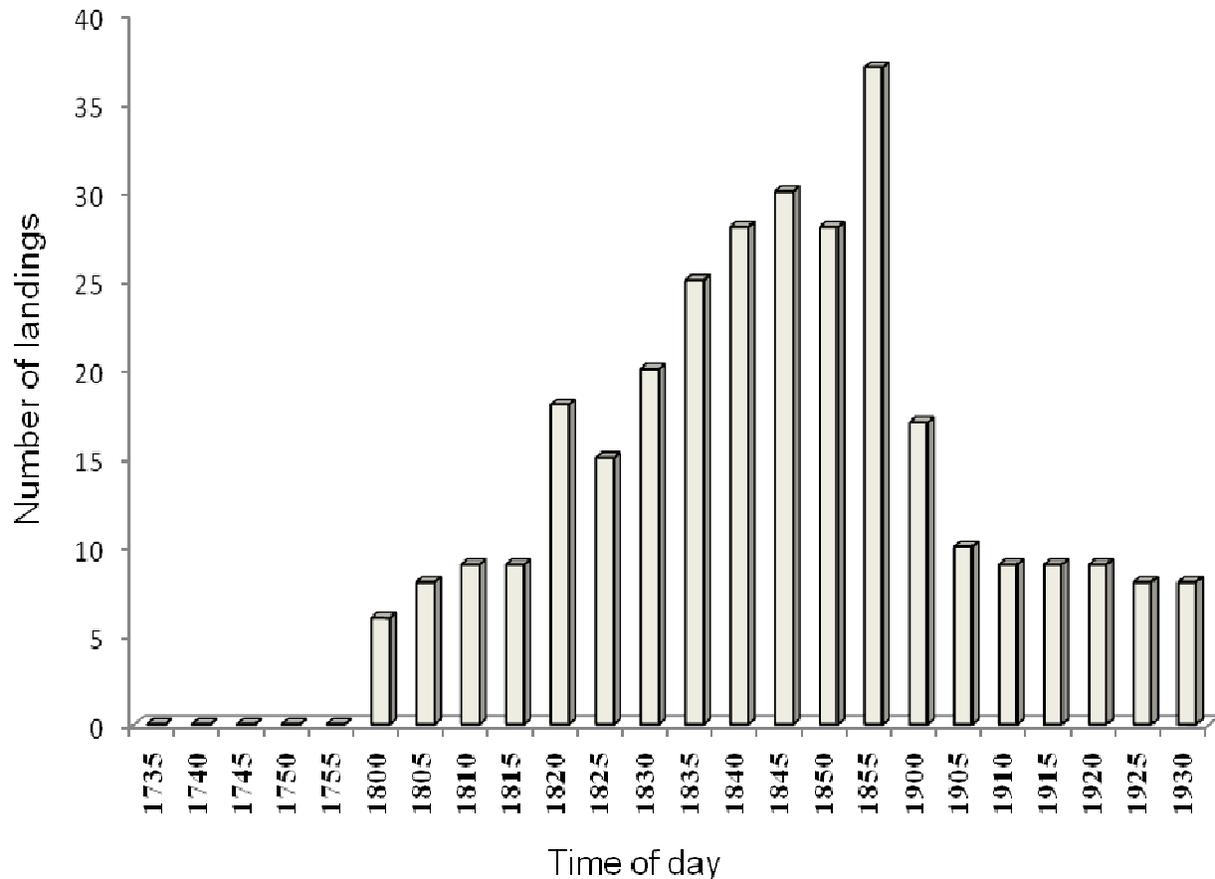
### **Attraction phase: Attraction between male and females**

Male was almost equally attracted to females and males

**Table 1.** Attraction between male and female of *B. papaya*.

Sex	Tested (Number)	fly Approach	
		Male	Female
Male	166	104 (41.6%)	62 (24.8%)
Female	175	121 (48.4%)	54 (21.6%)
Level of significance (P = 0.05)		*	*

Parenthesis denotes percentage of approaching by males and females.

**Figure 1.** Distribution of female attracted to wing fanning males at 5 min interval between 1730 and 1930 h.

wing fanning. Approaching of males were 42 to 48%, while female approach was only 22 to 25% (Table 1). Free flying males landed on the mini cages hung on chili and tomato plants, subsequently. They started wing fanning and attempted copulations similar to those male confined in mini cages. Females confined in mini cages seemed to be excited and walked around in an excited state, especially when males were fanning wings nearby. Free flying females showed no attraction to other females, but were significantly attracted to males confined on the plant. Attracted females flew to cluster on the males' mini cages and probed through the screen, with their ovipositors in movement, similar to the extension of the ovipositor by receptive females.

#### Attractiveness distribution over time

Most females remained inactive under leaves and on cage walls until an hour before sunset. As the light intensity started to decrease around 18:00 h, males started to fan wing, whereas the females began to approach these signaling males and exhibit probing responses. The number of landing was low at 17:30 to 17:55 h and gradually increased (Figure 1). Attractiveness was measured by the number of female landing, which increased between 18:20 and 18:55, reaching the peak of 36 landings per 5 min when most males in mini cages were noticed to fan their wings actively and stroke their abdomen. Such responses declined sharply at the

**Table 2.** Attraction of females to males possessing non-modified or modified wings.

Wing modification	Total tested		Female approaching male type	
	Male	Female	Non-modified male	Modified male
Clipped > 50%	50	200	110 (55%)	20 (10%)
Waxed	50	200	120 (60%)	20 (10%)
Level of significance (P = 0.05)			*	*

Parenthesis denotes percentage of approaching by females and males.

**Table 3.** Attraction of females possessing non-modified or modified sensory receptors to males.

Sensory receptor modification	Tested (number)	female	Female approaching normal male	
			Non-modified	Modified
Aristae removed	190		160 (80%)	30 (15%)
Antennae painted	170		150 (75%)	20 (10%)
Aristae and antennae removed	151		140 (70%)	11 (15%)
Level of significance (P = 0.01)			**	**

Total females tested for each sample of the 200 non-modified or modified sensory receptors. Parenthesis denotes percentage of approaching by males and females.

time of sunset (approximately 19:00 h) and about 15 min after sunset. The response number declined to 9 landings as most females began to leave the signaling males to settle on leaves or cage walls. However, a very small number of females, less than one female in each replicate, remained on the mini cages until 19:25 h, when it was completely dark.

### Effect of wing modification on attractiveness of females

Modified males (10%) were attracted to be significantly poor by females compared to non-modified males, though, these males seemed to fan their wings actively. Similarly, males (10%) with waxed wing bases were not attracted by females compared to non-modified males (Table 2). These males usually walked around or were inactive. It seemed that the prevention of wing fanning by waxing inhibited the abdominal stroking with their rear legs contrast to clipped wings male, which were able to stroke their abdomen at the time of wings fanning.

### Female sensory receptors in response to male

The responses of males toward females with any modification of their sensory receptors were reduced significantly (Table 3). Fewer females visited the modified sensory receptors' males than those non-modified males. A low magnitude of effect was noticed for both aristae and antennae removal, which suggested its weaker response towards attractiveness compared to other sensory receptors.

### Courtship phase: Roles of males wing fanning in mating

From experiment 1, the roles of male wing fanning in mating showed that males with less than 50% tips of their wings removed mated as frequently as non-modified males (Table 4). However, the males with > 50% of their wings removed were significantly less successful in mating than non-modified males. Similarly, males with waxed wing bases reduced mating significantly than non-modified males. More than 50% removal of wing and waxing showed similar magnitude (12 to 14%) of unsuccessful mating (Table 4). The results obtained from the 2<sup>nd</sup> experiment of the roles of males' wing fanning in mating (Table 5) supported the result of previous experiment (Table 4). Males with modified tip portion of their wings mated successfully as those of non-modified males. Clipping of > 50% of the wings and waxing of the wing bases similarly affected male mating success. Firstly, the males with > 50% of the wing removed were significantly less successful in mating than non-modified males. Similarly, males with the wing bases waxed were also significantly less successful in mating than non-modified males. Secondly, the males with > 50% of the wing removed mated significantly less frequently than those with the wing tip removed. Thirdly, the male with either > 50% of the wing removed or the wing bases waxed has similar result. The number of males tested was similar in all treatments. However, the low number of mating by males (n = 12) with more than 50% of their wing clipped off or their wing bases waxed is due to the failure of mating by either one. As noted earlier, the wing waxing bases inhibited both wing fanning and abdominal stroking with rear legs, but clipping the wing did not.

**Table 4.** Effects of wing modification males on mating success.

Wing modification	*Mated (number)	male	**Mated male (number)	
			Non-modified	Modified
Clipped < 50%	50		30 (60%)	20 (40%)
Clipped > 50%	47		40 (80%)	20 (14%)
Waxed	41		35 (70%)	6 (12%)
Level of significance (P = 0.01)			**	**

\*50 pairs of males tested for each modification. \*\*Each sample consists of two males possessing non-modified or modified wings. Parenthesis denotes percentage of approaching by males and females.

### Mechanism of female response

Two experiments were conducted under the mechanism of female response. The females, whose eyes were painted or whose sound receptors (aristae) were removed mated as those of non-modified females (Table 6) in experiment 1. However, when the pheromone receptor (antennae painted) was prevented, modified females mated significantly less than non-modified ones. A significant reduction in mating was also found in females whose entire antennae were removed. In the latter cases, both sound and pheromone receptors were removed in the females. The results of experiment 2 (Table 7) from mechanism of female response supported the previous experiment (Table 6). Females without visual or sound receptors mated similarly to those of non-modified females (Table 7: lines A, B and E). Based on lines B, C and H of Table 7, olfactory signals appeared to be more important than the acoustic signals in determining whether a female would mate or not. Pheromone and pheromone plus acoustic receptor modification also played a key factor in the success of mating (Table 7: lines C, D, F, G, I). Acoustic signals appeared less important only when the eliminated olfactory plus acoustic signal significantly reduced the mating of females by a greater magnitude than the effect of olfactory signal elimination alone.

## DISCUSSION

### Attraction phases

Sexual signals are produced by individual to attract its opposite sex for mating. This study revealed that the mature males of *B. papayae* were attracted poorly towards females or wing fanning males. However, females were strongly attracted to males, but not towards females. These findings were similar to those of the study on *B. cucurbitae*, in which the experiment was conducted in much smaller cages (25 x 25 x 25 cm) having a higher density of flies (Kobayashi et al., 1978). It suggested that the pheromone calling of males of *C. capitata* serves to attract other males for mating in addition to attracting females (Arita and Kaneshiro, 1983). Similar results were

found in *A. suspensa* and *B. tryoni* under field conditions (Fletcher et al., 1968; Perdomo, 1974). Males could still fan their wings normally even if a part of the wing was removed; however, it reduced the production of stimuli slightly. The removal of wing > 50% could affect the acoustic or olfactory stimulus. The wax procedure inhibited the wing fanning completely. Since, signaling males of *B. papayae* did not attract other males. Their preference depends upon other favorable environmental conditions. Preliminary data showed that if two or more males confined beside each other's fanned wings, it attracted more frequently and actively than those confined singly. Movements and interactions among males appeared to stimulate each other's fan wings producing signals in order to compete and attract females. The female approach to males gradually increased from 18:00 to 19:30 h, peaked at 18:55 h, and subsequently decreased to 19:00 h. These results suggested that there was a temporal distribution of either signal emission or female responsiveness, or both. The male attractiveness peaked at 18:55 h, lasted for a few minutes. This pattern suggested a strong dependence of a mechanism triggering sexual activity on factors that were changing during the time of dusk. Therefore, timing of sexual behavior in *B. tryoni* was controlled by light intensity (Smith, 1977).

Males that were unable to fan their wings were normally less attracted to females. Reduction of male attractiveness was affected by modifications of wing that might change in the signals that were not normally produced. Removal of wing > 50% might have affected the quality and amount of olfactory and acoustic signals produced by wing fanning. The results of this study slightly contradict with the findings of Keiser et al. (1973) who reported that normal sound is generated after removal of wing. Removal of wings could also affect the production of olfactory signal since small portions of the wing left intact may not be efficient in the dispersion of the pheromone. On the other hand, waxing of the wing bases may have totally prevented the production and dispersion of acoustic and olfactory signals as evident from the results. The significant reduction in the attractiveness of *B. papayae* males, whose wing fanning had been altered, certainly demonstrated that the normal wing fanning played a major role in the production of

**Table 5.** Mating males possessing non-modified or modified wings under all possible combinations.

Wing modification		*Mated male (number)	**Mated male	
Male-I	Male-II		Male-I	Male-II
Normal	Clipped- < 50%	30	18 (45%)	12 (30%)
Normal	Clipped- >50%	38	33 (82.5%)	5 (12.5%)
Normal	Waxed	30	27 (67.5%)	3 (7.5%)
Waxed	Clipped- < 50%	29	4 (10%)	25 (62.5%)
Waxed	Clipped- >50%	12	5 (12.5%)	7 (17.5%)
Clipped- < 50%	Clipped- >50%	35	5 (12.5%)	30 (75%)
Level of significance (P = 0.05)			**	**

\* 40 males tested for each combination; \*\*each sample consists of two types of males (male I and male II) possessing non-modified or modified wings placed with virgin females. Parenthesis denotes percentage of approaching.

**Table 6.** Effects of sensory receptor modification in females on mating.

Sensory receptor modification	Tested Female (number)	Tested Male (number)	*Mated Female	
			Non-modified	Modified
Eyes painted	49	43	24 (48%)	25 (50%)
Aristae removed	50	45	27 (54%)	23 (46%)
Antennae painted	46	44	32 (64%)	7 (26%)
Antennae and aristae removed	41	44	30 (60%)	30 (22%)
Level of significance P=0.05			**	**

\*Each sample consists of two females, one with non-modified and the other with modified sensory receptors placed with virgin males. Parenthesis denotes percentage of approaching.

**Table 7.** Mating females possessing non-modified or modified sensory receptors under all possible combinations.

Sensory receptor modification <sup>a</sup>		Female mated <sup>b</sup>	
Female-I	Female-II	Female-I	Female-II
A. None	Eyes	23 (57.5%)	17 (42.5%)
B. None	Aristae	25 (62.5%)	15 (37.5%)
C. None	Antennae	27 (67.5%)	13 (32.5%)
D. None	Antennae and Aristae	29 (72.5%)	11 (27.5%)
E. Eyes Aristae	Aristae	18 (45%)	22 (55%)
F. Eyes	Antennae	30 (75%)	10 (25%)
G. Eyes	Antennae and Aristae	33 (82.5%)	7 (17.5)
H. Aristae	Antennae	24 (60%)	16 (40%)
I. Aristae	Antennae and Aristae	29 (72.5%)	11 (27.5%)
J. Antennae	Antennae and Aristae	34 (85%)	6 (15%)
Level of significance (P=0.05)		**	**

<sup>a</sup> = Modifications include non-modified, eyes painted, aristae removed, antennae painted, and antennae and aristae removed. <sup>b</sup> = each sample consists of two types of females (female-I and female-II) possessing non-modified or modified sensory receptors, placed with males. Total females tested (40) for each combination. Parenthesis denotes percentage of approaching.

signals. These signals could be acoustic, olfactory or visual, or a combination of all.

The male wing fanning of *Bactrocera papayae* and the touch with the wings while stroking their abdomen during dusk appeared to generate sound. The buzzing and chick sound noticed to be intervening rhythmically with the

buzzing sound was generated by wing fanning and touch 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 stridulating of the wing on abdomen cilia (Jose et al., 1996). In addition to

production of wing sound, wing fanning might be associated with the release of a sex hormone in this species and an odor was released during the signaling of males which was reported by Drew (1989) and Kuba et al. (1984) for *B. cucurbitae* and *B. tryoni*. Increased wing fanning of males were directly related to generating males signal and mating which was closely correlated with the age of adults. Since the onset of mating assumes sexual maturation, the results showed that the onset of all sexual activities of *B. papayae* was coincidental with the attainment of maturity.

Females with experimentally impaired receptors sound and pheromone or both were significantly less attracted towards males than females with unmodified receptors. This result indicated strongly that both acoustic and olfactory stimuli necessarily attracted females. This study is the first of its kind, in that the role of acoustic stimulus in attracting *B. papayae* females was experimentally demonstrated. Webb et al. (1983a) conducted a study on *A. suspensa* in field cages to demonstrate the significance of male produced pheromones that were artificially administered. However, they reported the evidence of the presence of attracting pheromones in this species, but did not differentiate the significance of pheromone from acoustic signals on attraction of females. Females of *B. dorsalis* were attracted by both live males and rectal glands removed from males (Kobayashi et al., 1978; Schnez and Boush, 1971). These glands were suggested to be used for both production and storage of the pheromone because their attractiveness increased during the day and peaked during the period associated with sexual activity. The substance suggested to be a sex pheromone released as a smoke in *B. dorsalis* was found to be slightly attractive to females (Ohinata et al., 1982).

In this study, the influence of acoustic signals on attraction of females to signaling males is clearly shown. Certain sound components may be important in inducing female visitation, which are made primarily for attracting females without the pheromone receptors. However, most of the females with either type of sensory eliminations failed to approach the males.

### Courtship phase

The close range (average 5 cm) mating was reduced in males with > 50% of their wings removed or wing bases waxed. These results conclusively demonstrated that the normal wing fanning of males during courtship is important for a successful mating. Males mated better than they possessed sufficient wing area (only wing tips removed) and fanned their wings normally. Males with >50% removal of their wings still fanned their wings normally, although, they did not emit proper signals. On the other hand, males with only tips removal of their wings were successful as non-modified males. Wingless male of *B. dorsalis* mated successfully as winged males

(Keiser et al., 1973). Females were likely to be repeatedly experiencing attempted copulation by several competing males and were forced to mate eventually under a crowded condition of small cages. Hedge and Krishna (1997) reported similar result in *Drosophila malerkottiana*.

Females without either modified visual or sound receptors mated as effectively as females with non-modified receptors. *B. cucurbitae* females also mated successfully when their compound eyes were painted (Keiser et al., 1973). Therefore, it seems that the visual signals might have less importance in the mating behavior of *B. papayae*, because mating took place at dusk. Furthermore, males of this species possess neither an elaborate visual courtship display nor distinctively patterned wings, which could be utilized as visual signals during female encounters at close range. Apparently, acoustic signals play little or no role in eliciting signals copulation at close range. The male wing fanning might play a crucial role in dispersal of pheromones but the buzzing sound could also be produced incidentally. Therefore, sound may not be a significant factor for mating success in *B. papaya*, but it was believed that sound can play a role in the mating of other tephritids. The precopulatory song, produced by *A. suspensa* males during the insertion of the aedeagus, modifies behavior which results in female reception and longer mating durations (Sivinski et al., 1984). Surprisingly, females with the arista removed still responded to the precopulatory song normally and rejected mounted males as frequent as normal females (Sivinski et al., 1985a). They concluded that the arista are not only sound receptors in *A. suspensa*, but also they are mechanoreceptors present on the body, legs and or wings of the female. Their results suggest that an examination of the role of mechanoreceptor in *B. papayae* might prove fruitful. Various types of male sounds have been qualitatively described and correlated with male mating success (Webb et al., 1984). In contrast to this, this study was conducted directly to test whether or not acoustic signals play some role in successful mating.

Mating was reduced significantly when female antennae were painted. This clearly demonstrated that the chemical stimulus was crucial for mating by female. Therefore, in this study, *B. papayae* (tephritids) directly demonstrated the role of olfactory signal for mating. Previous studies in *A. ludens*, *A. suspensa*, *C. capitata*, *B. cucurbitae*, *B. latifrons* (Malaysian fruit fly), *B. tryoni* and *R. pomonella* reported only the presence of pheromone (Feron, 1959, 1962; Fletcher et al., 1968; Kobayashi et al., 1978; Little and Cunningham, 1987; Nation, 1972; Prokopy, 1975; Robacker et al., 1986). In this study, a significant reduction in mating success was detected when the entire antennae and arista were removed from females. Such a reduction in mating could be due to the entire lack of pheromone reception alone since sound was not a significant close range signal. This was similar to the mating inhibition that occurred in *C.*

*capitata* when both antennae were removed from females (Nakagawa et al., 1973).

Based on lines B, C, E, F and H of Table 7, pheromone appears to be more important than acoustic signals in determining whether a female would mate or not. However, on line J, the effect of pheromone plus acoustic signal elimination significantly reduces the mating of females, which is greater than the effect of pheromone elimination alone. The lack of acoustic signals alone is insufficient to significantly reduce female mating as shown by its lower magnitudes in the effect of heromone elimination (Table 7: lines B, C, E and F). However, the elimination of acoustic signals may have some degree of additive effect in one case (Table 7: line J). Even though antennal removal was thought to be a drastic operation, it might cause females to behave abnormally. However, they were physically active, but did not respond to signaling males as readily as normal females.

*B. papayae* female antennae were noticed to extend their ovipositors and probe through the screen of mini cages containing males. This probing response has also been reported from sexually excited females of *C. capitata* and *B. tryoni* females when exposed to the content of male pheromone gland (Feron, 1962; Fletcher and Ginnakis, 1973). It was used as an indicator of sexual response of *B. tryoni* females to pheromone. Similar pheromone was also noticed in *B. papayae* females when they were close to males in the mini cages. In view of the foregoing experimentation on *B. papayae* attraction and courtship, conclusively it can be said that visual, olfactory and acoustic signals play a very crucial role in attraction of opposite sexes which subsequently ends in a successful mating or courtship.

## REFERENCES

- Arita LH, Kaneshiro KY (1988). Pseudomale courtship behavior of the female Mediterranean fruit fly *Ceratitidis capitata* (Wiedermann). Proc. Hawaii Entomol. Soc., 24 (283): 205-210.
- Baker R, Herbert RH, Lomer RA (1982). Chemical components of the rectal gland secretions of male *Bactrocera cucurbitae* the melon flies. Experiments, 18: 232-233.
- Bateman MA, Boller EF, Bush GL, Chambers DL, Economopoulos AP, Fletcher BS, Heuttel MD, Moericke V, Prokopy RJ (1976). Fruit flies, In Delucchi VL, (ed.), studies in biological control. Cambridge Univ., N. Y., pp. 11-49.
- Bateman MA, Morton TC (1981). The importance of ammonia in proteinaceous attractants of fruit flies (family: Tephritidae). Aust. J. Agric. Res., 32: 883-903.
- Bellas TE, Fletcher BS (1979). Identification of the major components in the secretion from the rectal pheromone glands the Queensland fruit flies, *Bactrocera tryoni* and *Bactrocera neurohumerlis* (Diptera: tephritidae). J. Chem. Ecol., 5: 795-803.
- Berube DE (1978). Larval descriptions and biology of *Tephritis dilacerate* (Dipt.: Tephritidae), a candidate for biocontrol of *Sonchus arvensis* in Canada. Entomophaga, 22: 69-82.
- Clark, BHC (1971). Acoustic of insect song. Nature, 24: 255-259.
- Dickens JC, Hart WG, Light DM, Jang EB (1988). Tephritid olfaction: Morphology of the antennae of four tropical species of economic importance (Diptera: Tephritidae). Ann. Entomol. Soc. Am., 81: 325-331.
- Dodson GN (1978). Behavioral, anatomical and physiological aspects of reproduction in the Caribbean fruit fly *Anastrepha suspensa* (Loew). M. S. Thesis, Univ. of Florida, Gainesville. p. 68
- Drew RA (1989). The tropical fruit flies (Diptera:tephritidae) of the Australian and Oceanian regions. Memo. Queensland Museum, 26: 1-521.
- Feron M (1962). L'instinct de reproduction chez la mouche Mediterranee des Fruits *Ceratitidis capitata* Wied (Dipt. Trypetidae). Comportment sexual component de ponte. Rev. Pathol. Veg. Entomol. Agric., Fr. 41: 1-129.
- Feron M (1959). Attraction chimique du male de *Ceratitidis capitata* Wied. (Diptera: Tephritidae) pour la femelle. C. R. Acad. Sci. Ser. D., 248: 2403-2424.
- Fitt GP (1981). Responses by female Dacinae to male lures and their relationship to patterns of mating behaviour and pheromone response. Entomol. Exp. Appl., 29: 87-97.
- Fletcher LW, Claborne HV, Turne JP, Lopez E (1968). Difference in response of two strains of screw worm flies to male pheromone. J. Econ. Entomol., 61: 1386-1388.
- Fletcher BS (1977) Behavior response of Diptera to pheromones, allomones and kairomones. In Shrey HH, Mckelvey Jr. JJ (eds.), chemical control of insect behavior: theory and application. John Wiley and Son, NY. pp. 129-148.
- Fletcher BS, Giannakakis AM (1973) Factors limiting the response of females of the Queensland fruit fly (*Bactrocera tryoni*), to the sex pheromones of the male. J. Ins. Physiol., 19: 1147-1155.
- Giannakakis A, Fletcher BS (1985). Morphology and distribution of antennal sensilla of *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). J. Aust. Entomol. Soc., 24: 31-35.
- Hallberg E, Van der perris JN, Haniotakis GE (1984). Funicular sensilla of *Bactrocera oleae*: Fine structural characteristics. Entomol. Hell. 2: 41-46.
- Hee AKW, Tan KH (1998). Attraction of female and male *Bactrocera papayae* to conspecific males fed with methyl eugenol and attraction of females to male sex pheromone components. J. Chem. Ecol., 24(4): 753-764.
- Hedge SN, Krishna MS (1997). Size assortative in *Drosophila malerkotllana*. Anim. Behaviour, 45 (2): 419-426.
- Jose S, Briceno RD, Ramose D, Eberhard WG (1996) Courtship behavior of male *Ceratitidis capitata* (Diptera:Tephritidae) in captivity presented insect behavioural ecology. Fla. Entomol., 19(2): 130-143.
- Kellogg FE, Frizel DE, Wright RH (1962). The olfactory guidance of flying insects. iv. *Drosophila* Can. Entomol., 94: 884-888.
- Keiser I, Kobayashi RM, Schneider EL, Tomikawa I (1973). Laboratory assessment of 73 insecticides against the oriental fruit fly, Melon fruit fly, and Mediterranean fruit fly. J. Econ. Entomol., 66(4): 837-839.
- Keiser I, Kobayashi R, Chambers DL, Schneider EL (1973). Relation of sexual dimorphism in the potential stridulation and illumination of mating of oriental fruit flies of Mediterranean fruit flies in Hawaii Ann. Entomol. Soc. Am., 66: 937-941.
- Kobayashi RM, Ohinata K, Chambers DL, Fujimoto MS (1978) Sex Pheromones of the Oriental fruit fly and the melon fly: Mating behavior, bioassay method, and attraction of females by live males and by suspected pheromone glands of males. Environ. Entomol., 7: 107-112.
- Kuba H, Koyama J, Prokopy RJ (1984) Mating Behavior of Wild Melon Flies, *Dacus cucurbitae* COQUILLETT (Diptera:Tephritidae) in a Field Cage:Distribution and Behaviour of Flies. Appl. Entomol. Zool., 19: 367-373.
- Little HF, Cunningham RT (1987). Sexual dimorphism and presumed pheromone gland in the rectum of *Bactrocera latiphrones* (Diptera:Tephritidae). Ann. Entomol. Soc. Am., 80: 765-767.
- Manning A (1967). Antennae and sexual receptivity in *Drosophila melanogaster* females. Science, 158: 136-137.
- Monro J (1953). Stridulation in the Queensland fruit fly *Bactrocera* (strumeta) *tryoni* (Frogg). Aust. J. Sci., 16: 50-62.
- Nakagawa S, Farias GJ, Suda D, Chambers DL (1973). Mating behavior of the Mediterranean fruit fly following excision of the antennae. J. Entomol., 66: 583-584.
- Nation JL (1972). Courtship behavior and evidence for a sex attractant in the male Caribbean fruit fly, *Anastrepha suspense*. Ann. Entomol. Soc. Am., 65: 1363-1367.
- Ohinata K, Jacobson M, Kobayashi RM, Chambers DL, Fujimoto MS,

- Higa HH (1982). Oriental fruit fly and melon fly: biological and chemical studies of smoke produced by males. *J. Environ. Sci. Health (A)*, 17: 197-208.
- Perdomo AJ (1974). Sex and aggregation pheromone bioassays and mating observation of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) under field conditions Ph.D. dissertation, University of Florida, Gainesville. p. 127
- Pritchard G (1967). Laboratory observations on the mating behavior of the island fruit fly *Rioxa pornia* (Diptera: Tephritidae). *J. Aust. Entomol. Soc.*, 6: 127-132.
- Prokopy RJ (1975). Mating behavior in *Rhagoletis pomonella* (Diptera: Tephritidae). Virgin female attraction to male odour. *Can. Entomol. Soc.*, 107: 905-908.
- Roan CC, Flitters NE, Davis CJ (1954). Light intensity and temperature as factors limiting the mating of the oriental fruit fly. *Ann. Entomol. Soc. Am.*, 47: 593-594.
- Robacker DC, Hart WG (1987). Electroantennograms of male and female Caribbean fruit flies (Diptera:Tephritidae) elicited chemicals produced by male. *Ann. Entomol. Soc. Am.*, 80: 508-512.
- Robacker DC, Chapa BE, Hart WG (1986). Electroantennograms of Mexican fruit flies to chemicals produced by males. *Entomol. Exp. Appl.*, 40: 123-127.
- Rolli K (1976). Die akustischen sexualsignale von *Ceratitis capitata* wied. Und. *Bactrocera oleae* Gmel. *Z. Entomol.*, 81: 219-223.
- Schnleiz GA, Boush GM (1971). Suspected sex pheromone glands in three economically important species of *Bactrocera* J. *Entomol.*, 64: 347-349.
- Sivinski J, Webb JC (1985a). Sound production and reception in the caribfly, *Anastrepha suspensa* (Diptera:Tephritidae). *Fla. Entomol.*, 68: 273-278.
- Sivinski J, Burk T, Webb JC (1984). Acoustical courtship signals in the Caribbean fruit fly *Anastrepha suspensa* (Loew). *Ann. Behv.*, 32: 1011-1016.
- Smith WJ (1977). The behavior of communicating: an ethological approach. Cambridge Massachusetts: Harvard Univ. Press.
- Van der, JNC, Haniotakis, King BE (1984). Electroantennogram responses from olfactory receptors in *Dacus oleae*. *Entomol. Hell*, 2: 47-53.
- Webb JC, Sharp JL, Chambers DL, Mcdow JJ, Brenner JC (1976). The analysis and identification of sounds produced by male Caribbean fruit fly, *Anastrepha suspensa* (Loew). *Ann. Ento. Soc. Am.*, 69: 415- 420.
- Webb JC, Burk T, Sivinski J (1983a). Attraction of female Caribbean fruit fly *Anastrepha suspensa* (Loew). (Diptera:Tephritidae) to the presence of males and produced stimuli in field cages. *Ann. Entomol. Soc. Am.*, 76: 996-998.
- Webb JC, Calkins CO, Chamber DL, Schwienbacher W, Russ K (1983b). Acoustical aspects of behavior of Mediterranean fruit flies, *Ceratitis capitata*: Analysis and identification of courtship sounds. *Entomol. Exp. Appl.*, 33: 1-8.
- Webb JC, Sivinski J, Litzkow C (1984). Acoustical behavior sexual success in the Caribbean fruit fly *Anastrepha suspensa* (Loew). (Diptera: Tephritidae). *Environ. Entomol.* 13: 650-656.
- Zwölfer H (1974). Inneratliche Kommunikations systeme bei Bohrfliiegen. *Biol. unser. Zeit*, 4(5): 147-153.