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Evidence for a male-produced sex pheromone and behavioural responses of the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) to the pheromone in a horizontal wind tunnel

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Abstract

Evidence for a male-produced sex pheromone and behavioural responses of male and female *Bactrocera dorsalis* to the pheromone were investigated using a horizontal wind tunnel under laboratory conditions. Responses of males and females to different combinations of live flies and male rectal glands were assessed by monitoring three behavioural parameters namely the number of flies performing upwind straight and zigzagging anemotactic flights over at least 50cm to the treatment, total number of flies landing on the treatment and the total number of flies moved into the upwind section of the wind tunnel after 20 minutes. Significantly high female responses to live males were recorded in all three parameters considered. Male responses to either males or females were considerably low. It is evident from the results that female *B. dorsalis* are attracted to males and not vice versa. Neither of the sexes was attracted by their own sex and the lack of responses of virgin males to virgin females can be considered to be strong evidence for the absence of any female odour attracting males. Female responses to male rectal glands were at their highest during the 'dusk' period. Behavioral responses of females observed in the wind tunnel allow the conclusion to be drawn that the source of production or storage of the sex pheromone in *B. dorsalis* males is the rectal gland. In addition, these results confirm the

previously reported observations that mating activity in this species occurs only during the dusk period.

Key words: *Bactrocera dorsalis*, behavioral responses, male-produced sex pheromone, rectal glands, wind tunnel

Introduction

Existence of male-produced sex pheromones have been reported for many species of tephritid fruit flies (Fletcher, 1968; Katsoyannos, 1976; Nation, 1972; Ohinata *et al*, 1973; Pritchard, 1967; Prokopy, 1975). Kobayashi et al (1978) reported that a musty odour in a cage with oriental fruit flies during dusk, which they suspected to be the male pheromone or a substance associated with its release. Ohinata *et al* (1982) noted that a substance visible as 'smoke' is emitted by oriental fruit flies at dusk and these emanations were attractive to sexually mature females. The presence of a male pheromone in the melon fly was suggested and smoke emission was observed at dusk by sexually mature flies confined densely in a cage. During dusk, the males stridulated by rapidly drawing their wings over large bristles situated on the third abdominal tergite, producing a high pitched buzzing sound. This is considered to be a "mating call" (Monroe, 1953).

Fletcher (1969) reported that during courtship the males of *Dacus* (= *Bactrocera*) *tryoni* release a sex pheromone which is stored and secreted in glands associated with posterior ventral regions of the rectum. Females responded to extracts of these glands. Schultz and Boush (1971) investigated the suspected sex pheromone glands in three species of fruit flies including the oriental fruit fly. Nation (1981) systematically examined and confirmed the occurrence of male specific glands in the tephritid fruit flies in the genera *Anastrepha, Bactrocera* and *Rhagoletis*. Detailed morphological studies were conducted by Lee et al (1986) on rectums of oriental fruit flies from emergence to maturity to assess their role in pheromone production and storage. The rectal glands consisted of two main structures, a reservoir and a secretory sac.

The present study was carried out in order to establish evidence for a male-produced sex pheromone and behavioural responses of male and female *B. dorsalis* to the male sex pheromone using a horizontal wind tunnel.

Materials and Methods

B. dorsalis was reared under laboratory conditions (at a temperature of 29±20C, and 78± 2% RH) by using the method similar to that of Mitchell *et al* (1965). A twelve hour light period of 1500lux provided by fluorescent lamps and attenuated light was supplied for one hour each at dawn and dusk (20lux). The adult flies were maintained on a diet consisted of sugar and water supplemented with small quantities of yeast hydrolysate. Adult males and females were separated within twenty four hours after emergence and kept in different cages for bioassays. All experiments were carried out in a wind tunnel made of clear plastic tubing, described by Karunaratne (1998). Air flow in the wind tunnel was regulated at 0.1m/sec. Three behavioural responses such as the number of flies performing upwind straight and zigzagging anemotactic flights over at least 50cm to the treatment (tube containing live flies or rectal glands), the total number of flies landing on the treatment and the percentage of flies moving into the upwind section of the wind tunnel were recorded for 20 minutes. Each behavioural response monitored for each treatment was considered separately and analyzed using a one way analysis of variance and Duncan's multiple range test.

Behavioural responses of virgin males and females to different combinations of live flies

The test flies aged between 15-16 days were used in bioassays. These were introduced into the flight chamber in a glass jar covered with gauze and placed at the downwind section of the wind tunnel a few minutes before the onset of each bioassay. An open ended tube containing 'stimulus' flies, was placed at the upwind section of the wind tunnel as the treatment. The open ends of the tube were covered with gauze for air to pass freely through and facilitate the distribution of the released pheromone. Twenty virgin males and the same number of virgin females were used separately as the stimulus (treatment). An identical empty tube was used as the control. Virgin males and virgin females (treatment) in the glass tube were tested separately against mature virgin males and mature virgin females. Observations were made between 17:00-18:30h at 'dusk' conditions (15-20lux) as these were the most favourable conditions for the pheromone release and mating activity. Each treatment was replicated four times. Twenty five test flies, either males or females were used in each replicate.

Behavioural responses of virgin males and virgin females to male sex pheromone glands (rectal glands)

Observations were made of virgin females and virgin males separately to determine whether the males and females respond to the male sex pheromone (rectal glands) at different times of the day. Freshly dissected rectal glands of mature virgin males (13-18 days old) were used as the source of the pheromone. The rectal glands were removed under a dissecting microscope shortly before each experiment. This was done by grasping the aedeagus of the male with a pair of fine forceps and gently pulling the gland out, being careful to avoid puncturing the gland. To standardize the quantities, only the glands that were full of secretion were used for bioassays. Just before each test, five rectal glands were crushed on the centre of a filter paper (4.5cm) in a petridish. This was then placed on a tripod (10cm height) at the upwind section of the wind tunnel. An untreated filter paper was used against test flies as the control. Observations were made at 9:00, 12:00, 15:00 (1500lux) and 17:00 (20lux) hours. Twenty five test flies aged between 14-16 days were used and three replicates were made of each treatment.

Results and Discussion

A highly significant difference was obtained between the behavioural responses of the virgin females in the presence of live males and females (Table 1).

Table I. Behavioural responses of virgin females to live males and females

Treatment (Stimulus)	Response		
	Upwind anemotaxis	No. landing on source	Percent moving to upwind third section
Control	0.8 ± 0.2^{b}	0^{b}	11.1 ± 2.2^{b}
Virgin female	2.8 ± 0.3^{b}	0.8 ± 0.2^{b}	21.4 ± 3.8^{b}
Virgin male	11.0 ± 1.5^{a}	17.3 ± 1.4^{a}	76.5 ± 3.2^{a}

Mean values ± standard error; Means in the same column followed by similar letters do not differ significantly (DMRT, p>0.005)

Female responses to males were significantly higher than to the live females in all three parameters considered. When virgin males were tested against live males and females no significant differences were observed for any of the behavioural responses recorded (Table II). The results obtained from testing different combinations of live flies indicate that female *B. dorsalis* were attracted when males were presented as the 'stimulus' flies.

Table II. Behavioural responses of virgin males to live males and females

Treatment (Stimulus)	Response		
	Upwind anemotaxis	No. landing on source	Percent moving to upwind third section
Control	1.8 ± 0.3	3.5± 0.6	28.4 ± 6.9
Virgin female 3.3 ± 0.8 3.3 ± 0.8	6.5 ± 1.3	36.0± 4.9	Virgin male 3.5 ± 0.8 Virgin male

Mean values ± standard error; NS=Not significant (p>0.01)

In the presence of males, females became very active exhibiting a high number of movements and flights in the wind tunnel bioassay. They made upwind anemotactic flights towards the males and landed on the tube in which the treatment males were kept. The females which landed on the tube probed their ovipositors through the holes of rhe mesh towards the males. Flies that landed on the walls of the glass tube moved all over the tube exhibiting a searching behavior until they arrived on the mesh of the tube. During this time most of the males in the tube were engaged in wing vibration. Furthermore, when males were tested against live males and females, they were observed vibrating their wings rapidly. It is suggested that during this activity period a pheromone is released by males.

Tables III and IV show the behavioural responses of females to male rectal glands presented at different times of day. All three response parameters of females were relatively low between 9.00-15.00 hours and they did not differ significantly with those of the control. However, responses recorded at 17.00 hours were significantly higher than the rest. When the male sex pheromone was presented to males between 9.00-15.00 hours, the responses exhibited by them were very low and did not indicate significant differences from their respective controls.

Table III. Behavioural responses of mature virgin females to male pheromone (rectal glands) at different times of day

Time (Hrs)	Treatment (5 rectal glands)	Response		
		Upwind anemotaxis	No. landing on source	Percent moving to upwind third section
9.00 Control Control Treatment	0.0[a] 0.3 ± 0.3[a]	0.3 ± 0.3[a] 0.3 ± 0.3[a]	13.1 ±**4**.3[a] 12.0± **1**.7[a]	12.00 Control 12.00
Treatment 3.3 ± **0**.8[a] Treatment	3.3 ± **0**.8[a]	2.0± **0**.6[a]	22.9 ± 3.6[a]	17.00 Control 0.7 ± **0**.3[a] 0.3 ± **0**.3[a] 13.7 ± **1**.5[a] Treatment 9.3 ±**1**.2[b] 17.00
	Treatment	9.3 ±**1**.2[b]	10.7± **2**.3[b]	62.7 ± **11**.8[b]

Mean values ± standard error; Means in the same column followed by similar letters do not differ significantly (DMRT, p>0.005)

Relatively very low number of males landed on the pheromone source at the 'dusk' period (17.00 hours). However, the upwind anemotaxis and the movement of male flies to upwind section of the wind tunnel increased during this period. Even though these responses were significantly higher than the rest, the magnitude of the response was relatively low. It can be stated in general, that upon detection of pheromone odour produced by the males, virgin females demonstrated an oriented upwind flight via straight and zigzag anemotaxis to the male source and landed either on the tube containing the live males or filter paper containing crushed rectal glands. Male pheromone emissions (from live males and rectal glands) elicited upwind flight via upwind anemotaxis, and attracted more conspecific virgin females than males in wind tunnel assays. Generally, conspecific male response to male pheromone was low.

Table IV. Behavioural responses of mature virgin males to male pheromone (rectal glands) at different times of day

Time (Hrs)	Treatment (5 rectal glands)	Response		
		Upwind anemotaxis	No. landing on source	Percent moving to upwind third section
9.00	Control	1.0 ± 0.6	0.0	17.5 ± **5.5**
	Treatment	3.0 ± 0.6	1.3 ± 0.7	23.9± **1**.6
12.00	Control	1.3 ± **0**.6	0.0	12.8 ± **4**.5
	Treatment	2.0 ± 0.6	1.3 ± 0.7	21.8± 3.3
15.00	Control	0.3 ± **0**.3	0.3 ± **0**.3	13.6 ± **3**.8
	Treatment	2.0 ± **0**.6	0.3± **0**.3	19.9 ± **6**.2
17.00	Control	1.0 ± **0**.6	0.3 ± **0**.3	12.0 ± **2**.3
	Treatment	4.3 ± **0**.8	2.0± **0**.6	26.7 ± **1**.6

Probability		NS	NS	NS

Mean values ± standard error; NS=Not significant (p>0.01)

The release of sex pheromone by males to attract conspecific females is common in many species of tephritids including the Carribbean fruit fly, *Anastrepha suspensa* (Nation, 1972), the Mediterranean fruit fly, *Ceratitis capitata* (Ohinata *et al*, 1973), the apple maggot fly, *Rhagoletis pomonella* (Prokopy, 1975), the European cherry fruit fly, *R. cerasi* (Katsoyannos, 1976), and several other *Bactrocera* species (Kuba and Sokei, 1988; Kuba, 1991). Perkins *et al*. (1990) in their studies confirmed endogenous volatiles in the rectal gland of sexually mature *B. carambolae* males and regarded them as 'male pheromone'. Examination of the volatiles emitted by live *B. carambolae* males has revealed that these compounds were released into the air which coincided with courtship and mating periods of the flies, which is at dusk (Wee & Tan, 2005).

Wing vibration in *B. dorsalis* males apparently facilitates the evaporation of the pheromone by the production of local currents. In addition, the frequent drawing of legs across the posterior part of their abdomen and wings was observed in males during this time period. It is reported that pheromone release by *Bactrocera* males is achieved in a series of steps termed 'stridulation' (Monro, 1953) or 'spraying' (Kuba & Sokei, 1988). The pheromone is emitted from an internal abdominal gland via the anus and transferred with the hind tarsi to specialized setae that are only found on the wings of males. The pheromone is made airborne by rapid beating of the wings against abdominal bristles that are, again, uniquely male traits (Pike & Meats, 2003). Kuba & Sokei (1988) reported that pheromone clouds sprayed by *B. cucurbitae* males can be visually detected by focusing a beam of light at them during dusk when the males were vibrating their wings. When a male fly engages in calling behavior, sex pheromone droplets are excreted from his anus. This excretion is wiped off with the tarsus of its hind leg, and then is deposited on the cubital cell hairs on the wing. During wing vibration, the targal bristles on the 3rd abdominal segment are rubbed against the hairs of the cubital cell. Calling males sprayed clouds of pheromone with these actions (Kuba & Sokei, 1988)..

When the results (Table 1 and 2) are taken into account it is evident that female *B. dorsalis* are attracted to males and not vice versa. These corroborate the observations made by Kobayashi *et al* (1978) confirming the presence of a sex pheromone in the male, which controls the mating behaviour in *B. dorsalis*. Neither of the sexes was attracted by their own sex and the lack of responses of virgin males to virgin females can be considered to be strong evidence for the absence of any female odour attracting males. The results (Tables 3 and 4) also allow the conclusion to be drawn that the source of production or storage of the sex pheromone in *B. dorsalis* males is the rectal gland. In addition, these results confirm the previously reported observations that mating activity in this species occurs only during the dusk period.

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