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ANEMOTACTIC RESPONSES OF THE MALE ORIENTAL FRUIT FLY, *DACUS DORSALIS* TO 2,3,5 TRIMETHYL PYRAZINE IN A HORIZONTAL WIND TUNNEL

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Abstract

Anemotactic responses of the male oriental fruit fly *Dacus dorsalis* to 2,3,5 trimethyl pyrazine were studied using a horizontal wind tunnel. Various concentrations of the chemical compound had significant effects on the behavioural activities of the males but, very high concentrations of the chemical inhibited all their activities. Maximum number of flies landed on the source in response to 10 µl of the chemical. The flies showed an increase of flight activity (orthokinesis) in response to the stimulation. In moving air, the flies apparently located the chemical source using odour-modulated upwind anemotaxis. Two types of anemotaxis were observed in the present study,i.e. positive anemotaxis (straight upwind flights) and reversing anemomenotaxis (zigzagging upwind flights). Males performed both types when the odour was presented as a plume, but exhibited only zigzagging anemotaxis in the uniformly dispersed odour. A relatively lower concentration (1 µl) caused the flies to produce maximum number of zigzagging anemotactic flights whereas a higher concentration (10µl) elicited the highest number of straight flights in males. 2,3,5 trimethy l pyrazine had marked effects on males causing them to orient towards the source (attraction) with the aid of upwind anemotaxis and to land (arrestment) on it.

Key words: Orienate fruit fly, 2,3,5 trimethyl Pyrazine, anemotaxis, wind tunnel

1. Introduction

Tests to demonstrate the behavioural activity of an insect to a chemical compound are essential to prove that the compound is a pheromone component. Such behavioural tests or bioassays can also be invaluable for deducing the communicative function of a chemical identifed from an insect. They can, in addition, give information as to the mechanisms that are used by responding insects to locate the chemical source (Baker, 1985).

Since the earliest recognition that odours play an important role in the life of insects, many efforts have been made to investigate the various aspects of the insect-odour relationship. Laboratory investigations of the behavioural responses of tephritid fruit flies to attractive odours have been previously investigated using various types of bioassay.(Katsoyannos et al, 1980; Ohinata et al, 1973; Robacker & Hart, 1984). With the increased interest in sex pheromones, wind tunnels are being used almost entirely for investigating orientation mechanisms of many insects to such pheromones (Jones et al, 1981; Traynier, 1968).

2,3,5 trimethyl pyrazine has been identified as one of the major components of the male sex pheromone of the oriental fruit fly, *Dacus dorsalis* (Hebert, 1980; Bacon, 1985). This component was found to be highly attractive only to males, eventhough it is a constituent of the male pheromone. An investigation of locomotory responses of male oriental fruit flies to 2, 3, 5 trimethyl pyrazine has been carried out to determine the way which it modulates anemotactic behaviour in the flies. The bioassay technique of assessing the sex attractant involved the use of a low speed wind tunnel.

2. Materials and Methods

Insect material

Oriental fruit flies were reared in the laboratory by methods similar to those adopted by Tanaka (1969). They were maintained at a temperature of $26^0 \pm 2^0$ C and at 70± 10% relative humidity. A 12 hour light period of 1500 lux was provided by fluorescent lamps and attenuated light (20 lux) was supplied for one hour at dawn and one hour at dusk. Flies were maintained on a diet of dry sugar and water supplemented with small quantities of yeast hydrolysate.

Bioassay technique

The horizontal wind tunnel used for bioassays (Fig. 1) consisted of clear plastic tubing which, when inflated formed a cylindrical tunnel with a working section (30 cm diameter and 1.5 m long.) It was inflated with a single phase fan (24 cm diameter, 110 W), while a second fan (19 cm diameter, 86W,) drew air out of the tunnel through the exhaust tubing to the outside. Speeds of the two fans were maintained using a double variac voltage regulator. A honeycomb disc (30cm diameter) mad e of resin-treated paper and covered with gauze, was fixed into the tubing 50 cm downwind of the fan. A second disc similar in size was placed 1.5 m, further downwind to delineate the test chamber. The honeycomb served to even the turbulence in the air flow. The gauze prevented the flies passing through the mesh. Access to the chamber was provided by slits in the plastic wall. These were resealed with paper clips, while experiments were running. A white plastic sheet with transverse black stripes (2 cm wide) at 10 cm intervals beneath wind tunnel provided a uniform stationary ground pattern for the flying insects.

The wind tunnel was housed in a room with temperature maintained at 27 ± 1^0C, a relative humidity of 70±10% and a light intensity of 1500 lux within the tunnel. All the experiments in the wind tunnel were carried out with an average air flow of 0.1 m/s. Ammonium chloride smoke was used to measure the air flow speed

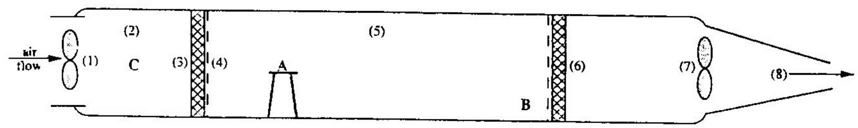

Figure 1 The Wind Tunnel. (1) Upwind fan, (2) Pre-chamber (3) Honey comb disc, covered with gauze (4) to reduce turbulence, (5) Flight chamber (6) down wind honey-comb disk, (7) fan carries air via exhaust tube (8) to outside. A = Position A for odour source, the odour forms a distinct plume in the flight chamber. B = Position B, flies are released from a jar placed down wind in the flight chamber. C = Position for odour source, the odour permeates the whole air stream.

in the tunnel. This was done by placing a source of concentrated hydrochloric acid and ammonia at position A (Figure 2), and recording the time taken for the inflow of the smoke particles over a certain distance.

The flies in a glass jar covered with gauze were introduced to the flight chamber and placed at position B, in the downwind area of the tunnel.

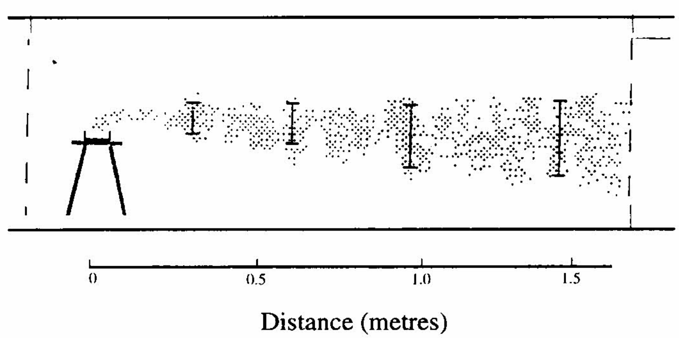

Figure 2 The shape of the odour plume in the wind tunnel

The chemical source to be tested was placed 20 cm downwind of the honeycomb at position A (Fig. 1) to produce a plume of odour and at position C to create a uniform odour distribution in the flight chamber. In each case, the source was placed in a petridish (8.5 cm diameter), which rested on a 15 cm high tripod.

Analysis of behavioural responses monitored in the wind tunnel

Three types of behaviour were monitored over each observation period to study the anemotactic responses of the males.

Activation

Flight activity was monitored to determine whether 2,3,5 trimethyl pyrazine has an activating effect (orthokinesis) on flies. All flight patterns observed during the experiment (upwind, downwind, horizontal, vertical and across the wind tunnel) were recorded and number of flights/ fly was determined. Data were transformed to the square root (Sokal & Rohlf, 1981),

Upwind orientation

This was monitored to determine whether the chemical substance stimulates the flies to move upwind. This behaviour was measured in the following ways.

(i) Upwind anemotaxts (zigzagging and straight upwind flights)

Counts were taken of zigzagging and straight upwind flights occurred continuously over at least,50cm in the flight chamber. Mean numbers of flights/25 flies were determined and a $\sqrt{x+1}$ transformation was applied to the data.

(ii) Movement to upwind third

The percentage of flies that had moved into the upwind third section of the flight chamber (number of flies landing on the upwind mesh, + number of flies landing at position A + number of flies in the upwind third of the wind tunnel) was obtained. An arc sin $\sqrt{x}$ transformation was applied to the data.

Landing at odour source

This was monitored to determine whether the locomotory anemotactic responses stimulated are effective on the flies in locating the odour source and then landing (arrestment) on it. Flies landing on the source to the duration of the observation period were counted. A $\sqrt{x+1}$ transformation was applied to the data.

Each behavioural response monitored was considered separately and a one-way analysis of variance (ANOVA) carried out. Wherever analysis of variance indicated a significant difference between responses to different treatments, Duncan's multiple range test (DMRT) was carried out at a p=0.01 level of significance to identify the significant treatments.

Experimental

The following experiments were carried out between 8.00 a.m. and 12.00 noon under day light conditions (1500 lux). Each test was carried out for twenty minutes, using twenty five males which were, 14- 17 days old . Required doses of the chemical compound were prepared by injecting appropriate amounts into the cavity of rubber bungs by means of a microsyringe. An empty rubber bung was used as the control in each test.

(i) Anemotactic responses of males to six doses of 2,3,5 trimethyl pyrazine (0.01,0.1,1, 10, 20, 50µ1) presented in rubber bungs were tested in the wind tunnel. This experiment was replicated four times.

(ii) Behavioural activities of males in response to 2,3,5 trimethyl pyrazine were observed further by presenting 10µ1 of the chemical at position C (Figure 1) to produce a homogeneous (dispersed) pheromone cloud. An untreated bung was placed at position A during the observation period. This experiment was replicated six times.

3. Results

The effects of concentration on behavioural responses of males are summarized in Table I and Figure 3. One- way ANVOA showed that all the responses were significantly affected by the treatments, 10µ1. of the chemical elicited highest responses in males in all the behavioural parameters measured. When the two types of anemotaxsis were considered separately, the highest number of zigzagging anemotaxis was observed with 1µ1, whereas 10µ1 elicited the highest number of straight upwind anemotaxis in the flies. When analysis of covariance was carried out to test whether the flight activity has any effect on males landing on the source of various concentrations, it did not show any significant effect (Table II). On the other hand, upwind anemotaxis (zigzagging and straight flights) had a marked effect on number of flies landing on the source of various concentrations ($F=3.08$ $p<0.05$).

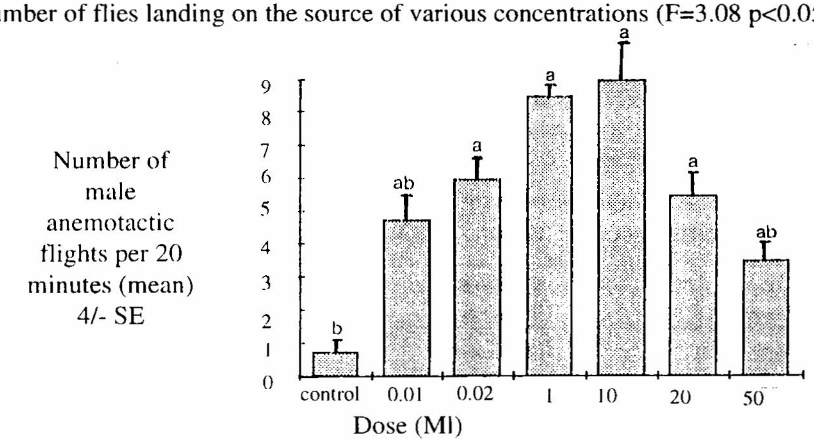

Figure 3. Upwind anemotaxis of males in response to various doses of 2,3,5 trimethyl pyrazine in a wind tunnel bioassay.

Table I Anemotactic responses of male oriental fruit flies to different concentrations of 2,3,5 trimethy pyrazine in a wind tunnel

	Response				
		Upwind Anemotaxis			
Treatment	Number of flights per male (Mean±SE)	Zigzagging flights (Mean±SE)	Straight flights (Mean±SE)	Landing on the source (Mean±SE)	Percent moving to upwind third (Mean±SE)
	d	b	b	b	b
Control	4.4±0.5	0.0	0.8±0.5	0.8±0.57	17.6±5.3
	cd	ab	ab	b	ab
0.01	6.9±1.0	1.3±0.5	3.5±0.6	2.0±0.7	33.5±4.3
	bc	ab	ab	b	ab
0.1	8.5±0.4	2.0±0.7	4.0±0.7	2.8±0.6	32.8±3.6
	ab	a	ab	b	ab
1	10.6±1.4	4.0±0.4	4.5±0.3	2.0±0.4	36.0±5.5
	a	ab	a	a	a
10	13.3±0.81	2.8±0.5	6.3±1.4	8.8±2.2	56.0±6.9
	abc	ab	ab	b	b
20	9.9±0.7	2.0±0.4	3.5±0.9	2.3±0.8	20.0±1.6
	bc	ab	ab	b	b
50	9.4±0.3	1.0±0.4	2.5±0.6	1.3±0.7	19.2±3.4
F(6,21)	13.52	3.6	3.29	4.19	8.4
Probability	p<0.005	p<0.05	p<0.05	p<0.01	p<0.05

Means in the same column followed by similar letters do not differ significantly.
Mean values ±standard error with four replicates.

The analyzed results are shown in Table III where 10μl of the chemical was presented to the males as a plume of odour and as a uniform odour cloud separately. In both tests (plume and uniform odour clouds) the insects showed high activation. Only straight upwind anemotaxis was observed in the uniform odour cloud, whereas both zigzagging and straight upwind anemotaxis were observed in the plume of odour. Although the number of flies landing on the upwind mesh was significantly higher in the uniform cloud than in the plume of odour ($p<0.01$, DMRT), the percentage movement of flies into the upwind third of the wind tunnel in the uniform odour cloud was not significantly different from that of odour plume.

Table II Effects of flight activity and upwind anemotaxis on the landing of flies on a source of different concentrations of 2,3,5 trimethyl pyrazine

Analysis of Covariance	Source of Variation	d.f.	F-value	Probability
Flight activity on landing on source	Treatments*	6	2.04	NS
	Error	17		
Upwind anemotaxis on landing on source	Treatments*	6	3.1	p<0.05
	Error	17		

*Represents seven treatments including the control
d.f - Degrees of freedom
NS-Not Significant

Table III Anemotactic responses of male oriental fruit flies to 10 ul of 2,3,5 trimethyl pyrazine in a wind tunnel presented as a plume and a uniformly dispersed odour source.

Treatment	Response					
			Upwind anemotaxis			
	Number of flights per male (Mean±SE)	Zigzagging flights (Mean±SE)	Straight flights (Mean±SE)	Landing on the source (Mean±SE)	Landing on the upwind mesh (Mean±SE)	Percent moving to upwind third (Mean±SE)
Control	3.6±0.5 b	0.0 b	0.5±0.2 b	0.3±0.2 b	0.5±0.2 b	11.8±1.2 b
Plume of odour	12.3±0.7 a	3.2±0.4 a	5.0±0.8 a	7.0±0.8 a	0.8±0.3 b	50.1±5.3 a
Uniform odour	10.9±1.1 a	0.0 b	6.7±0.6 a	0.5±0.3 b	6.3±1.0 a	61.3±6.7 a
F(2,15)	36.44	38.33	7.1	31.9	33.25	35.91
Probability	$p<0.005$	$p<0.005$	$p<0.01$	$p<0.005$	$p<0.005$	$p<0.005$

Means in the same column followed by similar letters do not differ significantly ($p>0.01$, DMRT) Mean value±standard error for six replicates.

4. Discussion

Studying the insect behaviour in response to odour sources in a wind tunnel is very useful as the horizontal air stream allows the insect to use upwind anemotaxis for guidance towards the source in the way that the same insect could use it from a distance in the field (Kennedy, 1977). Furthermore, odour modulated anemotaxis has been verified as a major mechanism used by flying insects to discover the location of an odour source (David et. al, 1983). The evaporating odour molecules form an elongated and irregularly shaped plume moving in a downwind direction. An insect that orients anemotactically turns its body axis into the wind when it is stimulated by the odour.

According to Kennedy et al (1981), a flying insect find its way to the odour source using two anemotactic manoeuvres. One method is direct upwind flight in response to the onset or increase of the pheromone stimulus as long as the stimulus is maintained. The other is cross-wind flight with switching between left and right of the wind line (zigzagging) which occur only in response to the loss or decrease of the pheromone stimulus. This zigagging flight pattern that cuts across the pheromone plume is called reversing anemomenotaxis because it is oriented at an angle to the wind. The zigzagging flight is probably a behavioural response to the loss of the pheromone stimulus at the edge of the plume. The response tends to bring the insect back into the plume again.

In the present wind tunnel study, males exhibited both types of upwind anemotaxis in response to the various concentrations of 2,3,5 trimethyl pyrazine. The upwind anemotaxis increased with the increase of concentration up to a maximum at 10μl, and then decreased with the increase of concentration of the chemical. When the two types of anemotaxis were considered separately, both responses increased with the increase of concentration but a relatively lower concentration caused the flies to produce maximum zigzagging anemotaxis whereas a higher concentration elicited the highest number of straight anemotactic flights in males. Carde &Hagman (1979) stated that in pheromone plumes, zigzagging of the gypsy moth, *Lymantria dispar* becomes more pronounced when concentrations are relatively low. Ralatively high concentration caused the males to produce more straight upwind flights. The increase of straight flights could have been due to the narrowing of the gaps within the plume caused by the increase of concentration (Marsh et al, 1978). The width of the 'active space' also increases with concentration (Baker, 1985), causing the males to fly largely within the plume area. With the increase of the concentration, the male flight activation (orthokinetic effect) increased.to a maximum at 10μl and then decreased with further increase in the concentration. The combination of other orientation mechanisms (zigzagging and straight anemotaxis) with the orthokinetic reaction may be serving to guide the flies towards the odour source.

When 10μl of the chemical was presented to the males as a homogeneous cloud, they showed increased flight activation. The flies exhibited straight upwind flights but no ziggzagging flights were observed. It is apparent from the present study, that 2,3,5 trimethyl pyrazine not only increased the activity in male *Dacus dorsalis* but also modulated direct upwind anemotactic responses when the odour source was presented as a uniform cloud. This was supported by the observations of the high numbers of males landing on the upwind mesh as well as moving into the upwind third of the wind tunnel. In addition to evoking direct upwind anemotaxis, this chemical component, when presented in a plume form induced an upwind zigzagging anemotaxis along the plume to the source. These observations accord well with the observations of *Drosophila melanogaster* (Kellog et al, 1962), *Anagasta kuehniella* (Traynier, 1968) and *Ceratitis capitata* (Jones et al, 1981).

The activities of the male oriental fruit flies suggest that two distinct forms of anemotaxis are involved in the behavioural mechanism to locate the chemical source. In the field it is possible that the flies responding to a plume of odour, fly directly upwind when they are at a distance from the source where the odour is uniform, but when the plume narrows near the source, the flies then switch to the zigzagging anemotaxis.

Finally, it can be concluded that 2,3,5 trimethyl pyrazine has an activating effect on males (orthokinesis). It acts as an attractant, causing them to orientate towards the source with the aid of upwind anemotaxis. Furthermore, this chemical compound acts as an arrestant, causing the flies to aggregate on the odour source as a result of orientation movements.

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