EFFECTS OF ULTRASONIC ON PRODUCTION AND PERCEPTION OF AGGREGATION PHEROMONE IN *TRIBOLIUM CONFUSUM* (DUVAL).

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INTRODUCTION

The confused flour beetle, *Tribolium confusum* (Duval), is a serious economic pest of stored products in many areas of the world, and it is a secondary pest of stored grain, (Cronwell, 1966).

Quite apart from the economic importance of *T. confusum* as a pest of flour and other cereal products, is its significance as a model in population studies (Park, 1962).

Suzuki *et al.* (1975), while working with *T. confusum* and with *T. castaneum*, identified seven unsaturated hydrocarbons (1-tetradecene, 1- pentadecene, 1-hexadecene, 1-heptadecene, 1, 6-pentadecadiene, 1, 8- heptadecadiene, and heptadecatriene) which, in admixture, have repellent qualities and which therefore act as alarm pheromones.

Any attempt to device control measures, using pheromones, or to allow for their influence in population models, calls for an understanding of the effects of environmental and physiological factors. Many factors affect the production and perception of pheromones by insects. Accordingly, the present study examines the effects of habituation behavior and age, on the response and perception of aggregation pheromones in this species under ultrasonic treatment.

Hinton, (1942), differentiated the sexes of the confused flour beetle *T. confusum* by the presence of a sub basal setiferous sex patch on the ventral side of male femur, a structure which is absent in the female.

Males of *T. castaneum, T. confusum*, *Prostephanus truncates* (Horn) and *Rhyzopertha damenica* (Fabricius) (Bostrichidae), produce aggregation pheromones attractive to both sexes (Oceallachain and Ryan, 1977, Suzuki; 1980; Williams *et al.*, 1981 Hodges *et al.*, 1984).

T. castaneum males produce the aggregation pheromone, 4,8- dimethyldecanal (Suzuki, 1980), from setiferous sex patch on the ventral side of the femur which mediates behavioural responses in both sexes (Faustini *et al.*, 1981,1982).The attractancy of the synthetic pheromone has led to the production of commercial lurs for use in trapping.

MATERIAL AND METHODS

The beetles were reared under lab-condition at 25±5C^{0}and 65RH. ± 5%.The diet used was wheat flour (extracted 70%). Diet contains 5% yeast powder, this to prevent a possible olfactory response to yeast particles sticking their bodies (Loschiavo, 1965).Insects were sexed as pupae based on the structure of the genital lobes (Ho, 1969), the females possessing larger and more flexible lobes than the males. Male and female pupae were held in separate jars containing food media until adult eclosion.

Beetles male were mounted into drying silver colloidal paint, in order to display the setiferous sex patch of the pheromone site, it was necessary to place the entire insect into a hexane bath for ca. 30 min to dissolve the setiferous secretion. Preparations were coated with ca. 200A° of 60-40 gold (Faustini *et al.*, 1981).Observations were made with a Scanning electron microscope.

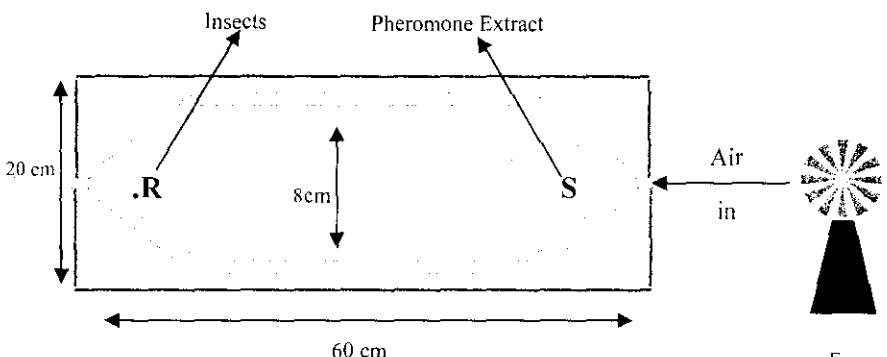

Fig. 1: Diagram of the olfactometer showing fan, pheromone source (s) & release point (R).

The pheromone precursor was extracted, by soaking sample in normal hexane (10cm) for 5 hours at 0C°, then homogenized. The homogenate was filtered, and the residue was thoroughly washed with normal hexane according to the method of (Grant *et al.*, 1975).

The olfactometer used was similar to that described by Jones (1977) and consisted of 60 cm long glass with 8 cm high (fig.1). 30 Adult beetles of mixed sexes were released on surface of the wheat flour at R (side).

The orientation of *T. confusum* to aggregation pheromone was observed in an olfactometer in which odour gradients (at S side) were set up still. The electric fan was used as a source of air current positioned in front of the pheromone. One hour was allowed for the pheromone to diffuse into the olfactometer and set up a concentration gradient before testing. After introducing the pheromone source (S side in fig.1), the beetles were released at 50 cm from the source; they were removed from the chamber by a suction tube after each test. An orientated response was recorded for beetles moved from the releasing point towards the odour source.

Beetles were habituated by exposing them for 3, 9 and 12 hours to the extracted aggregation pheromone from 50,70 and 100 males were exposed to ultrasound range of 24000Hz to 45000 Hz from small unit so that the waves were directed into it from a distance of about 20 cm. at the normal laboratory conditions ($25C^{o}\pm 5\ C^{o}$ and 65% RH. $\pm$ 5%).

The response of *T. confusum* was studied, four concentrations (0.5, 1.0, 2.0and 8.0 ml) were used from 50, 70 and 10 males, respectively, increasing the pheromone concentration stimulated greater response in *T. castaneum* suggesting that their receptor cells are probably capable of discriminating between different odour concentrations (Visser, 1986).

Beetles were released at 50 cm from the source. Each concentration was replicated three times.

For studying the effect of age on pheromone production, extracts were prepared from newly emerged male ages 3, 5 and 7 days. Each male age group extract was tested in three replicates. Production of male pheromone reaches a peak five days after emergence (Oceallachain and Ryan, 1977).

Analysis of variance was used to analyze the data; means were separated by Duncan's (1951) multiple range test using SPSS Computing Program.

RESULTS AND DISCUSSION

Morphological Description of the pheromone production site could be seen in Fig. 2A. Fig. 2B for the male front leg shows the ventral setiferous sex patch of the pheromone production site in *T. confusum* male beetles. Fig. 2C shows the dorsal view of male front leg. The setae are particularly sparse within the center of the setiferous sex patch (Fig. 2 D) but appear dense in arrangement at its periphery. Ventral view of the male femoral sex patch (Fig. 2 E) shows the opening of sex pheromone gland. The

same observations were detected by Faustini *et al.* (1981) in their studies on *T. castaneum* for the site of Aggregation pheromone production.

Response and habituation

Results presented in Table (1) show a few response (2.8), when males number was 50 after 9 hours, whereas no response after 12 hours by the active ultrasonic- emitting devices. The highest level of response (63) was after three hours in untreated group. However, both males and females exhibited a sequence of behavioral responses which included extension of the prothoracic legs, after accompanied by rapid head and body movements and antennal protraction followed by quick zig- zag walking movements towards the pheromone source. On reaching the source, they moved around it and they attempted to climb the pheromone source, probing intermittently with their antennae. Obeng- Ofori and Coaker, (1990) reported that prolonged exposure to aggregation pheromones has a marked effect on male *Tribolium* spp. In a population where males predominate, their olfactory receptors become adapted so that positive orientation is no longer possible. They may then become more responsive to other stimuli, such as those inducing flight and dispersal to other attractive odour or food sources.

Reduction in aggregation pheromone response (treated) occurred in insects treated, without recovery after 9 hrs; but increased to the same pheromone (treated) by untreated insects without restoration after 3 hrs. The same trend of response was happened by insects exposed to untreated pheromone (Table 1)..

The response of *Tribolium* spp. to the pheromone source was similar to that reported in other species (Abdel- Kader and Barker, 1979; Faustini *et al.*, 1981).

The percentage response of *T. confusum* to different pheromone concentrations is shown in Table (2). It is clear from the recorded data that the response of treated beetles was significantly affected. The male response started with low level and increased with the increase of concentration to reach maximum level of response (2.9 and 2.7%) at (2.0 and 8.0 ml) respectively comparing to (47 and 54.8%) at (2.0 and 8.0 ml), respectively in the control. Obeng-Ofori and Coaker, (1990), reported that increasing pheromone concentration stimulated greater response in *T. castaneum* suggest that the receptor cells are probably capable of discriminating between different odour concentrations.

TABLE (I)

Effect of ultrasound on the habituation behavior to the aggregation pheromone in *Tribolium confusum.*

Treatment	Tested Sex	%of beetles attracted to source of extracted pheromone								
		3hrs			9hrs			12hrs		
		50	70	100	50	70	100	50	70	100
1	Female and male (treated).	5.5c	6.3c	8.4 c	2.8c	3.7. c	5 c	0b	0b	0 c
2	Female and male (untreated)	35b	19b	22.3 b	4b	7b	7.7b	0b	0b	0 b
3	Female and male (untreated).	51a	55a	63a	37a	42a	48a	43a	39a	38 a

1- Pheromone extracted from treated (ultrasound) male.
2- Pheromone extracted from treated (ultrasound) male.
3- Pheromone extracted from untreated (control) male.
50, 70 and 100: number of males from which aggregation pheromone extracted.
Small letter represent significant differences between treatments and percentage response to aggregation pheromone to some hours at p >0.01 according to Duncan test.

TABLE (II)

Response of *T. confusum* beetles to different concentration of aggregation pheromone extracted from males treated with ultrasound.

Treatment	Tested Sex	%of beetles attracted to source of extracted pheromone											
		Concentration of aggregation pheromone											
		0.5 ml			1.0 ml			2.0ml			8.0 ml		
		50	70	100	50	70	100	50	70	100	50	70	100
1	Female and male (treated)	0c	0c	0c	1.4c	1.9b	1.9b	1.5b	1.9c	2.9b	2.0c	1.9c	2.7c
2	Female and male (untreated)	2.6b	2.6b	4.3b	.2	.6c	2.2b	1.3c	2.3b	2.6b	5.1b	5.3b	5.3b
3	Female and male (untreated)	11.5a	17a	24.3a	18.6a	25a	36.6a	30.5a	41a	47a	41a	44a	54.8a

1- Pheromone extracted from treated (ultrasound) male.
2- Pheromone extracted from treated (ultrasound) male.
3- Pheromone extracted from untreated (control) male.
50, 70 and 100: number of males which extracted aggregation pheromone.
Small letters represent significant differences between treatments and percentage response to aggregation pheromone by using different concentration from aggregation pheromone at p >0.01 according to Duncan test.

The data obtained on the response of beetles at different ages to aggregation pheromone showed in Table (3). The response however, started at low level (29.8%) for young beetles (3- day old). It increased as they became older. The maximum response (48%) was reached at 5- day old males pheromone extract.

The percentage of beetles response exposed to ultrasound differs significantly. The analysis of data indicated significant decrease levels of response.

Oceallachain and Ryan, (1977) and Obeng- Ofori and Coaker, (1990) reported that male secreted no pheromone when 1- day old, increasing gradually to a maximum at 10- 12-day old, after eclosion. The ability of *Tribolium* to respond to pheromone immediately after emergence is consistent with their ability to mate 17-26 hours later (Dawson, 1964).

The effect will be though preventing the two sexes from coming together for mating and reproduction.

Mating behavior and reproduction of moths associated with field crop were affected by ultrasound (Payne and Shorey, 1968; Barker and Carde, 1978). Acharya and Mc Neil (1998) and Ismail *et al.* (1990) showed that males of the European corn borer, *Ostrinia nubilalis* (Hubner), the true army worm, *Pseudaletia unipuncta* (Haworth) and *Callosobruchus maculatus* (F.), flying upwind in response to female sex pheromone in a wind tunnel aborted the upwind flight when exposed to simulated aerially hawking bat sounds (50 kHz, 50-100 pulses/s, and 90 dB sound pressure level (dB SPL). Females of both species stopped calling when exposed to howking bat sounds (Acharya and McNeil, 1998). Therefore, ultrasounds that simulate those produced by hawking bats could alter reproductive activities of moths.

In the present study, the male produced aggregation phermone from sub basal perceived by olfactory receptors in both sexes. Ultrasonic may affect olfactory function which may after cluse, how this receptors serve the insect and may be responsible to reduce the biological activity of the insects. Gharieb and El- Degwi (2002) reported that the effect of sterile dose of gamma radiation on olfactory receptors may reduce the biological activity of insects. The synthesis of the male aggregation pheromone in *T. confusum* could therefore lead to a useful tool for monitoring and controlling populations in stored grain. It can be concluded that the effect of ultrasound on aggregation pheromone production and perception is an important factor that can be recommended in the control programme of *T. confusum*. The effect will be though preventing the two sexes from coming together for mating and Reproduction.

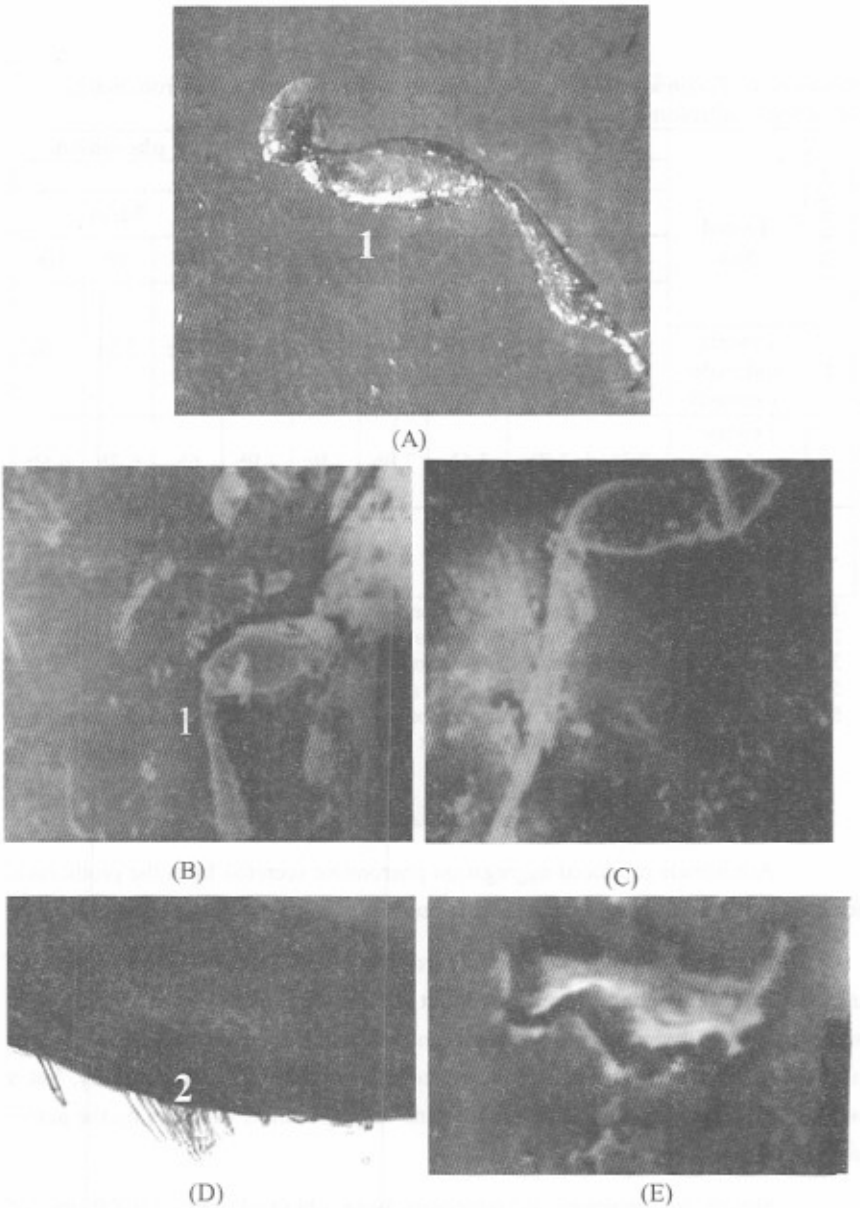

Fig. 2 Ventral view of the male front leg by binocular (130 x) (A). Ventral view of the male front leg, 1-showing sex patch S.E.M.(B). Dorsal view of the male front leg (C). Ventral view of the location sex patch on a 10 day- old male (2 noticed the glandular hairs) (D). Ventral view of the male femoral sex patch, (3 note the opening of sex pheromone gland) (E). (50X).

TABLE (III)

Response of *T. confusum* beetles to aggregation pheromone extract from males treated with ultrasonic at different ages.

Treatment	Tested Sex	%of beetles attracted to source of extracted pheromone								
		Age of male after eclosion								
		3days			5days			7days		
		50	70	100	50	70	100	50	70	100
1	Female and male (treated).	1.2c	1.4c	1.8c	2.0c	2.3c	3.0c	2.7c	2.3c	3.5c
2	Female and male (untreated.	2.3b	2.7b	3.6b	7.0b	7.9c	7.9b	6b	6.7b	6.6b
3	Female and male (untreated).	29.8a	37.3a	37.8a	37a	42a	48a	33a	34a	36a

1- Pheromone extracted from treated (ultrasound) male.
2- Pheromone extracted from treated (ultrasound) male.
3- Pheromone extracted from untreated (control) male.
50, 70 and 100: number of males from which aggregation pheromone extracted.
Small letters represent significant differences between treatments and percentage response to aggregation pheromone extracted from Different male ages at $p > 0.01$ according to Duncan test.

SUMMARY

Adult male produced aggregation pheromone secreted from the prothoracic femoral setiferous sex patch of *Tribolium confusum.*

Pheromone production and perception was studied under effect of ultrasound waves produced from small unit in relation to habituation, concentration and male age. A living source of pheromone habituates the responding beetles. The habituation decreased gradually and reflexes the response to monotonously, but a weak response happened after 9 hours but no response after, 12 hours by the active ultrasound- emitting devices.

Significant increases in responses were obtained with 2.0-8.0 mg of pheromone extract than from those with the lower concentrations in all treatment. The response started at low level (29.8%) for young beetles (3- day old), and increased as they became older. The maximum response (48%) was reached at 5-day old. The percentage of beetles response exposed to ultrasound differs significantly. The analysis of data indicated significant decreased levels of response.

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