Table 2. Density of adult *S. madanensis* on unit weight of anchovy and atmospheric humidity and temperature during 1976

	Density y	Relative humidity x	Temperature z
January	0	63.0	26.3
February	0	64.0	26.65
March	1,850	69.5	28.10
April	6,450	75.5	28.55
May	5,975	77.0	28.35
June	7,175	74.5	27.75
July	5,800	84.0	26.50
August	10,450	82.0	26.50
September	11,925	79.0	26.90
October	11,850	83.0	26.75
November	12,350	87.0	26.40
December	15,625	74.0	26.90

Table 3. Correlation coefficient between the population density and relative humidity and atmospheric temperature during the 2 years

	1975	1976
εy	41,190	89,450
εy^2	169,661,610	8,001,302,500
εx (rel. hum.)	9,605	912.5
εx^2	922,560.25	832,656.25
εz (temperature)	320.1	325.65
εz^2	102,464.01	106,047.9225
εxy	3,333,816.5	7,110,587.5
εzy	1,083,727.65	2,419,377.5
Correlation coefficient between density of mite and relative humidity	0.0032 (not significant)	0.0041 (not significant)
Correlation coefficient between density of mite and atmospheric temperature	−0.0039 (not significant)	−0.0003 (not significant)

each month are given in tables 1 and 2. The correlation coefficient values between the density of the mites and the known variables of atmospheric temperature and humidity are given in table 3.

The correlation coefficient values obtained for both the years in respect of the seasonal density of the mites and the atmospheric factors humidity and temperature indicate that these variables and the seasonal fluctuation in the population intensity of the mite are not correlated. Fluctuations in the population density of the mite evidenced in nature can then be attributed to the influence these atmospheric factors may have on the developing stages of the mite, absence of predators etc. The results of the present study are also suggestive that a prediction on the magnitude of population of this obnoxious mite, that may be present from season to season or from place to place, is not possible based on parameters like atmospheric humidity and temperature.

Specific differences in tsetse fly sounds and their behavioural significance

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Summary. Differences in the sounds of 4 species of tsetse flies have been demonstrated. It has also been shown that tsetse flies perceive and react to sounds made by other members of the species.

Sex differences in the mating, pre- and postfeeding sounds of *Glossina morsitans* have been demonstrated and shown to have characteristic spectrograms for the frequencies 0–100 kHz[1]. The present authors have made recordings of the premating, pre- and postfeeding sounds of *G. morsitans orientalis* (Vander.), *G. palpalis* (R-D), *G. tachinoides* Westwood and *G. austeni* Newstead, in the University of Salford Recording studio, using a 'Shure' microphone type SM76 and a 'Uher' Tape Recorder type 4200, at a recording speed of 19 cm/sec. The frequency response of the tape recorder within ±3 dB was 4 Hz to 20 kHz.

The prefeeding sounds are made as the proboscis is driven into the host and is a short song with bursts of about 0.25 sec duration. The postfeeding sounds were produced as and after the proboscis was withdrawn when the fly was gorged with blood. Semigorged flies seldom produced sounds. The bursts of postfeeding sounds are of 2–30 sec duration and show slight variations in pitch and tone. They may be produced for up to 30 min after feeding has finished, especially if the flies are disturbed by vibration. The sounds are produced by the rapid contraction of the flight muscles of the pterothorax and are not produced if the axillary wing sclerites are damaged or coated with wax. Removal of the wings and/or halteres and coating of the spiracles with wax does not inhibit sound production. Stereoscan photographs show that when the wings are flexed over the abdomen, the relative positions of the axillary sclerites are changed and the wing bases disengaged from the click mechanism.

The sound recordings obtained were analyzed with the aid of an oscillograph to give the wave form and with a real

Records of number of speciments of *G. austeni* producing sounds under the conditions indicated

No. of flies present in cage	No. of experiments	Males: No. of flies producing sounds	Males: Mean No. producing sound per fly	Females: No. of flies producing sounds	Females: Mean No. producing sound per fly present
1	30	25	0.83	27	0.90
2	30	45	0.75	44	0.70
3	20	32	0.53	40	0.50
4	20	38	0.48	46	0.57

The difference between the results for the experiments with 1 and 4 flies per cage are statistically significant. For males: $\chi^2_{(1)} = 4.6$ for which $0.05 > p > 0.02$. For females: $\chi^2_{(1)} = 8.0$ and $0.01 > p > 0.001$.

time ⅓ octave analyzer (Bruel and Kjaer type 3347) to obtain volume-frequency histograms. The apparatus employed is sensitive to frequencies up to 20 kHz and has a flat response for 0–16 kHz but is less sensitive to higher frequencies. Although Erickson and Moller[2] claim that tsetse flies produce sounds with harmonics up to 100 kHz, our recordings failed to record any harmonic over 12 kHz and are, therefore, faithful recordings for frequencies up to 20 kHz.

A comparison of the oscillograms and volume-frequency histograms reveal specific differences between the pre- and postfeeding and premating sounds of the four species as are shown in the oscillograms and in the volume-frequency histograms (figure 1). Other similar records show each individual fly has its own characteristic postfeeding sounds in spite of sound variations made by specific individuals. The characteristic sound of each sex of the species concerned are illustrated in the accompanying figures.

In order to try and determine the responses of tsetse flies to sound produced by other individuals of the same species, tsetse flies were placed in a geigy cage, 15×9×5 cm, and fed on lop-eared rabbits. The number of flies in the cage was 1, 2, 3 and 4 and the number producing sounds after feeding was noted. The experiments were repeated until the summation of results was statistically reliable (table).

Fed flies with the axillary sclerites waxed to inhibit sound production and contained in a geigy cage failed to stimulate movement of nearby unfed flies, but sounds produced by fed flies with unwaxed axillary sclerites, did.

Gorged flies were kept in a geigy cage, surrounded by dark polythene and securely sealed to prevent any pheronome produced by these flies being perceived by flies in other cages. When these gorged flies emitted sounds, unfed flies in nearby cages moved at random.

5 unfed flies, 2–3 days old and of the same species and sex, were placed in a geigy cage and the number of flies moving recorded at 10-sec intervals, together with the number with unsheathed proboscis. When a polythene covered geigy cage containing gorged flies was slightly shaken to stimulate these flies to produce postfeeding sounds, it was

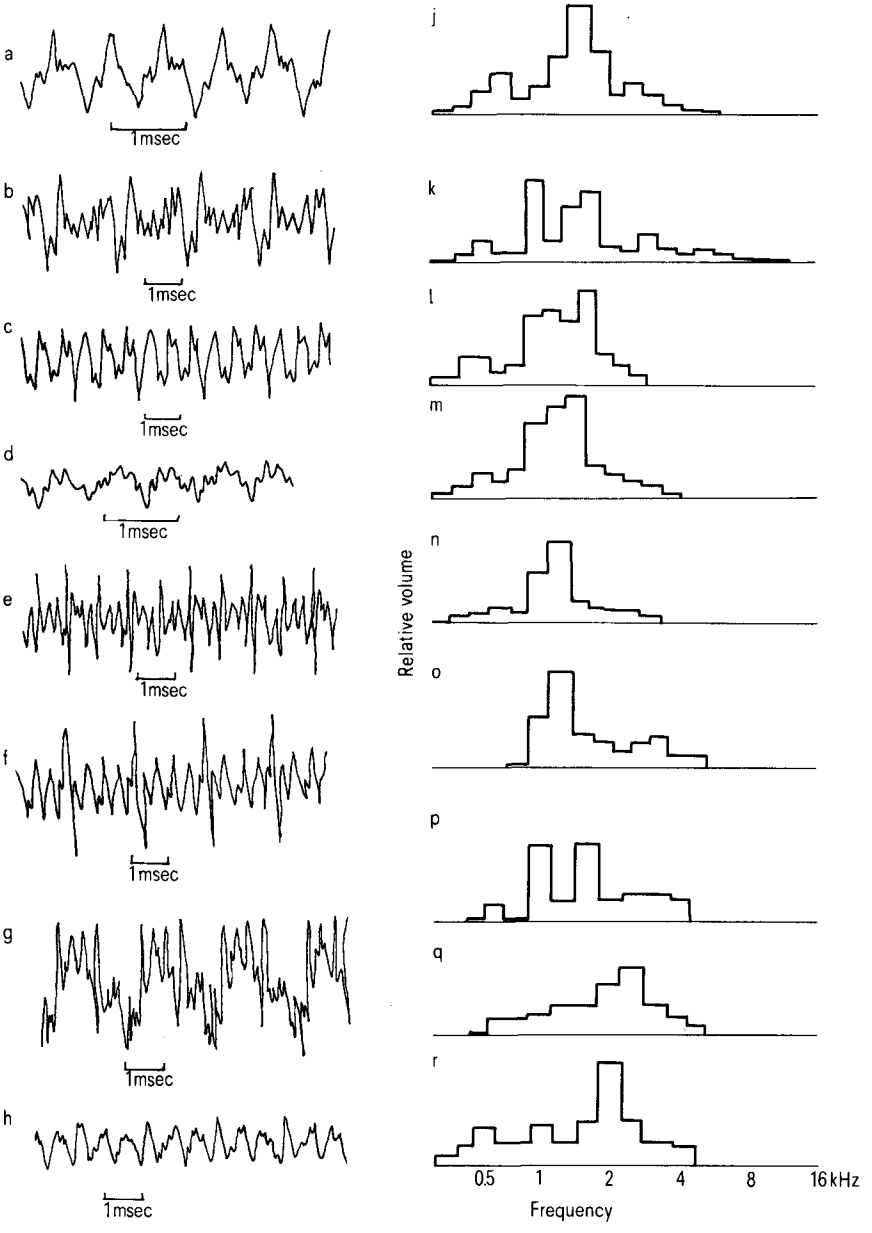

Fig. 1. *A–F* Oscillograms of postfeeding sounds of tsetse flies.

A Females of *Glossina morsitans; B* females of *Glossina palpalis; C* females of *Glossina austeni; D* males of *Glossina morsitans; E* males of *Glossina palpalis; F* males of *Glossina austeni.*

G–H oscillograms of the mating sounds of tsetse flies.

G Glossina palpalis; H Glossina austeni.

J–R Relative volume/frequency histograms of the sounds of tsetse flies. Y: relative volume; X: frequency in kHz.

J Females of *glossina tachinoides; K* females of *Glossina palpalis; L* females of *Glossina austeni; M* males of *Glossina morsitans; N* males of *Glossina tachinoides; O* males of *Glossina palpalis; P* males of *Glossina austeni.*

Q–R Mating sounds of tsetse flies.

Q Glossina palpalis; R Glossina austeni.

observed that the unfed flies in another cage kept at a distance of 3 cm moved at random in all directions with unsheathed proboscis for a period up to about 60 sec. The experiments were repeated a sufficient number of times for the summated records to be statistically reliable and are given in figure 2. Flies with intact antennae or with the arista removed responded to the sounds of other individuals of the same species, but flies with no flagellum to their antennae did not do so. This seems to indicate that the organs for perceiving the sounds are situated in the flagellum. These experiments were performed at 25 °C and at a relative humidity of 80%.

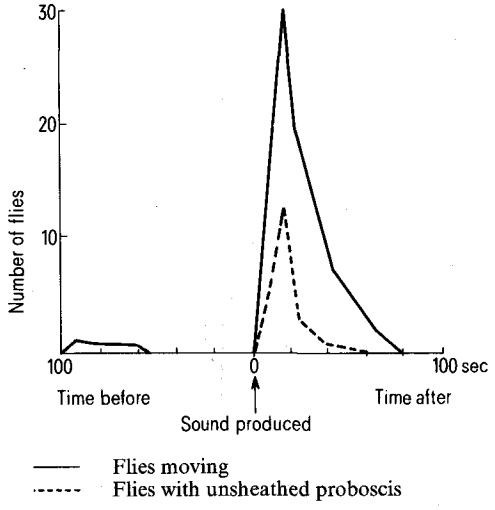

Fig. 2. Graph of response of tsetse flies to sounds produced by *Glossina morsitans*.

The tsetse flies failed to respond to the sounds of another species. The facts show that tsetse flies hear and respond to sounds produced by members of their own species.

It has been observed that tsetse flies are attracted to a host by radiant heat emitted from the skin, but feeding may be inhibited when the fur exceeds a critical length[2]. It would follow, therefore, that if a tsetse fly gorged successfully on an area of skin, the postfeeding sound could stimulate other flies which have failed to become gorged, to move away from those areas of skin where feeding is difficult. This would result in unfed flies eventually accumulating in those areas where blood could easily be obtained and/or where they could find a mate.

1 M. Abdillahi, Ph. D. thesis, University of Salford (1974).
2 E. C. Erickson and A. R. Møller, J. acoust. Soc. Am. *57* (4), 984 (1975).

Dithiols simulate endotoxin in the *Limulus* reaction[1]

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Summary. Dithiothreitol, dithioerythritol and bacterial lipopolysaccharides increase optical absorbance and clot *Limulus* lysate. Purification of dithiothreitol from possible endotoxin contamination by vacuum sublimation or chromatography does not abolish the reaction with lysate. The dithiols reported active here represent the smallest molecules capable of simulating endotoxin in the *Limulus* test.

The *Limulus* amebocyte lysate (LAL) method is the most sensitive assay for the measurement of endotoxin in serum, radiopharmaceutical and biological products[2-9]. A number of proteolytic enzymes, synthetic polynucleotides and peptidoglycans have the ability to mimic endotoxin in the *Limulus* crab assay procedure[10-12]. Here we report that certain low mol. wt thiols have the same property.

Materials and methods. LAL was reconstituted from lyophylized material obtained from Sigma Chemical Co. Endotoxin-free water was prepared by distilling water over $KMnO_4$ in an allglass apparatus, or purchased from Abbott Laboratories. Whenever possible, disposable plasticware was employed and glassware was carefully washed and heated overnight at 180 °C. Oxidation of DTT was done according to Cleland's method[13]. LAL test was performed according to the method described by Watson et al.[14].

P-200 chromatography. In order to show that the active material was not in the high mol. wt fraction, and thus not contaminated with endotoxin, solutions containing thiols sublimed and not sublimed, were chromatographed on acrylamide P-200, BioRad Corp. 0.2 ml of the sample was chromatographed on an endotoxin-free 1.0 × 25 cm column of BioGel P-200, 50-100 mesh, which had been equilibrated with endotoxin-free 0.5 M NaCl. Fractions of 0.5 ml were collected, using the same eluant. All fractions were checked for reactivity with LAL. The void volumes were determined, using dextran blue obtained from Pharmacia.

We eliminated the possibility of contamination of thiols by degraded or retarded endotoxin by chromatographing a mixture containing 1 ng LPS and 0.2 ml of 10^{-2} M DTT. After such separation all LAL-active material in the thiol fraction was volatile in vacuo at 80 °C (0.005 mm).

Results. The table shows that significant reaction of LAL occurs in the presence of 10^{-4} M DTT or DTE. Such concentrations determined the gelation of LAL in 24 h at 37 °C. Samples of thiols both before and after sublimation showed the same activity with LAL. No activity would be sublimed from LPS. Mercaptoethanol, monothioglycerol or L-cysteine showed no reaction at such concentrations. Figure 1 represents the dose-response curve for LPS and

Activation of *Limulus* lysate by dithiols

Molarity	Absorbance at 360 nm			
	DTT		DTE	
	Not sublimed	Sublimed	Not sublimed	Sublimed
10^{-2}	1.77	1.56	1.90	1.6
10^{-3}	1.49	1.22	1.28	1.22
10^{-4}	0.99	1.08	1.01	0.87
10^{-5}	0.34	0.23	0.03	-

The table shows OD of *Limulus* lysate after 1 h incubation at 37 °C and after subtraction of a blank of 0.1-0.2 A 360. (1 ng/ml *E. coli* endotoxin gave 0.42 units increased absorbance.)