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Responses of tsetse flies (Diptera: Glossinidae) to natural and synthetic ox odours

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Abstract

In Zimbabwe, studies were made of the levels of known tsetse attractants present in natural ox odour. Typically an ox (400 kg) produced phenol (0.1 mg/h), 3-methylphenol (0.09 mg/h), 4-methylphenol (0.7 mg/h), 3-ethylphenol (0.01 mg/h), 4-ethylphenol (0.02 mg/h), 3- and 4-n-propylphenol (0.02 mg/h), 1-octen-3-ol (0.01 mg/h), carbon dioxide (140 l/h), acetone (5 mg/h) and butanone (0.3 mg/h). Of these, only phenol, 4- and 3-methylphenol and carbon dioxide were always detected in ox odour. Studies were made of the numbers of *Glossina pallidipes* Austen and *G. morsitans morsitans* Westwood attracted to natural ox odour and synthetic odour, the latter consisting of blends of identified attractants dispensed at the doses naturally present in ox odour. Natural ox odour caught twice ($P < 0.05$) as many *G. pallidipes* and 1.5 ($P < 0.05$) times as many *G. m. morsitans* as the synthetic blend suggesting the presence of an unidentified attractant in ox odour. Passing ox odour through filters indicated that all attractants can be trapped on a combination of charcoal and sodalime filters but the unidentified attractant(s) may pass through a sodalime filter, and break through a charcoal filter used for more than 6 h. Increasing the dose of ketones in the synthetic odour from 2 to 100 mg/h doubled the catches at the source. Increases in ketone levels in hosts, induced by starvation or possibly trypanosomiasis, may increase attraction of tsetse to such animals.

Introduction

In southern Africa, most tsetse (*Glossina* spp. (Diptera: Glossinidae)) are attracted to an ox in response to the host's odour (Vale, 1974a). Active components identified so far include carbon dioxide (Vale 1974a), acetone (Vale, 1980), 1-octen-3-ol (henceforth termed octenol) and butanone (Vale & Hall, 1985) and a number of phenols (Vale *et al.*, 1988a). Traps and insecticide-impregnated targets baited with blends of these chemicals are now widely used to control tsetse (Vale *et al.*, 1988b; Willemse, 1991).

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Vale & Hall (1985) compared the numbers of tsetse attracted to natural ox odour and a source of odour containing carbon dioxide, acetone and octenol at the levels present in natural ox odour. Twice as many tsetse were attracted to the source of natural odour and they concluded that ox odour contained unidentified attractants. Their studies suggested that at least two unidentified attractants were present in ox odour: a volatile one that passed through a charcoal filter, and a relatively involatile attractant that was trapped. Since then, a volatile attractant, butanone, and a number of less volatile ones, various phenols, have been identified (Hassanali *et al.*, 1986; Vale *et al.*, 1988a). It is not known whether these attractants explain completely the difference between natural and synthetic ox odour.

In the present paper we describe three types of study undertaken to determine whether any further unidentified attractants are present in ox odour. Firstly, studies were made of doses of known attractants in ox odour and, following on from this, comparisons were made of the numbers of tsetse attracted to natural ox odour and a synthetic odour containing all the known attractants at their natural concentrations. Secondly, studies were made of the effect of adding large doses of known attractants to the natural and synthetic ox odours; previously evidence for the existence of unidentified attractants was based, in part, on the observation that octenol synergizes natural ox odour but not synthetic odours (Vale & Hall, 1985). Thirdly, studies were made of the effect of passing ox odour through sodalime and charcoal filters designed to remove known attractants. Evidence for the existence of unidentified attractants, and an indication as to their chemical nature, is then inferred from the responses of tsetse to these filtered odours.

Materials and methods

All field studies were undertaken near Rekomitjie Research Station in the Zambezi Valley of Zimbabwe where *G. pallidipes* Austen and *G. morsitans morsitans* Westwood are present.

Catching methods

To gauge the numbers of tsetse attracted to various odours, an electric net (Vale, 1974b), 1.5 × 1.5 m, was placed 1 m downwind of the odour source. The net was mounted on a corrugated tray coated with polybutene. Tsetse that struck the net were killed or stunned and fell onto the tray where they became stuck. Tsetse orientate imprecisely to an odour source unless it is marked by a visual stimulus (Vale, 1974a). Consequently, a target, consisting of a panel of black cloth, 75 × 75 cm, was sewn on to the centre of the electric net.

Natural ox odour

Natural odour was obtained by placing 1-6 oxen in a roofed pit and exhausting the air from the pit at 2000 l/min via a ventilation shaft (25 cm diam.) fitted with a 12 V co-axial fan (Vale, 1974a). The pit was cleaned daily to minimize the accumulation of phenolic materials present in ox excreta. In some experiments ox odour was passed through charcoal and/or sodalime filters, in which case a specially modified, 240 V centrifugal fan was used. In experiments where both filters were used simultaneously, the sodalime preceded the charcoal filter. The charcoal filter consisted of a cylinder (21 cm o.d., 11 cm i.d. and 20 cm long) containing activated charcoal (6-12 mesh; 2 kg). The filter was changed after 6 h of use unless stated otherwise. The sodalime filter consisted of a drum (85 × 55 cm wide) containing 80 kg of medical-grade sodalime. The sodalime was wetted with 4 l of water immediately before starting an experiment. The performance of the filter was maintained by replacing 16 kg of the filter with fresh sodalime after 3 h of use; the routine for replenishing the sodalime was organized so that none was used for > 15 h.

Synthetic ox odour

Blends of carbon dioxide, acetone and butanone were dispensed at various rates by the methods of Vale & Hall (1985). Octenol and various phenols were dispensed from sealed sachets of low density polyethylene, the wall thickness (0.15-0.3 mm) and surface area (5-50 cm^2) of the sachet being varied to produce different release rates (Laveissière *et al.*, 1990).

Air sampling for attractants

Carbon dioxide.

Air was drawn at 300 ml/min via a tube (3 mm i.d.) inserted through a port (1 cm diam.) in the pit ventilation shaft. The concentration of carbon dioxide was measured using an infra-red gas analyser (EGA, ADC Ltd, Hoddesdon, UK) modified to operate over a range of 0.01-1.0% carbon dioxide. The output from the analyser was recorded continuously by a data logger (1200 Squirrel, Grant Instruments, Cambridge, UK) and the data were subsequently downloaded onto a personal computer for analysis. The logger recorded the mean levels of carbon dioxide at 1 minute intervals.

Phenols and octenol

To measure the release rate of octenol and various phenols, air was drawn at 2-4 l/min for 2 h through filters containing 100 mg of Porapak Q (Waters, Milford, MA 01757, USA; 50-80 mesh) inserted through the sampling ports in the ventilation shaft of a pit containing 4 oxen. The filters were subsequently extracted with dichloromethane and analysed for octenol and various phenols by capillary gas chromatography (GC) and GC-linked to mass spectrometry (GC-MS) (Bursell *et al.*, 1988).

Ketones

Acetone and butanone were measured by drawing air from a face mask attached to the muzzle of an ox or from a ventilated pit containing 6 oxen. The air was passed through collection filters packed with carbonized molecular sieves (ORBO-90 or ORBO-91, Supelco Inc. Bellefonte, PA, USA) at 100 ml/min for 30 min. Immediately after collection the filters were stored in a deep freeze and sent by courier to the National Resources Institute (NRI) for analysis where they were analysed within seven days of collection. Each filter was extracted with two aliquots (2 ml) of propyl acetate containing 1% N,N-dimethyl formamide. Extracts were analysed by capillary gas chromatography on a Poraplot Q fused silica column (Chrompack, 10 m × 0.32 mm i.d.) with helium carrier gas (inlet pressure 1 kg/cm^2), split injection (20 ml/min split) at 200°C, flame ionization detector at 250°C and injection volume 1.0 µl. The oven was held at 160°C isothermal for 7 min, programmed at 40°C/min to 220°C, then held isothermal for 10 min. Acetone and butanone were quantified by comparison of peak areas with those of external standards, and > 90% was desorbed in the first aliquot of solvent. The OSHA desorption and analysis procedures recommended for use with these collection filters (Anon., 1988) were not suitable for measurement of the very low concentrations of acetone and butanone encountered in this work.

Calibration

Prior to measuring the ketones produced by an ox, the efficiency of the entrainment method was estimated by measuring acetone and butanone released at known rates. These results were then used to calibrate the estimates of ketones produced by the oxen. In the laboratory, bottles (44×20 mm) containing acetone or butanone and fitted with caps with 1-6 mm diameter holes were placed in a low-speed (0.08 m/s) wind tunnel. The rate of release of ketones from these bottles was measured by either entrainment using the ORBO filters and subsequent gas chromatography or by weighing the bottles at various intervals.

Similar studies were carried out in the field. Bottles releasing acetone and butanone at 314-374 mg/h and 239-279 mg/h, respectively, were placed in a ventilated pit and the rate of release of the ketones estimated by either weighing the bottles at intervals or by entrainment. In studies of acetone or butanone produced by oxen in a ventilated pit, butanone (166-289 mg/h) or acetone (368 mg/h), respectively, were dispensed within the pit as an internal standard.

Experimental design and analysis

All field experiments were carried out during the 3 h preceding sunset when tsetse are most active (Hargrove & Brady, 1992). In comparisons of different blends of odours, the various treatments were incorporated into a series of replicated Latin squares consisting of days× sites×treatments. Only one site was used in studies of filtered odour and so treatments were compared using a randomized block design; groups of adjacent days were regarded as different blocks and treatments were randomly allocated to days within these blocks. The catches (n) were normalized using a $\log_{10}(n+1)$ transformation and subjected to analysis of variance. Differences between more than two means were assessed by a least significant difference test. The detransformed means are reported accompanied by their 95% confidence intervals or the transformed standard errors so that more detailed comparisons can be made by transforming the counts back to the log scale.

Experiments and results

Chemical analysis of natural odour

Phenols and octenol

In samples of odour from a ventilated pit containing 4-6 oxen (table 1, Experiment 1), phenol, 4- and 3-methylphenol and carbon dioxide were always present but octenol and the other phenols were sometimes not detected: octenol was detected unambiguously in just 18% ($n=11$) of the samples.

Ketones

Calibration. In the laboratory studies, the range of release rates measured by weighing the ketone dispensers was 29-270 mg/h for acetone and 11-164 mg/h for butanone. The release rates, estimated by entrainment using ORBO-90 filters, gave estimates 1.6 (range 1.1-2.0) times greater for acetone and 3.0 (2.4-3.9) times greater for butanone than the rate derived from weighing the bottles. For the ORBO-91 filters, the rates by entrainment were 1.0 (0.7-1.9) and 1.8 (1.4-2.4) times greater, respectively.

In the field studies, the ORBO-90 filters gave estimates 1.5 (range, 1.1-2.1) times greater for acetone and 2.4 (1.4-4.0) times greater for butanone than the release rate indicated by weighing the bottles. For the ORBO-91 filters the rates were 0.6 (0.4-0.8) and 0.8 (0.4-1.1) that of the weighed release rates, respectively. Consequently the concentrations of acetone and butanone in natural odour measured using ORBO-90 filters were corrected by dividing the estimates for acetone by 1.5 and 2.4 for butanone and the estimates from the ORBO-91 filters were corrected by dividing the estimates by 0.6 for acetone and 0.8 for butanone.

Using these correction factors, the mean rates of butanone or acetone released as internal standards were estimated by entrainment and found to be within 14% of the rate estimated by weighing, indicating that this method

Table 1. Release rates of attractants from various odour sources and an estimate of the mean rate of production of each attractant from a single ox. For experiments 1 and 3 the rate for a single ox is calculated by dividing the measured release rate by the number of oxen. For experiment 2, the release rate is calculated assuming that the oxen expired 40 l/min of air.

Odour	Oxen	n	Release rate (mg/h or l/h)	Range (mg/h or l/h)	1 ox rate (mg/h or l/h)
Experiment 1: ventilated pit					
Phenol	4	13	0.429	0.145-0.752	0.108
3-methylphenol	4	13	0.354	0.164-0.699	0.089
4-methylphenol	4	13	2.914	1.657-4.541	0.729
3-ethylphenol	4	13	0.054	0-0.229	0.014
4-ethylphenol	4	13	0.097	0-0.501	0.024
3- & 4-n-propylphenol	4	13	0.081	0-0.348	0.020
Octenol	4	13	0.056	0-0.477	0.014
Carbon dioxide (l/h)	6	20	846	480-1200	141.0
Experiment 2: face mask					
Acetone	1	22	0.9	0-10.3	0.9
Butanone	1	22	0.19	0-4.3	0.19
Experiment 3: ventilated pit					
Acetone	6	15	43.4	0-110	7.2
Butanone	8	8	1.0	0-8.4	0.18

should produce reliable estimates of the amounts of ketones produced by oxen. For all measurements, at least one sample was taken on each of the two types of collection filters.

The limits for detection and reasonably reliable quantification of acetone and butanone in the GC analysis were approximately 0.25 ng and 0.5 ng injected, respectively, i.e. 0.5 µg and 1.0 µg per sample, respectively. With the ORBO 90 filters, this corresponds to minimum detection levels for acetone and butanone of 0.3 mg/h and 0.35 mg/h in direct sampling of ox breath with a face mask, and 2.0 mg/h and 2.5 mg/h per ox in sampling from the ventilated pit containing six oxen.

Ox breath. Acetone and butanone were detected in only 45% ($n=22$) and 18% of samples, respectively. The mean rates of production of acetone and butanone (table 1, Experiment 2) were 1 mg/h and 0.19 mg/h, respectively. The rate for acetone is similar to that reported by Vale & Hall (1985).

Whole odour. The rate of production of ketones was estimated from six oxen placed in a ventilated pit. In 27% ($n=15$) of cases no acetone was detected and in 88% ($n=8$) of samples, no butanone was detected. The results (table 1, Experiment 3) show a higher mean rate of production of acetone (7 mg/h) compared to the estimates from ox breath while the rates of butanone (0.2 mg/h) from the two sources are virtually identical.

Comparison of synthetic and natural odour

Comparisons were made between the numbers of tsetse attracted to sources of either ox odour or a blend of synthetic attractants dispensed at the concentrations present in natural odour. The synthetic ox blend (henceforth termed SO) consisted of carbon dioxide (2 l/min), octenol (0.2 mg/h), acetone (2 mg/h), 4-methylphenol (0.4 mg/h) and 3-n-propylphenol (0.04 mg/h). Each dose, measured by direct weighing of the dispensers or by entrainment, was based on the present (table 1) and previously reported (Hall *et al.*, 1984; Vale & Hall, 1985) estimates of the rates of production of attractants by one ox. The two odours (natural or SO) were compared in six separate experiments at different seasons. The results (fig. 1) show that the natural odour attracted consistently more tsetse than did SO. Pooling the data of all six experiments, the difference was significant for both sexes of both species but there was no significant interaction between odour and experiments. For *G. pallidipes*, natural odour caught 1.9 times as many females ($P < 0.001$ for difference between means) and 2.0 times as many males ($P < 0.001$). For *G. m. morsitans*, the natural odour caught 1.5 times as many females ($P < 0.05$) and 1.5 times as many males ($P < 0.01$).

Clearly SO is not an exact mimic of natural odour. This is either because natural odour contains unidentified attractants, or because the doses used in SO differ from natural. SO is based on field measurements of natural ox odour, although the results (table 1) indicated that the dose of attractants produced by an ox is variable. The attractants in ox odour were not measured routinely except for carbon dioxide, which ranged within ± 10% of the assumed rate. It is therefore possible that the oxen used in the field experiments were producing unusually high amounts of attractants. Moreover, although 4-methylphenol and 3-n-propylphenol can account for all the activity of the phenolic

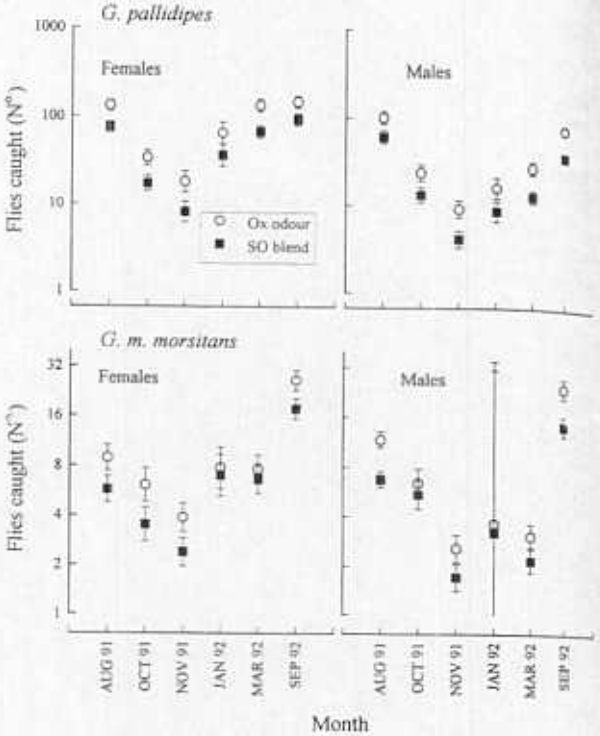

Fig. 1. Mean daily catch of tsetse (± S.E.) from a target baited with either natural ox odour or SO. Means are based on six to twelve replicates of each treatment.

attractants in ox odour, another four phenols (3- and 4-ethylphenol, 3-methylphenol, 4-n-propylphenol and phenol) are present naturally in ox odour and could act as attractants (Vale *et al.*, 1988a). Similarly SO did not contain butanone which, although present at very low levels, could also be a significant attractant. To determine whether these factors explain the difference between SO and ox odour, studies were made of the effect of adding large doses of known attractants to ox odour or SO.

Addition of known attractants to ox odour and SO

Acetone (500 mg/h) or butanone (500 mg/h) or octenol (2 mg/h) were added to the SO at ca. 100 times the levels produced by an ox (table 1; Hall *et al.*, 1984; Vale & Hall, 1985) and the various phenols were added at ca. 10 times the mean natural dose. For each attractant, studies were made of the effect of adding the attractant to SO or natural odour from one ox. The large numbers of treatments precluded the incorporation of all the treatments in a single experiment. Therefore the catches of various treatments were expressed as a proportion of a standard. For studies of natural odour the standard consisted of a target baited with natural odour and for studies of SO the standard consisted of a target baited with SO.

Adding octenol or butanone to ox odour or SO significantly increased the catch of tsetse 2-3 times, whereas adding phenols to either odour had no significant effect except for male *G. m. morsitans* (fig. 2). Ox odour caught twice as many *G. pallidipes* as SO in the presence of octenol

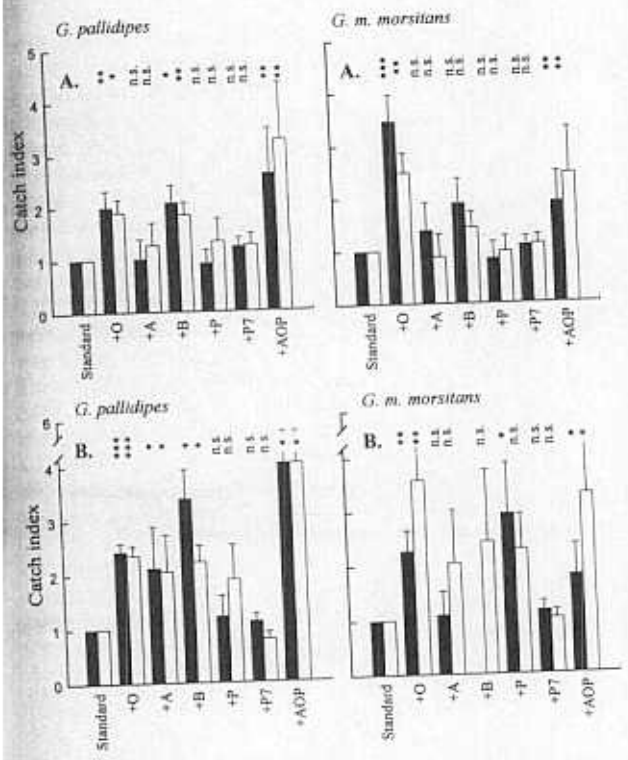

Fig. 2. Catch (+S.E.) of male tsetse (solid bars) or female tsetse (open bars) attracted to either ox odour or SO ± various. For attractants added to ox odour (A.) or SO (B.), the catches are expressed as a proportion of a standard consisting of the catch from a target baited with a single ox or SO, respectively. Indices are based on 8–12 replicates of each treatment. Asterisks indicate that the catch index is significantly different from unity at the $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***) level of probability or not significantly different (n.s.). Attractants consisted of: +A=acetone (500 mg/h), +O=octenol (2 mg/h), or +P=4-methylphenol (4 mg/h) and 3-n-propylphenol or +P7=4-methylphenol (4 mg/h), 3-methylphenol (0.9 mg/h), 4-ethylphenol (0.8 mg/h), 3-ethylphenol (0.7 mg/h), 4-n-propylphenol (0.6 mg/h), 3-n-propylphenol (0.5 mg/h) and phenol (1.0 mg/h).

or phenols (table 2). Acetone added to SO increased the catch significantly but had no significant effect when added to ox odour (fig. 2), and the difference between SO and ox odour was apparently reduced in the presence of high doses of acetone or butanone (table 2). Hence the difference between SO and ox odour does not appear to be materially altered by the presence or absence of super-normal doses of octenol or phenols though the difference is reduced by high doses of ketones (table 2).

Direct comparisons between ox odour and SO in the presence of a blend of acetone, octenol and phenols were carried out in six different experiments, each of 6–12 replicates, at different times of year and at various sites. The results (fig. 3) show that for male and female *G. pallidipes*, natural odour caught more tsetse than the SO blend in 11 out of 12 comparisons. For *G. m. morsitans*, the difference was less consistent. Pooling the data from all six experiments for analysis (table 2) shows that the means are significantly different ($P < 0.05$) for male and female *G. pallidipes* and female *G. m. morsitans* but not for male *G. m. morsitans*. There was no significant interaction between experiments and treatments. For both species the overall difference between the SO and natural odour blends was less than that observed in the absence of acetone, octenol and phenols (fig. 1). These data confirm that ox odour attracts significantly more tsetse than SO but suggest that the difference could be due, in part, to differences in the dose of ketones.

Dose responses to ketones

The SO blend produced 2 mg/h of acetone and no butanone. This is very similar to the dose based on samples analysed from ox breath but some 5 mg/h less than the estimate derived from whole ox odour. To investigate this further, studies were made of the numbers of tsetse attracted to SO plus various doses of acetone and/or butanone. For each treatment the mean catch was expressed as a proportion of the catch from a target baited with the odour from one ox. This catch index was related to the doses of acetone and/or butanone using a weighted regression with

Table 2. The detransformed mean catch of tsetse from targets baited with ox odour or SO plus various added odours. The number of replicates (n), the transformed standard errors (S.E.) and the probability that the paired means are different at the $P < 0.05$ (*), $P < 0.01$ (**) levels of probability, or not significantly different (n.s.).

Species	Odour added	Females					Males			
		Ox	SO	n	P	S.E.	Ox	SO	P	S.E.
G. pallidipes	O	246.1	115.2	10	**	0.054	156.7	73.2	**	0.065
	A	32.5	20.0	18	n.s.	0.080	14.4	12.7	n.s.	0.074
	B	25.4	15.7	10	n.s.	0.089	10.9	9.7	n.s.	0.085
	P	22.8	13.5	10	n.s.	0.119	7.3	3.8	n.s.	0.097
	P7	184.2	72.6	8	**	0.068	96.3	42.5	**	0.054
	AOP	277.8	193.3	53	*	0.030	109.4	84.6	*	0.032
G. m. morsitans	O	32.9	14.9	10	**	0.059	30.5	17.9	*	0.057
	A	3.7	3.9	18	n.s.	0.073	3.6	2.8	n.s.	0.054
	B	3.9	2.7	10	n.s.	0.076	1.4	2.3	n.s.	0.073
	P	2.8	3.2	10	n.s.	0.086	1.3	2.0	n.s.	0.084
	P7	28.1	17.0	8	*	0.061	25.5	15.6	**	0.057
	AOP	14.6	11.1	53	*	0.030	8.0	6.5	n.s.	0.034

Added odours consist of: A=acetone (500 mg/h), O=octenol (2 mg/g), or P=4-methylphenol (4 mg/h) or 3-n-propyl-phenol or P7=4-methylphenol (4 mg/h), 3-methylphenol (0.9 mg/h), 4-ethylphenol (0.8 mg/h), 3-ethylphenol (0.7 mg/h), 4-n-propylphenol (0.6 mg/h), 3-n-propylphenol (0.5 mg/h) and phenol (1.0 mg/h). See text and Table 1 for composition of SO and ox colour, respectively.

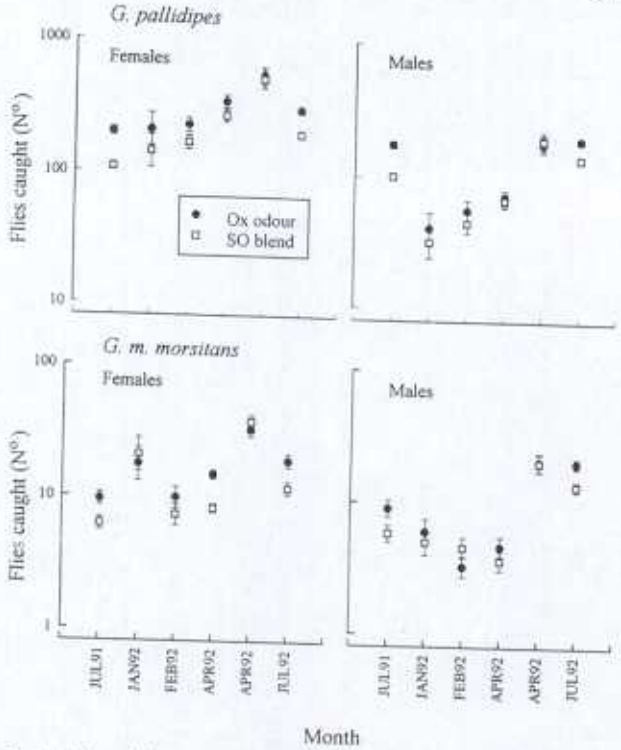

Fig. 3. Mean daily catch of tsetse (±S.E.) from a target baited with acetone (500 mg/h), octenol (2 mg/h), 4-methylphenol (4 mg/h), 3-n-propylphenol (0.5 mg/h) plus either natural ox odour or SO. Means are based on six to eight replicates of each treatment.

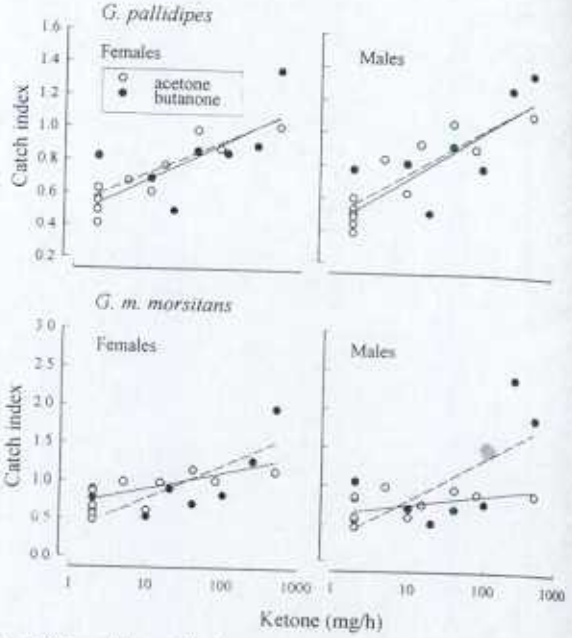

Fig. 4. Mean daily catch of tsetse attracted to a source of SO with variable doses of acetone (solid circles) or butanone (open circles) and regression lines for acetone (broken line) or butanone (solid line). Catch index is the catch expressed as a proportion of the catch from a target baited with the odour of a single ox based on six to eight replicates of each treatment.

the weights equal to the reciprocal of the variances of the index.

The results (fig. 4) show that increasing the dose of acetone or butanone increased the catch of tsetse. The doses of acetone and butanone were subjected to various transformations, the best fit being obtained with a $\log_{10}$ transformation. There was no significant difference between the dose responses to acetone and butanone ($P > 0.1$). The regression coefficient for the pooled data was 0.278 (0.0367, S.E.) indicating that ten-fold increments in the dose of ketones increased the catch of tsetse 1.9 times. There was no significant interaction between the dose responses of various species and sexes. The data (fig. 4) show that blends of SO producing approximately 100 mg/h of butanone and/or acetone attract as many tsetse as natural odour. This is some four times greater than the highest reported natural dose of acetone (Vale & Hall, 1985) and some 10-50 times greater than the mean rate of production reported in the present work.

To determine whether there is any interaction between acetone and butanone, the numbers of tsetse attracted to SO producing 5 mg/h acetone and 10 mg/h butanone were compared with the number attracted to the odour from a single ox. This blend produced a mean daily catch of 50 *G. pallidipes* and 7 *G. m. morsitans* compared to 69 and 13 respectively, for natural odour. The difference between the natural and synthetic blend is similar to that for blends containing 15 mg/h acetone or 15 mg/h of butanone (fig. 4) indicating that there is no great interaction between these two ketones. It seems that the difference in the numbers of tsetse attracted to natural odour and SO cannot be attributed to any slight difference (5 mg/h) in the dose of acetone or butanone in the two odours.

Filtering ox odour

The various comparisons of SO and natural odour implied that there is an unidentified attractant present in ox odour. To obtain indications of the chemical nature of this attractant, studies were made of the effect of passing natural odour through filters of charcoal and sodalime.

Efficacy of filters

Two types of charcoal filter were used: a 'new' filter in which the charcoal was used for less than 6 h and then replenished with fresh material, or an 'aged' filter in which the charcoal had been used for 6-40 h. The new filter trapped all known attractants apart from carbon dioxide and the aged one trapped the less volatile attractants such as the phenols and octenol, while allowing more volatile materials such as acetone to pass (Vale & Hall, 1985).

To assess the efficacy of the new filter, air from a pit containing either 4-6 oxen or dispensers of butanone (290 mg/h) and acetone (275 mg/h) was passed through charcoal or sodalime filters at 2000 l/min. Samples of air from before and after the filters were analysed for all known attractants. No octenol or phenols were detected in air after it had passed through the charcoal filter, ketones were reduced by >90% and there was no apparent effect on the concentration of carbon dioxide. The sodalime filter had no discernible effect on the concentration of octenol or either

of the ketones but reduced the concentration of phenols by ca. 90% and carbon dioxide from ca. 0.8% to background levels or below (0.04-0.00%)

In some experiments, synthetic odours were added to filtered ox odour to replace odours removed by the filter. In these cases, the dispensers were placed on the ground adjacent to the pit vent rather than the usual position within the pit. An experiment was carried out to determine whether altering the dispensing position affected the numbers of tsetse attracted to an odour. A blend of carbon dioxide (120 l/h), acetone (50 mg/h), octenol (0.4 mg/h), 4-methylphenol (0.8 mg/h) and 3-n-propylphenol (0.1 mg/h) was dispensed entirely within the pit or at the pit outlet. There was no significant effect of dispensing position on the catch of tsetse. For instance, the mean catch (12 replicates) of *G. pallidipes* and *G. m. morsitans* was 232 (194-277, 95% C.I.) and 7 (4-11), respectively, when the odour was dispensed in the pit compared to 225 (189-269) and 6 (4-10) when dispensed outside the pit.

The sodalime filter generally reduced the concentration of carbon dioxide to less than ambient (0.04%) and also had a perceptible aroma. An experiment was therefore undertaken to determine whether these aspects of the sodalime filter had an effect on the olfactory responses of tsetse. Acetone (500 mg/h), octenol (2 mg/h), 4-methylphenol (4 mg/h) and 3-n-propylphenol (0.5 mg/h) were dispensed at the pit vent and the air from an empty pit was vented either directly, or via a sodalime filter. There was no significant effect of passing pit air via the sodalime filter. For *G. pallidipes* the mean catch (six replicates) was 14 (7-27, 95% C.I.) with the filter compared to 15 (7-28) without it.

Effect of filtering ox odour

Studies were made of the effect of passing the odour of a single ox through charcoal and sodalime filters. All treatments were compared with the catch from unfiltered odour from a single. ox. The results (fig. 5) for *G. pallidipes* show that passing ox odour through an aged, or, a new charcoal filter reduced the catch by ca. 40% and 70%, respectively. Passing ox odour through filters of new charcoal and sodalime reduced the catch by 80%, to a level similar to that with no odour, suggesting that all the attractants present in ox are removed by a combination of new charcoal and sodalime filters. Adding physiological doses of octenol, 4-methylphenol and 3-n-propylphenol to ox odour passed through a new filter increased the catch to ca. 60% of ox odour, and adding the same octenol-phenol blend to odour passed through an aged filter increased the catch to ca. 130% ox odour. The catch indices for odours passed through the new charcoal filter were consistently less than those for the aged filter, suggesting that there are relatively volatile attractants present in ox odour that pass through a charcoal filter used for more than 6 h. The results for *G. m. morsitans* show a similar pattern except that there is not such a clear distinction between the new and aged filters for females.

In a second experiment, studies were made of the effect of filtering ox odour but with a blend of acetone (500 mg/h), octenol (2 mg/h), 4-methylphenol (4 mg/h) and 3-n-propyl-phenol (0.5 mg/h) added to the filtered odour after the filter. The removal of these attractants by the filter would thus be obviated by their replacement, and any differences in the catch would be due to the removal of carbon dioxide and/or

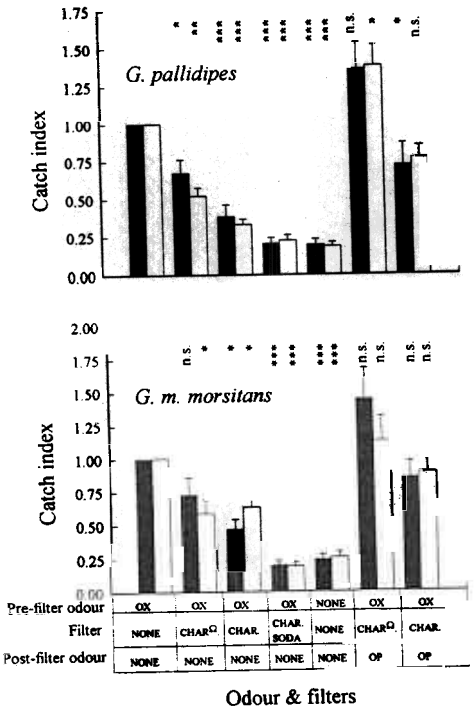

Fig. 5. Mean daily catch (+S.E.) of male tsetse (solid bars) or female tsetse (open bars) attracted to a source of odour from a single ox passed through filters of new charcoal (CHAR), old charcoal ($CHAR^{\Omega}$) or sodalime (SODA) with or without a blend of octenol and phenols (OP) added after the filters. The catches are expressed as a proportion of the catch from unfiltered ox odour based on 6-12 replicates of each treatment. Asterisks as for figure 2.

any unidentified attractants. Comparisons were made of the catch of a target baited with the artificial odour plus: 1. ox odour; 2. ox odour passed through a charcoal filter; 3. ox odour passed through a sodalime filter; 4. ox odour passed through charcoal and sodalime filters; 5. ox odour passed through charcoal and sodalime filters with carbon dioxide (8 l/min) added after the filters.

The catches from the various treatments were expressed as a proportion of the catch from that with unfiltered ox odour (1). The data were analysed using one-tailed tests of significance since the effects of the filters were expected, if anything, to reduce the catch of tsetse, and similarly the catch with filtered ox odour was likely to be greater than that with no additional odour.

The results (fig. 6) for *G. pallidipes* show that passing ox odour through the charcoal filter reduced the catch by 10-20%, but not significantly ($P > 0.05$). Passing ox odour through the sodalime filter, reduced the catch by 40-50% ($P < 0.025$) but to a level significantly greater than that obtained with no ox odour ($P < 0.05$ for both sexes). Passing the odour through both filters reduced the catch to a level similar to that with no ox odour ($P > 0.05$). The reduction in the catch produced by the sodalime filter is due to the removal of carbon dioxide from ox odour. The significant difference between the catch with the sodalime filter and that with no ox odour suggests that there is an unidentified attractant present in ox odour that passes through the sodalime filter, since adding carbon dioxide to

ox odour that had passed through charcoal and sodalime filters produced a catch similar to that produced with a charcoal filter only. The dose of carbon dioxide used was comparable to that produced by the four oxen, which also implies that the sodalime was not removing any unidentified attractants. The results for *G. m. morsitans* are broadly similar except that adding carbon dioxide to ox odour that had been passed through charcoal and sodalime filters produced a catch greater than that produced by unfiltered ox odour.

Discussion

Practical implications

The present estimates of the amount of octenol (ca. 0.01 mg/h), carbon dioxide (2 l/min) and acetone (1-8 mg/h) in ox odour are similar to previous results (Hall *et al.*, 1984; Vale & Hall, 1985). The present study also shows that butanone is present in natural ox odour at only very low levels (< 1 mg/h) and all the identified phenolic attractants, previously identified from ox urine (Bursell *et al.*, 1988), are naturally present in ox odour (table 1).

Natural ox odour attracted twice as many *G. pallidipes* as a blend of carbon dioxide, acetone, 4-methylphenol and 3-n-propylphenol dispensed at their natural doses. There was a smaller but still significant difference between the odours even when supernormal doses of acetone, octenol and phenols were added to both blends. It is therefore highly unlikely that the difference between the synthetic odour and natural odour is due to differences in the doses or ratios of known attractants. The difference is more probably due to the presence of unidentified attractant(s) in ox odour.

Some indications of the chemical nature of the unidentified attractant(s) can be deduced from the filter experiments. All the attractants present in ox odour can be trapped on a combination of sodalime and new charcoal filters. Ox odour passed through a new charcoal filter (used for less that 6 h) attracted about half those attracted by ox odour passed through an aged filter (used for 6-40 h) suggesting that the new filter trapped a relatively volatile attractant present in ox odour. Acetone and butanone were trapped by the new charcoal filter but Vale & Hall (1985) showed that acetone would break through a charcoal filter used for more than 6 h. It seems unlikely, however, that the difference in the catch between the new and aged charcoal filters can be explained entirely by acetone and butanone breaking through. These are only present at 5 mg/h and adding this low dose of acetone has an almost imperceptible effect on the catch (fig. 4; Vale & Hall, 1985; Torr, 1990). It seems more likely that the increase is due to a relatively volatile, unidentified attractant breaking through a charcoal filter used for more than 6 h.

Filter experiments in which a background odour of acetone, octenol and phenols was present showed that passing ox odour through a sodalime filter reduced the catch, as would be expected by the removal of all the carbon dioxide. Intriguingly, however, this filtered ox odour still attracted significantly more tsetse than did the background odour alone. The quantities of acetone, octenol and phenols that passed through the sodalime filter are small relative to the background odour and would not account for the difference between the background and filtered-plus-background odours. A more likely explanation is that the unknown attractant passed through the sodalime filter.

There appear to be a number of similarities between the unidentified attractant and acetone. They are relatively volatile, pass through sodalime filters and the difference between SO and natural ox odour was less apparent when acetone was present at 100 times its natural level. Den Otter *et al.* (1988) showed that 3-nonanone and 4-heptanone elicit electroantennographic responses although field studies show that neither has a behavioural effect (Torr, unpublished data). Vale (1980) also investigated a variety of ketones of which only acetone acted as an attractant.

Comparisons of natural odour and SO indicate that the unidentified component(s) can at least double the efficacy of the attractants. It thus seems certain that a similar increase in the performance of traps and targets to control tsetse can also be expected by the identification and use of the unknown attractant. However, several features of the other known attractants suggest that the effect of the unknown attractant(s) could be greater than this.

First, several of the identified attractants are present at very low levels in natural odour. Hence baiting a target or trap with a physiological dose of acetone (5 mg/h), or butanone (0.2 mg/h), or octenol (0.02 mg/h) does not generally have any clear or significant effect on the catch of tsetse (Vale & Hall, 1985; Torr, 1990). If these attractants were selectively filtered from ox odour there would be little change in the numbers of tsetse attracted to the odour, suggesting erroneously that these are not important attractants. It is only when these are dispensed at supernormal doses that their effect is apparent (Vale & Hall, 1985). The clear effect of removing the unidentified attractant in the present study suggests that it is an important component of ox odour and it seems likely that once the attractant has been identified catch increases greater than a doubling will be possible.

Second, in the comparisons of ox odour and SO, repellents such as 2-methoxyphenol (Vale *et al.*, 1988a) and various carboxylic acids (Vale, 1977, 1980) known to be present naturally in ox odour were not present in SO. The presence of natural repellents in the natural blend but not in SO will lead to an underestimate of the likely efficacy of the unidentified attractant. Adding physiological doses of octenol and phenol to replace these attractants removed by the aged filter produced catches of tsetse greater than that from ox odour (figs 5 & 6). This may be due to the filter removing repellents present in the natural odour. Another possible explanation is that the blend of octenol and phenols added to the filtered odour is greater than the amounts naturally present. Natural odour typically produced 0.01 mg/h of octenol but frequently none was detected. Therefore the 0.05 mg/h produced in SO may be a slight but significant overestimate of the natural dose.

A problem with simply measuring the numbers of tsetse attracted to different odours is that the variance is so large that one odour generally has to attract twice as many tsetse as a second odour for one to be able to demonstrate a clear and significant difference. For instance, in comparisons of SO and natural odour with a background odour, the natural odour caught 1.5 times as many *G. pallidipes* as SO. Although the difference was consistent between experiments it was only significantly different in two of the six comparisons. As a result, experiments have to be repeated many times to obtain a clear indication of the effect of an

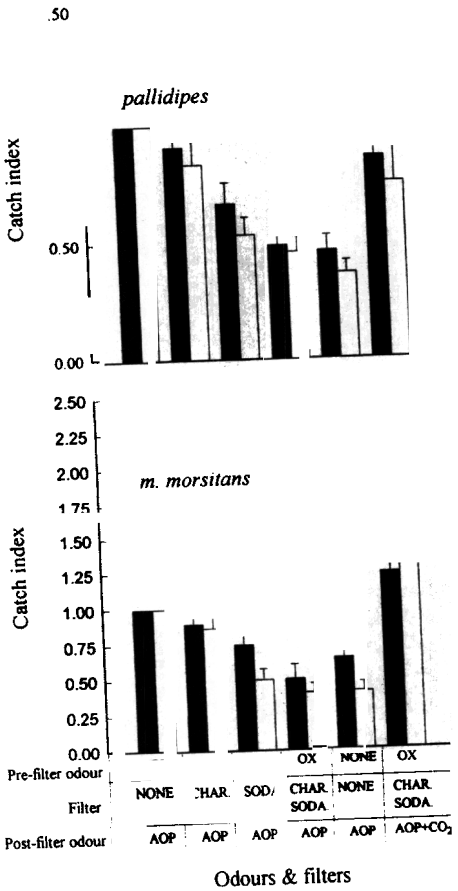

Fig. 6. Mean daily catch (+S.E.) of male tsetse (solid bars) or female tsetse (open bars) attracted to a source of acetone (500 mg/h), octenol (4 mg/h), 4-methylphenol (4 mg/h) and 3-n-propylphenol (0.5 mg/h) and the odour from four oxen passed through charcoal (CHAR) or sodalime (SODA) filters with or without carbon dioxide added after the filters. The catches are expressed as a proportion of the catch from unfiltered ox odour based on eight replicates of each treatment. Asterisks as for figure 2.

odour. Physiological doses of natural attractants frequently do not elicit large increases in catch (Vale & Hall, 1985; Torr, 1990) and the differences between the natural and synthetic odours demonstrated here are at the limits of the techniques for demonstrating differences between odours. A more satisfactory approach may be to develop a bioassay based on specific behavioural responses to given odours. One possibility could be based on apparent differences in the activation of tsetse in the presence of natural and synthetic odours (Bursell, 1987; Torr, 1988a, 1988b).

Biological implications

Several of the attractants present in ox odour act synergistically. For instance, 4-methylphenol and 3-n-propylphenol (Vale *et al.*, 1988a), and acetone and carbon dioxide (Torr, 1990) act as synergistic pairs. Vale & Hall (1985) found that adding octenol to ox odour increased the catch of *G. m. morsitans* and *G. pallidipes* 2.5 and 1.5 times, respectively, but when added to carbon dioxide the increases were only 1.14 and 1.11 times. They suggested that octenol is synergized by an unidentified chemical in ox odour. In the present study there is no clear evidence for octenol having a greater effect with natural odour than with the synthetic, since adding octenol to either ox odour or SO increased the catch of *G. pallidipes* and *G. m. morsitans* 3 times (fig. 2).

It is remarkable that tsetse employ such a large number of chemicals to locate their hosts, there being at least 12 different attractants naturally present in host odour. This large repertoire may be necessary due to the inherent variability of the odours produced by mammalian hosts. The chemical assays showed that only carbon dioxide, phenol and 4- and 3-methylphenol were always found to be present in natural ox odour. Yet despite this variability there was remarkably little variation in the attractancy of ox odour as indicated by the fact that in the six separate comparisons of natural odour with SO, natural odour was 1.7 - 2.6 times more attractive than SO and there was no significant interaction between the treatments and the experiments. This variation is small given that meteorological conditions, sites and oxen varied between these experiments. Tsetse may be able to overcome the inherent variability of ox odour by being receptive to different chemicals that can substitute for each other. For instance several of the phenols substitute for each other to a degree and acetone and butanone are complete substitutes for each other.

It is also intriguing that the only attractants always found in ox odour are also naturally present in the environment. There is a constant background of carbon dioxide, and phenols are likely to be released from urine sprayed onto vegetation where they retain their activity (Vale *et al.*, 1988a). Indeed, the phenols are only produced from microbial action on aged urine (Okech & Hassanali, 1990) and the phenols detected here are presumably released from soiled areas of the host's skin. Hence the most consistent attractants identified to date do not appear to be particularly good indicators of the proximity of a host.

It is noteworthy that small increases in the dose of acetone and butanone produced detectable increases in the numbers of tsetse attracted to a source of SO. Vale (1981) noted a change in the attractiveness of cattle subjected to brief periods of starvation. This may have been due to increases in the amount of acetone produced by an ox; Vale & Hall (1985) reported a relatively high rate of 24 mg/h of acetone in the breath of an ox that had been starved for a week. Bayliss & Nambiro (1993) did not find any evidence that cattle infected with *Trypanosoma congolense* (Protozoa) attracted more tsetse than uninfected animals. However, the cattle that they studied were not in the very late stages of trypanosomiasis where animals lose weight rapidly and become so debilitated that they cease feeding. Such animals may be particularly attractive to tsetse which would have important implications for the epidemiology of trypanosomiasis.

Dedication

We dedicate this paper to the late Professor Ellinar Bursell whose work contributed to its origins and whose inspiration and enthusiasm provided an example to us all.

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