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Methods for dispensing odour attractants for tsetse flies (Diptera: Glossinidae)

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Abstract

Methods for dispensing tsetse attractants using sealed polyethylene sachets and bottles were studied in the laboratory and field. 1-Octen-3-ol (octenol), 4-methylphenol and 3-*n*-propylphenol were dispensed singly or as blends from sachets 25–200 cm^2 in surface area and with a wall thickness of 0.06–0.32 mm; butanone was dispensed from polyethylene bottles. The release rates of attractants, assessed gravimetrically or by GC analysis of volatiles released, were independent of the amount present. The rates were related directly to surface area, inversely related to wall thickness and increased exponentially with temperature. With blends of the attractants, the release rates of the two phenols were directly proportional to the concentration present, but that of octenol showed an exponential dependence. A similar exponential effect was seen with blends of the attractants and an involatile diluent. For mixtures of chemicals, the ratio of the released components was not affected significantly by temperature, sachet size or wall thickness. Release rates from polyethylene sachets and bottles in the field varied 100-fold according to temperature differences related to the time of day, season, and degree of insolation. Day-degree models to predict the losses of attractants from a polyethylene sachet in shade or in full sunlight were highly correlated ($r^2 = 0.84$ and 0.81 respectively) with observed losses. The practical implications of these findings are discussed.

Introduction

An increasingly important method of controlling human and animal trypanosomiasis employs traps or insecticide-impregnated targets baited with synthetic attractants to lure and kill tsetse flies (Green, 1994). Developments in bait technology over the past decade have resulted in a 10–1000-fold increase in the cost effectiveness of traps and targets (Vale, 1993a), mainly by improving the visual attractiveness and the efficiency (Vale & Hargrove, 1979) of traps and targets and by identifying potent olfactory attractants. The most important attractants for practical purposes are acetone, butanone, 1-octen-3-ol (Vale & Hall, 1985a,b) and various phenols (Owaga *et al.*, 1988; Bursell *et al.*, 1988). Combinations of these have been used to control tsetse in Zimbabwe (Vale *et al.*, 1986), Zambia (Willemse, 1991) and Kenya (Dransfield *et al.*, 1990).

There have also been significant reductions in the cost of the technology by, for example, constructing traps and targets from cheap materials (Vale, 1993b). Improvements can also be achieved by developing a cheap and robust system to dispense attractants over long periods, thereby reducing the need for expensive maintenance visits to replenish odour baits (Barrett, 1994). Such a system should be capable of dispensing attractants at any required dose and blend appropriate to the pest species and operational needs. In the present paper we describe the performance of a dispensing system consisting of sealed polyethlyene sachets and bottles.

The responses of *Glossina pallidipes* Austen and *G. morsitans morsitans* Westwood (Diptera: Glossinidae) to

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octenol, phenols, acetone and butanone are well established (Vale & Hall, 1985a,b; Vale *et al.*, 1988; Torr, 1990; Torr *et al.*, 1995) as is the successful use of sachets to dispense these odours (e.g. Hargrove & Langley, 1990; Vale, 1991 *et seq.*). Accordingly, the present paper reports mainly on the physical performance of dispensers in the laboratory and field.

Materials and methods

Laboratory studies were carried out at the Natural Resources Institute (NRI) in Chatham, UK, and the Department of Veterinary Services, Zimbabwe. Field studies were performed at Rekomitjie Research Station (16°18'S; 29°23'E, altitude 510 m) in the Zambesi Valley of Zimbabwe where *G. pallidipes* and *G. m. morsitans* occur.

Attractants

Studies were made of the attractants 1-octen-3-ol (henceforth termed octenol) (International Flavours and Fragrances, Duddery Hill, Suffolk, UK), 4-methylphenol (Aldrich Chemical Company Ltd., Gillingham, Dorset, UK), 3-*n*-propylphenol (Palmer Research, Clwyd, UK), acetone and butanone. These chemicals are used as baits for *G. pallidipes* and *G. m. morsitans* in Zimbabwe and for other tsetse species (Green, 1994).

Dispensing systems

The dispensing systems consisted of containers of low-density polyethylene of differing shape, surface area and thickness. The containers were partially filled with single components or blends of attractants and then sealed so that the chemicals diffused out of the container through the polyethylene.

Laboratory studies

Laboratory measurements were made in a room held at the required temperature (±1°C) with six air changes per hour. Release rates for dispensers containing single components were measured by maintaining duplicate dispensers in a wind tunnel (8 kph windspeed) and weighing them at intervals. For dispensers containing mixtures, release rates of the individual components were measured by quantitative gas chromatographic (GC) analysis (Bursell *et al.*, 1988) of volatiles emitted by the dispenser and trapped on Porapak Q (Waters, Milford, Massachusetts, USA; 50–80 mesh). In early studies, the dispenser was held in a silanized, 2-litre, round-bottomed flask, and air was drawn in through an activated charcoal filter (15×2 cm; 6–8 mesh) and out through a filter containing Porapak Q (5×1 cm; 2.5 g). The filter was extracted with dichloromethane (25 ml) and an appropriate internal standard (e.g. decyl acetate) added before GC analysis. In later experiments, the dispenser was maintained in the windtunnel and the exhaust air sampled (2 l/min for 2 h) with a Porapak Q filter (100 mg). The trapped volatiles were removed with dichloromethane (3×0.5 ml) and analysed by GC against an appropriate internal standard.

Field studies

Initial laboratory studies identified those dispensers that released attractants at appropriate doses and duration and which might thus be suitable for field use. Following this, studies were made of the performance of dispensers under natural field conditions.

Physical performance

Release rates from dispensers in the field were estimated by weighing at least two replicates at 2 h intervals. Simultaneous measurements were made of shade temperature and solar radiation using an automatic weather station (type WS01, Delta-T Devices, Newmarket, UK) within 300 m of the various dispensers. In some experiments, the temperature of sachets was measured by attaching a surface temperature probe (Probe type EU, Grant Instruments, Cambridge, UK) to the dispenser. All environmental variables were measured at 10–15 min intervals using either Delta-T or Squirrel 1200 (Grant Instruments, Cambridge, UK) dataloggers. Data were then downloaded onto a PC for analysis. Daily temperatures were also recorded from maximum and minimum mercury-in-glass thermometers held in a standard Stevenson screen.

Biological performance

Studies were made of the responses of tsetse to attractants released from some dispensers. Experiments were carried out during the 3 h preceding sunset when tsetse are most active (Hargrove & Brady, 1992). Epsilon traps (Hargrove & Langley, 1990) or F3 traps (Flint, 1985) were baited with the dispensers and the catches were compared using a Latin-square design of treatments × sites × days.

Statistics

The significance of differences in the release rates of attractants from different types of dispensers was assessed by ANOVA and the relationship between environmental variables and release rates were analysed by ANCOVA. In studies of the responses of tsetse to different baits, the catches (n) were transformed to $\log_{10}(n+1)$ and then analysed by ANOVA. All analyses used GLIM4 which fits statistical models using a maximum likelihood method (Crawley, 1993).

Experiments and results

Preliminary laboratory studies

Polyethylene vials

Vials (36×8×1.5 mm) loaded with 4-methylphenol (100 mg) in dioctylphthalate (Aldrich Chemical Co. Ltd; 0.5 ml) released the attractant at 0.003 mg/h at 27°C. Replacing the dioctylphthalate by the chlorinated hydrocarbon Cereclor S45 (ICI, Cheshire, UK) increased the release rate to 0.013 mg/h. Previous work (Vale *et al.*, 1988) has shown that these rates are 0.1–0.001 times below the minimum effective dose.

Polyethylene tubing

Larger dispensers were constructed from pieces of polyethylene tubing plugged or heat-sealed at each end. Initial release rates for the components of a 4:1:2 mixture of 4-methylphenol, 3-*n*-propylphenol and octenol (4 g) from a tube (12.5 cm long, 7 mm i.d. × 10 mm o.d.). were 0.8 mg/h, 0.1 mg/h and 0.23–0.3 mg/h respectively at 27°C, but after 60 days the rates declined by 80%. This decline was associated with hardening of the polyethylene, not seen in unfilled tubing, and neither of these effects were prevented by adding Cereclor to the contents. Release rates of tubes loaded with the individual components showed a similar decline.

Polyethylene sachets

Thin-walled sachets were constructed from polyethylene layflat tubing (Packrite, Harare, Zimbabwe.) with 0.15 mm thick walls and a surface area of 50 cm^2 using a heat sealer. The sachets were filled with 4 ml of 4-methylphenol, 3-*n*-propylphenol or octenol. Release rates remained constant over the experimental period of 80 days at 0.64 mg/h, 0.4 mg/h and 1.1 mg/h respectively. Release rates of sachets containing an 8:1:4 mixture of 4-methylphenol, 3-*n*-propylphenol and octenol declined only slightly over the 218 days of the experiment from 0.38, 0.022 and 0.13 mg/h at 27°C for the three components respectively on day 7 to 0.27, 0.024 and 0.10 mg/h on day 218 as the relative amounts of the more volatile 4-methylphenol and octenol decreased.

Polyethylene bottles for ketones

Studies were made of the feasibility of dispensing ketones from sealed polyethylene containers. Acetone and butanone diffused out of the small sachet dispensers (walls 0.15 mm thick, 50 cm^2 surface area, 27°C) at 5–7 mg/h at 27°C. However thin-walled sachets, large enough to contain acetone or butanone sufficient for several months, would not be robust for operational use and so studies were made of various larger, thick-walled polyethylene bottles. In preliminary studies it was found that butanone diffused more readily than acetone through polyethylene and consequently studies concentrated on dispensers for butanone since for *G. pallidipes* and *G. m. morsitans*, acetone and butanone are equally effective attractants (Torr *et al.*, 1995). A number of low-density polyethylene and polypropylene bottles with a wall thickness of *c.* 1 mm and a volume of 200–500 ml were investigated by placing the bottles in a wind tunnel and measuring the rate of release of butanone for 90–150 days. The most promising bottles consisted of a polyethylene 500 ml vaccine pack (Bettix, Bolton, UK) which released butanone at 19 (±1.1, S.E.) mg/h at 27°C or a 500 ml polyethylene bottle (TT containers, Sevenoaks, UK) which released butanone at 9 (±0.1) mg/h at 27°C and 21 (±0.7) mg/h at 33°C respectively.

These preliminary studies indicated that a mixture of octenol and phenols could be dispensed in the field using a thin-walled polyethylene sachet and that butanone could be dispensed from a polyethylene bottle. Accordingly, more detailed laboratory and field studies were undertaken to investigate the effect of surface area, thickness and age of sachet, the blend ratio and ambient temperature on the rate of release of octenol, phenols and butanone from these dispensers.

Laboratory studies on sachet dispensers for octenol and phenols

Unless stated otherwise, the sachets used in the following studies were 50 cm^2 in surface area (5 × 5 cm) and had walls 0.15 mm thick.

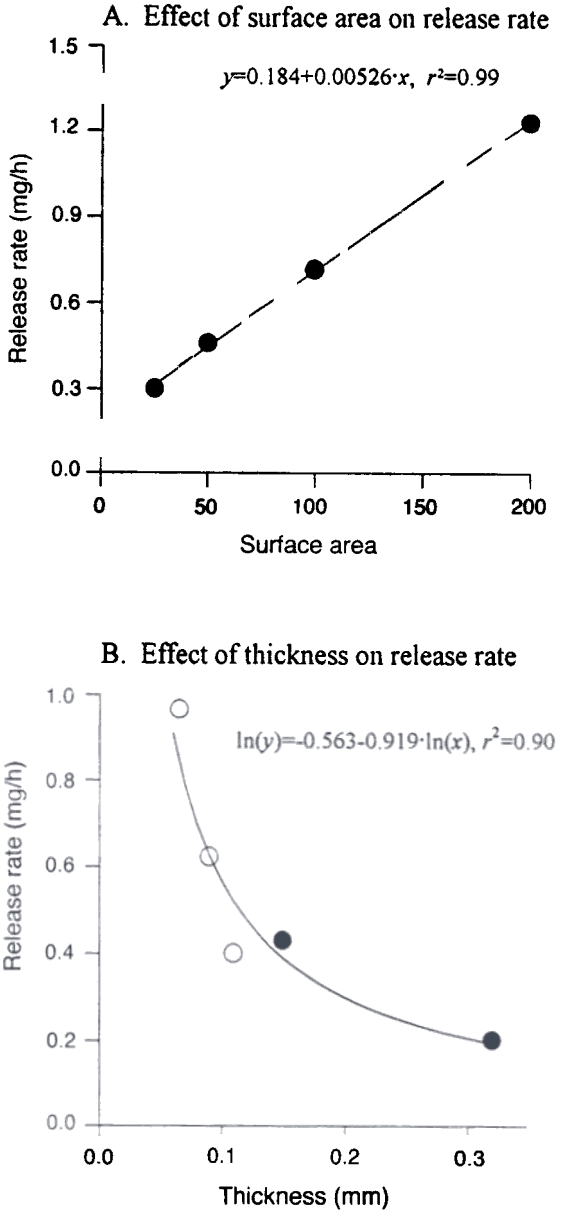


Fig. 1. Effect of surface area (A) and sachet thickness (B) on the release rates of attractant from 50 cm^2 sachets. Attractant consisted of an 8:1:4 blend of 4-methylphenol, 3-*n*-propylphenol and octenol. Solid and open dots indicate polythene supplied from Packrite (Harare, Zimbabwe) and Transatlantic Plastics, (Southampton, UK), respectively. Sachets were maintained at 27°C and release rates were estimated gravimetrically over seven days.

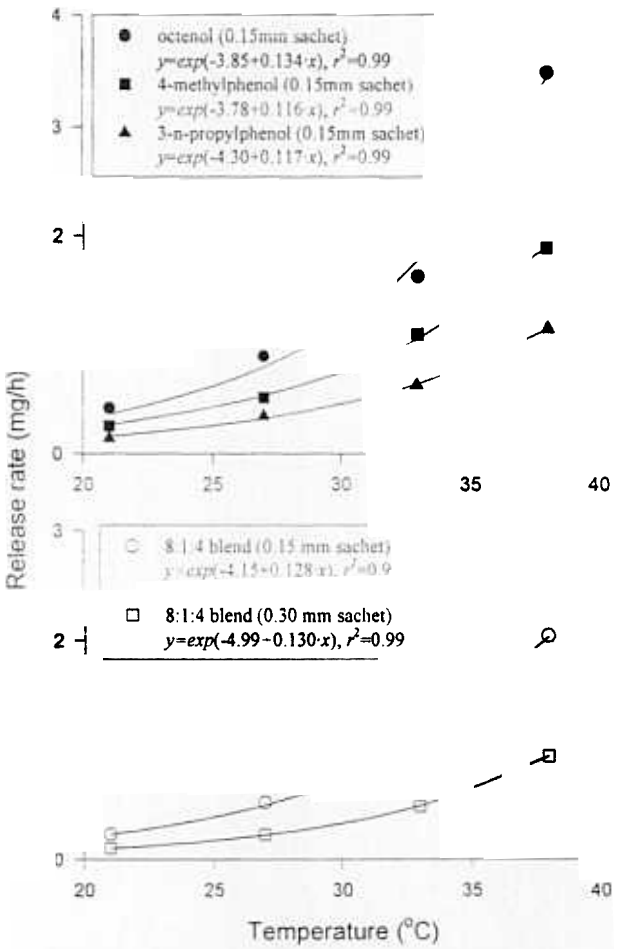


Fig. 2. Effect of temperature on the release rate of attractants from various 50 cm² sachets maintained in the laboratory. Release rate estimated gravimetrically.

Effect of sachet surface area and thickness

Increasing the surface area of a sachet increased the release rate of the components (fig. 1a) and increasing the thickness decreased the release rate (fig. 1b). Changes in thickness and surface area were shown to have no significant effect on the ratios of the components released from blends.

Effect of temperature

Increasing the temperature from 21° to 38°C produced an exponential increase in the release rate of chemicals (fig. 2), but there was no significant effect on the ratios of the components released from blends. Release rates for the 8:1:4 blend of 4-methylphenol, 3-*n*-propylphenol and octenol were not affected (<10%) by placing the sachet in a bag made of the cotton drill cloth used to construct targets (Vale *et al.*, 1986).

Effect of blend ratio

Studies were made of how the release rates of octenol, 4-methylphenol and 3-*n*-propylphenol were related to their percentage composition in blends of the three components

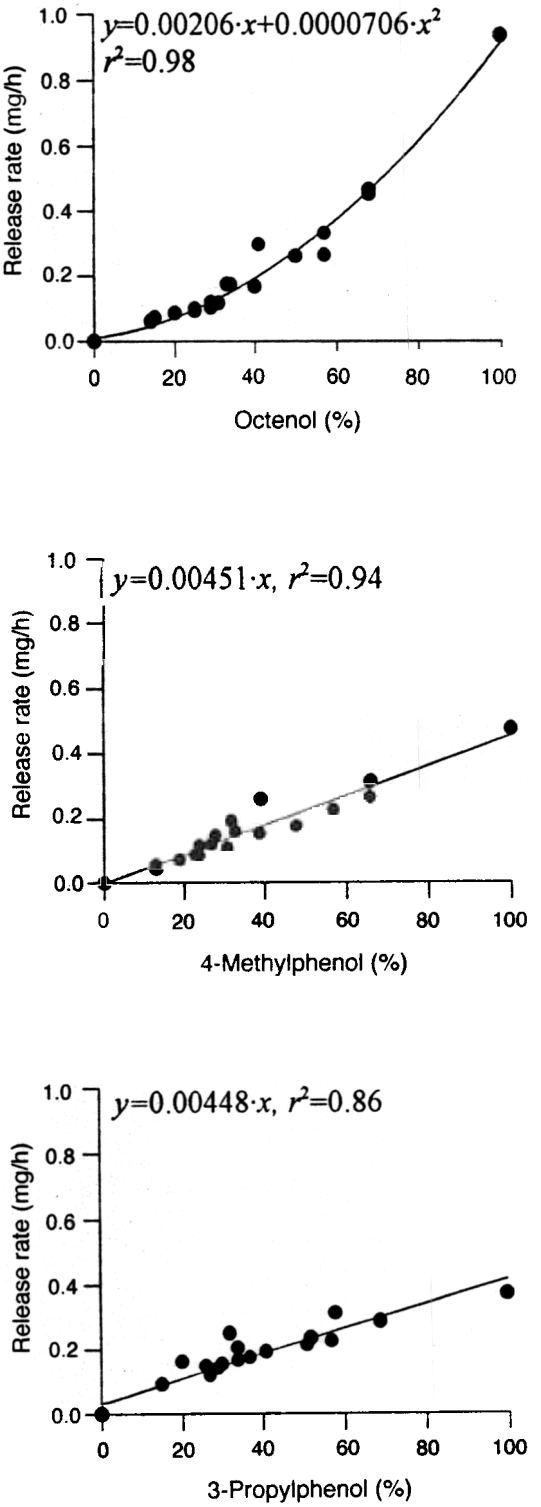


Fig. 3. Effect of changes in the percentage of octenol, 4-methylphenol and 3-*n*-propylphenol on their release rates from a polyethylene sachet (50 cm², walls 0.15 mm thick) at 27°C. Release rates were obtained indirectly by measuring the weight loss of a sachet and by GC analysis of entrained samples to estimate the fraction of the weight loss attributable to each component.

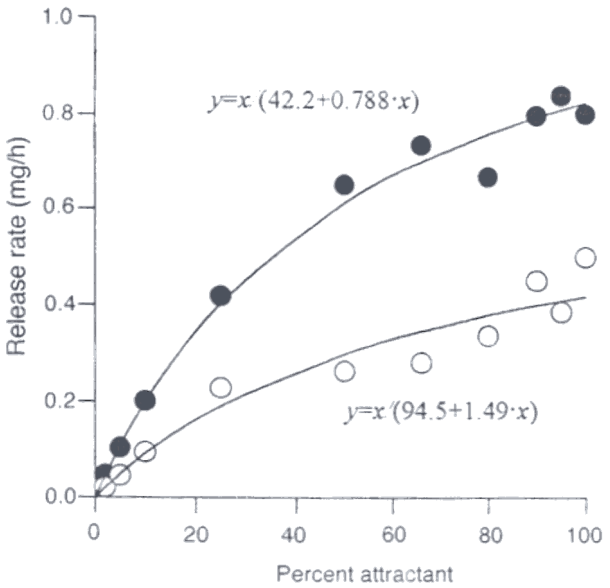


Fig. 4. Rate of release of octenol (●) or 4-methylphenol (○) from sachets (50 cm², 0.15 mm thick) containing various concentrations of these attractants in dioctylphthalate and held at 27°C. Release rates were estimated gravimetrically. Lines fitted by maximum likelihood using a gamma error with a reciprocal link to give expressions of the general form $y = x/(a + bx)$. Standard errors of a and b for octenol were 1.631 and 0.04033, and for 4-methylphenol 8.550 and 0.1982, respectively.

by measuring release rates from sachets containing 22 different blends maintained in the laboratory wind tunnel. The data (fig. 3) showed that the release rates of the two phenols varied linearly with the percentage composition, but that of octenol increased exponentially with increasing percentage of octenol in the blend.

Studies were made of controlling release rate by adding dioctylphthalate as an involatile diluent for the attractants. Sachets were filled with 2 ml of various dilutions of octenol or 4-methylphenol and these were placed in a wind tunnel for up to 52 days. Release of dioctylphthalate was undetectable by measurement of weight loss, and rates of release of the attractants were measured by loss in weight of the sachets. As the attractant diffused out of the sachet, the concentration decreased. Accordingly, only data for the first seven days were considered since there was no significant change in the release rate over this period. The results (fig. 4) showed that there was a curvilinear relationship between concentration and release rate which could be fitted by an inverse linear curve.

Table 2. Detransformed mean catch (transformed mean in brackets) of *Glossina m. morsitans* and *G. pallidipes* from traps baited with acetone (500 mg/h) and a new or a year-old old sachet (0.15 mm thick, 50 cm²) containing an 8:1:4 blend of 4-methylphenol, 3-*n*-propylphenol and octenol.

Odour bait	*G. m. morsitans*	*G. pallidipes*
Acetone + new sachet		
Acetone + old sachet		
Acetone only		
No odour		
Transformed S.E.		

Effect of age

Laboratory studies were made of the effect of sachet age on the release rate of octenol and phenols from blends of the three attractants. Sachets containing eight blends of 4-methylphenol, 3-*n*-propylphenol and octenol (4 ml total) in the ratios: 32:1:32, 32:1:16, 16:1:16, 16:1:8, 8:1:8, 8:1:4, 4:1:4 and 4:1:2 were maintained in a wind tunnel at 27°C for up to 218 days and the release rates of the three components were measured by entrainment after 7, 37, 85, 159 and 218 days. Results after 7 days and after 218 days when approximately 65% of the contents had been released, are shown in table 1. The release rates of 4-methylphenol and octenol decreased with time and the release rate of 3-*n*-propylphenol increased as the relative amount of this less volatile component increased. Thus release rates of the components of a 8:1:4 mixture of 4-methylphenol, 3-*n*-propylphenol and octenol were in the ratio 18:1:6 initially and 11:1:4 after 218 days at 27°C.

Field studies were undertaken to determine whether the change in blend affected the performance of sachets. Traps were baited with either new or 12-month old sachets (50 cm², 0.15 mm thick) which had been filled initially with *c.* 10 g of an 8:1:4 blend of 4-methylphenol, 3-*n*-propylphenol and octenol. The results (table 2) showed that there was no significant difference in the catch of traps baited with old or

Table 1. Release rates (mg/h) of 4-methylphenol (4MP), 3-*n*-propylphenol (3PP) and octenol (Oct) from eight different blends contained in 50 cm², 0.15 mm thick sachets at 27°C, measured by entrainment after 7 d and after 218 d when approximately 65% of the contents had been released.

Initial ratio[1]	4MP		3PP		Oct		Ratio	
	7 d	218 d	7 d	218 d	7 d	218 d	7 d	218 d
32:1:32	0.29	0.21	0.004	0.007	0.24	0.18	63:1:57	31:1:26
32:1:16	0.38	0.27	0.005	0.010	0.12	0.12	83:1:26	28:1:12
16:1:16	0.28	0.22	0.010	0.011	0.23	0.17	29:1:24	20:1:15
16:1:8	0.38	0.25	0.010	0.011	0.13	0.11	39:1:13	23:1:10
8:1:8	0.28	0.22	0.016	0.020	0.23	0.18	17:1:14	11:1:9
8:1:4	0.38	0.27	0.022	0.024	0.13	0.10	18:1:6	11:1:4
4:1:4	0.28	0.18	0.033	0.044	0.23	0.15	9:1:7	4:1:3
4:1:2	0.36	0.22	0.042	0.050	0.11	0.09	9:1:3	5:1:2

[1]Initial ratio of 4-methylphenol, 3-*n*-propylphenol and octenol (4 ml) in sachet.

new sachets, both increasing the catch of *G. pallidipes* significantly.

Physical performance in the field

Effect of temperature

Studies were made of the effects of temperature on the release rates of 4-methylphenol, 3-*n*-propylphenol and octenol dispensed either singly or as an 8:1:4 blend from a sachet. The results (fig. 5) showed that the release rates for the three components increased exponentially with temperature, in line with the laboratory data (fig. 2). Field release rates were consistently 10–15% lower than corresponding rates in the laboratory, except for the 8:1:4 blend in a 0.15 mm thick sachet where the field release rate was greater.

Although the release rate for the 8:1:4 blend increased exponentially with temperature and a simple exponential model was a good fit ($r^2 = 0.96$), the measured release rates at high temperatures (>45°C) were systematically less than the fitted line. Fitting a further polynomial term ($y = -4.289 + 0.1774x - 0.001016x^2$) to the model reduced the deviance significantly ($P < 0.001$), suggesting that the release rate is not a simple exponential function of temperature for this blend.

The release rate of butanone from a plastic bottle (Bettix 500 ml vaccine pack) also showed an exponential increase with increase in temperature, although this seemed to be more variable than with the sachets (fig. 5).

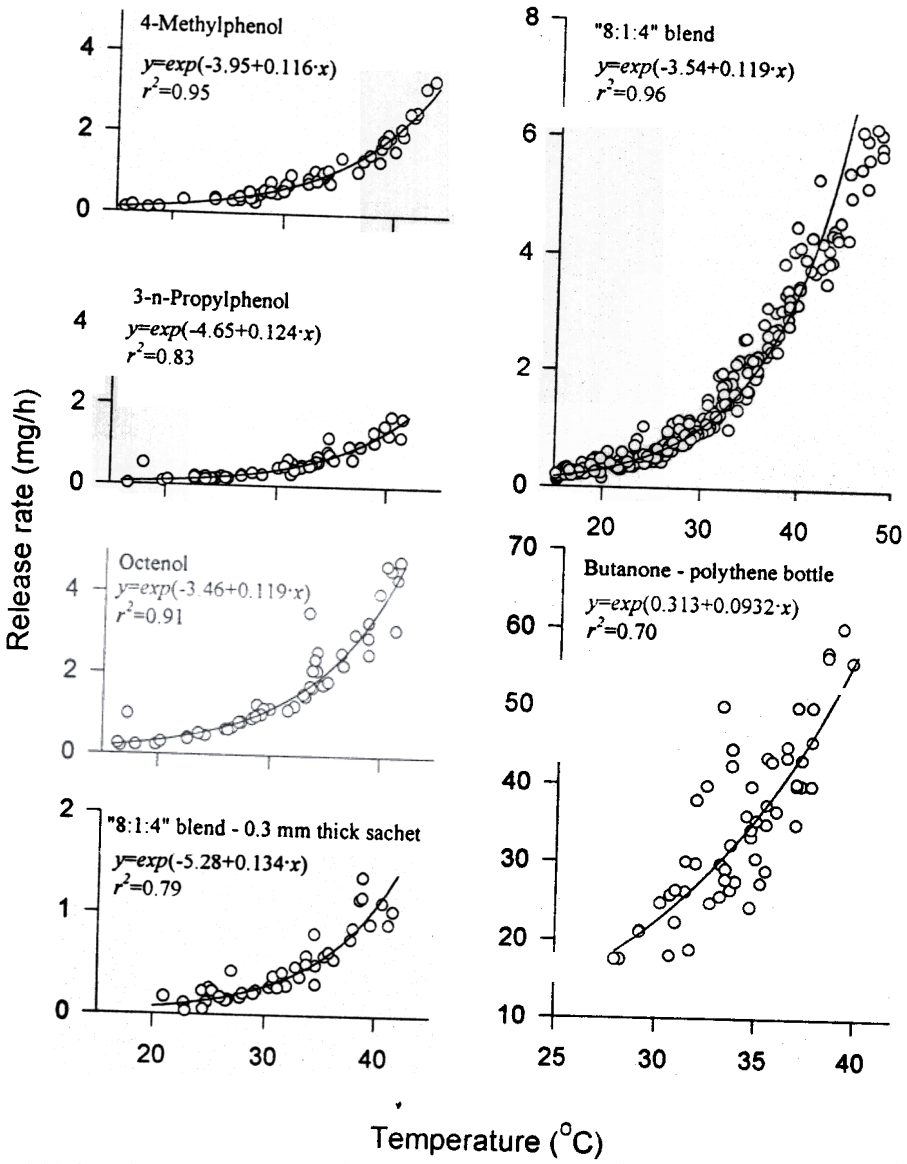


Fig. 5. Effect of temperature on the release rate of attractants from sachets (50 cm²) in the field. All sachets were 0.15 mm thick unless stated otherwise. Weight loss was estimated gravimetrically and temperature is the mean temperature in the 2 h between weighing.

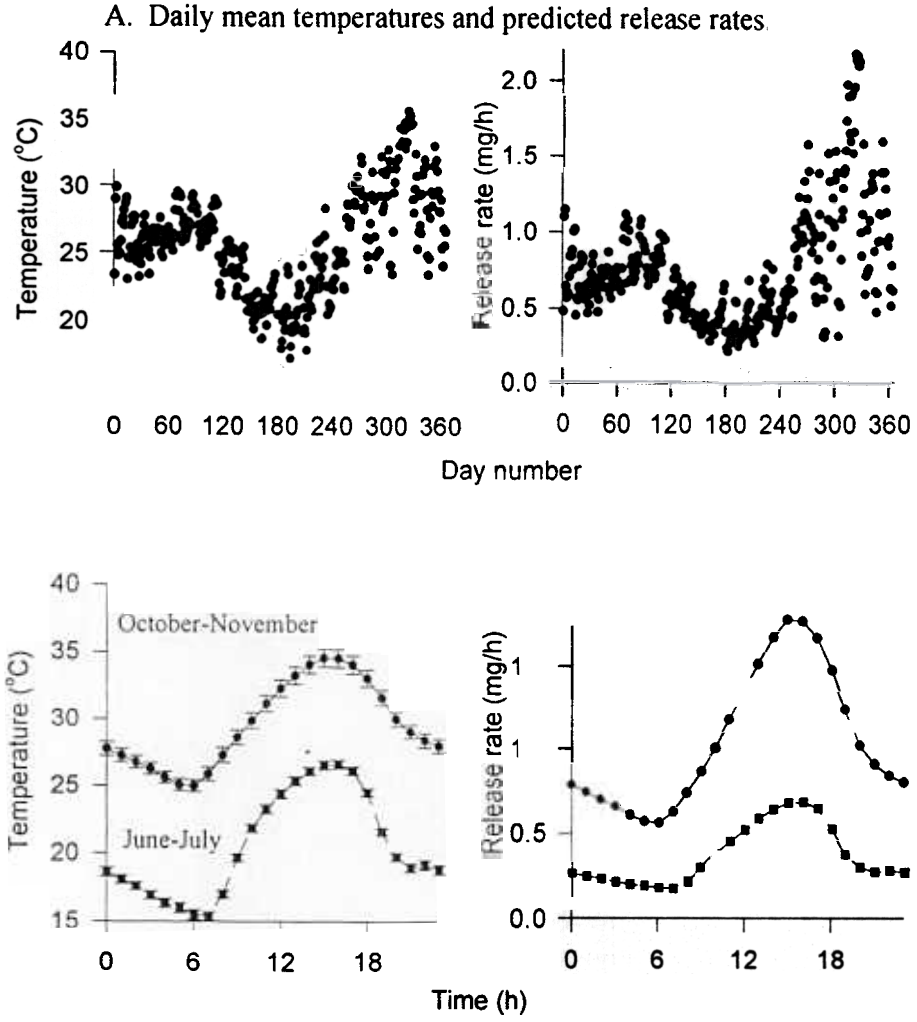


Fig. 6. Daily mean temperature and the hourly mean temperatures for the cold (June–July) and hot (October–November) seasons at Rekomitjie for 1994. The predicted release rates from a standard sachet (50 cm^2, 0.15 mm thick) containing an 8:1:4 blend of 4-methylphenol, 3-*n*-propylphenol and octenol, using the appropriate regression from fig. 5.

Diurnal and seasonal effects on release rates from sachet dispensers

Diurnal and seasonal fluctuations in temperature and the effect of shade and insolation could produce large changes in the dose of attractant from sachets. To investigate this, meteorological data from Rekomitjie for 1994 were analysed to assess their effect on release rates. The results (fig. 6) show that the mean daily temperature varied between *c.* 16°C and 37°C and the mean hourly temperature varied between 25° and 36°C in the hot season (October–November) and 15–26°C in the cold season (June–July). The rate of release of an 8:1:4 blend of 4-methylphenol, 3-*n*-propylphenol and octenol from a sachet was estimated for each hour of 1994 using the fitted model for this blend and sachet (fig. 5). From these hourly estimates, the mean daily release rates were calculated and the results (fig. 6) showed that the release of attractants from the sachet would have varied between 0.22 and 2.17 mg/h over the year. For the mean hourly temperatures, the corresponding mean release rates would have varied between 0.18–0.69 mg/h in the cold season and 0.57–1.78 mg/h in the hot season (fig. 6).

In the field, targets and traps may be placed in shady or open sites and consequently odour dispensers may be insolated or shaded. Moreover, sachets are generally placed in pockets in the fabric of a trap or target. The pockets of Epsilon and F3 traps are sewn into the blue-coloured portion of the trap whereas for targets the pockets are generally black. Thus dispensers may be exposed to a variety of different microclimates according to the siting of the trap or target and the colour of the cloth pocket. To investigate the effect of these variables, studies were made of the rate of release of attractants from sachets in blue or black cloth held in shade in a Stevenson screen or in direct sunlight. The bags containing the sachets were suspended facing East–West.

In one experiment, five sachets, each containing 4 ml of an 8:1:4 mixture of 4-methylphenol, 3-*n*-propylphenol and octenol and placed in cotton drill bags (8×8 cm) dyed phthalogen blue, were suspended in shade or direct sunlight and weighed at intervals through the year. The results (fig. 7)

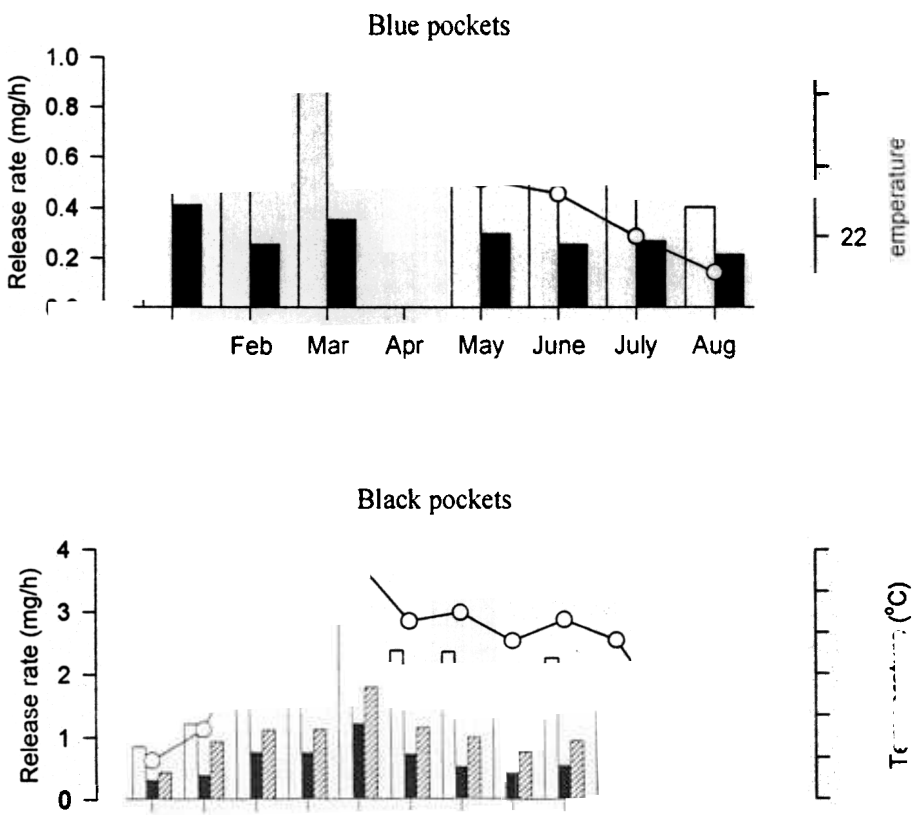
showed that the mean release rate of the sachets exposed to direct sunlight was 0.60 mg/h (0.17, SE) compared to 0.28 mg/h for those in the shade.

In a second experiment, five sachets, each containing 10 g of a 12:1:6 blend of 4-methylphenol, 3-*n*-propylphenol and octenol and placed in a black bag (8×8 cm), were suspended in the shade, or full sun or beneath a small pitched roof of waxed cardboard. The sachets were weighed at monthly intervals to measure mean monthly rates of release over a year. Sachets in full sun were depleted in less than a year and were therefore replaced with new ones at 3–5 month intervals. The results (fig. 7) showed that the rate of release decreased in the order full sun > card roof > shade. The cumulative loss of attractant from the sachets in full sun over one year was 17.4 g compared to 4.8 g from sachets in shade. The largest mean loss of attractant from the sachets in full sun was 2.4 g per month during November–December 1994 compared to 0.8 g per month for the sachets in shade. The black bag in full sun had a higher release rate than the blue bag at comparable temperatures, presumably because the blue bag reflects more radiation than the black one and is thus subject to less insolation.

The release rate of butanone from a bottle dispenser also increased in full sun. The mean release rate from a dispenser (Bettix 500 ml vaccine pack) placed in a black bag in full sun was 106 mg/h (range 38–234). These rates occurred when the shade temperature was 32.5–39.5°C, giving predicted rates of 28–54 mg/h for a dispenser in the shade (fig. 5).

The surface temperature of a sachet in a black pocket in full sun was measured continuously for 24 h periods on one day of each month from July 1994 to July 1995. The results (fig. 8A) showed that the black pocket in full sun was *c.* 8°C hotter during the day and *c.* 1.6°C cooler at night than the shade temperature. Comparisons were also made of the temperature of black and blue pockets in full sun for seven days during July–September 1995. The results (fig. 8B) showed that there was no significant difference in the temperature at night but during the day the black pocket was *c.* 2°C hotter than the blue one. This accords with previous indications (fig. 7) that in full sun the sachets in black pockets have a higher release rate than sachets in blue pockets. Pooling the data for the bags, the black and blue bags were on average 3.0°C and 2.1°C warmer than the mean shade temperature.

To understand more fully seasonal and diurnal variations in release rate, continuous measurements were made of air temperature and black bulb temperature between 7 April 1995 and 5 February 1996. The results (fig. 8C) showed that



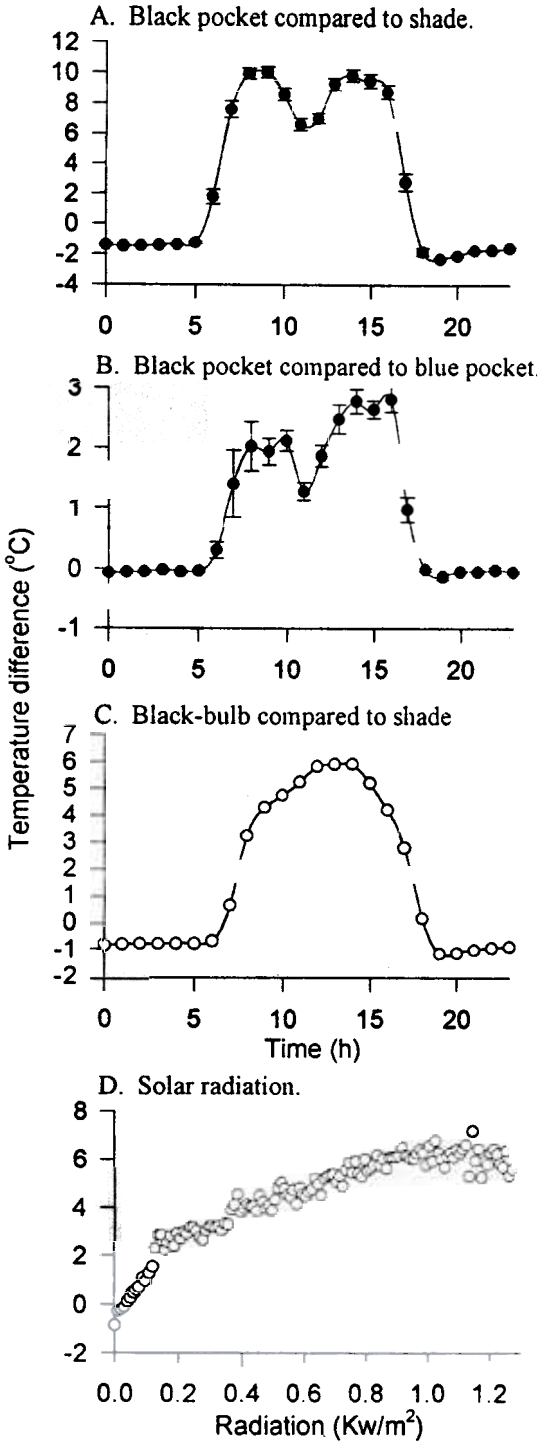


Fig. 8. Mean (±S.E.) differential in surface temperature of Standard sachets (containing 8:1:4 blend of 4-methylphenol, 3-*n*-propylphenol and octenol) in:— black pocket in either full sun or full shade (A) or in full sun and in either black or blue pockets (B). Also, mean differential between black-bulb and shade temperatures (C) and the relationship between solar radiation and difference between black bulb and shade temperatures (D).

the black bulb temperature exhibits a similar pattern to the temperatures of the black and blue pockets (fig. 8A,B), being *c.* 1°C cooler at night and up to 6°C hotter during the day. The black pocket exhibited a midday dip in temperature (fig. 8A) which was probably due to the bags being suspended vertically facing East-West so that at midday only the uppermost edge of the bag was insolated. The black bulb on the other hand is spherical and the surface area exposed to the sun would not have varied with time of day. The general similarity between the patterns of the sachet and black bulb temperature suggests that the temperature profile of the black bulb is a reasonable model of the thermal behaviour of a sachet in direct sunlight.

The mean differential between the shade and black-bulb temperatures was 1.6°C (range, −2.5 to 12.6°C). The differential was not affected markedly by the time of year. For instance, in June 1995 the mean air temperature varied between 15.7°C and 27.2°C compared to 27.6°C and 37.4°C in October. The mean differential between shade and black bulb temperature remained remarkably similar, being 1.6°C (range, −2.5 to 8.6°) in June and 1.7°C (2.1 to 9.7°C) in October. The temperature difference was, as expected, affected by solar radiation (fig. 8D), the temperature difference between the shade and sun temperature being greater towards the middle of the day and on days where there was less cloud cover.

A model of sachet performance

For practical purposes, it is necessary to predict the effective life of a sachet. For any given sachet, the release rate is a function largely of temperature. Consequently, studies were made of the reliability of a day-degree model to predict the longevity of a sachet.

The performance of a sachet containing a 12:1:6 blend of 4-methylphenol, 3-*n*-propylphenol and octenol was modelled. This sachet is currently being used widely in tsetse control operations in Zimbabwe. The biological performance of this sachet was not significantly different from the previous 8:1:4 blend: the detransformed mean catch (12 replicates) of *G. pallidipes* from a trap baited with acetone plus a 12:1:6 sachet was 66 (1.826 ± 0.0662, transformed mean ± SE) compared to 52 (1.725 ± 0.0662) for the 8:1:4 sachet.

Release rates for the 12:1:6 sachet were measured at 2 h intervals as for fig. 5, and the equation relating temperature in °C (x) and release rate in mg/h (y) for the 12:1:6 sachet was found to be:

$$y = exp(0.146x - 4.48) \quad (r^2 = 0.88) \qquad (1)$$

Two models, based on either hourly or daily measurements of temperature were considered. For the hour-degree model the hourly loss of attractant from a sachet was calculated by using equation (1) and substituting the mean hourly estimates of shade temperature from the automatic meteorological station at Rekomitjie. A second model was based on daily measurements of maximum and minimum temperature from the Stevenson screen at Rekomitjie. The daily loss of attractant was estimated using equation (1) and substituting the mean temperature calculated for each day. The daily temperature was estimated as being the mean of the maximum and minimum and it was assumed that the sachet was at this temperature for 24 h each day.

Predicted weight losses were compared with the observed loss of attractants from 12:1:6 sachets in black pockets placed in the shade (fig. 7). The sachets were weighed at 25–35 day intervals between 13 July 1994 and 11 July 1995 and the 12 observed losses were compared with the predicted ones. The results (fig. 9) show that both models overestimated the amount of attractant released by the sachet by *c.* 10%. The observed total loss of attractant over the 12 months of the experiment was 4.837 g compared to 6.089 g and 5.493 g for the models. However, an improved fit to the observed weight losses could be obtained by subtracting 0.2°C from the observed hourly temperatures ($r^2 = 0.95$) or 0.8°C ($r^2 = 0.84$) for the daily temperatures (fig. 9). The gross simplifications of the day-degree model did not have a marked effect on the predictive accuracy of the model. The adjustment to the temperature in the sachet model is probably related to the observation that sachets are 1–2°C cooler than the air temperature at night (fig. 8).

Studies were made to determine whether a simple correction could be made to the day-degree model to fit the observed loss of attractants from sachets in the sun (fig. 7). The best fit ($r^2 = 0.81$) was made by adding 7.6°C to the daily mean temperature (fig. 9).

Thus a simple day-degree model, using equations relating temperature and release rate for the various attractants (figs 2 and 5) with daily measurements of maximum and minimum temperature, is an accurate predictor of dispenser performance. The general formula to estimate the cumulative loss of attractant from a sachet containing a 12:1:6 blend of 4-methylphenol, 3-*n*-propylphenol and octenol over *n* days is:

$$Y = \sum exp(a + b(c + x_t))*24 \qquad (2)$$

where: Y = cumulative loss of attractant in mg/day; $a = -4.4780$; $b = 0.1456$; $c = -0.8$ (for sachets in shade) or 7.6 (for sachets in the sun); and x_t = mean daily temperature for day t.

Discussion

Sachet dispensers

The present study shows that the tsetse attractants, 4-methylphenol, 3-*n*-propylphenol and octenol can be dispensed either singly or as a blend from sealed polyethylene sachets, 0.06–0.3 mm thick. Release rates of single components from the sachets remained constant until the contents were exhausted with no sign of degradation of the polyethylene under field conditions, whereas with thicker polyethylene tubing the release rate declined with age and this was associated with a hardening of the polyethylene even in the laboratory. To release the attractants at a required dose, the sachet surface area or thickness can be varied, the release rate being directly related to surface area and inversely related to thickness. Thus at 27°C, doubling the surface area or halving the thickness of a sachet doubles the release rate. For most practical research purposes, the simplest method of controlling dose is to place single chemicals in sachets of suitable size and thickness. For example, Torr *et al.* (1995) simulated natural ox odour by dispensing a range of different phenols, octenol, acetone and butanone from sachets 25–50 cm^2 in surface area and with walls 0.15–0.3 mm thick.

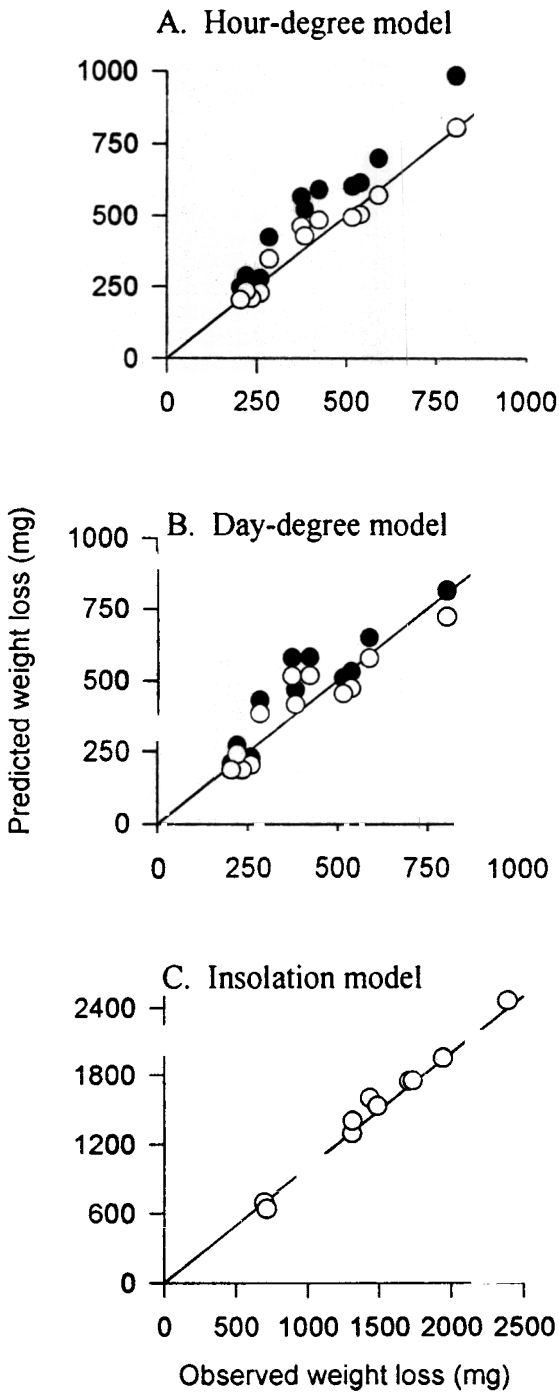


Fig. 9. Scatterplot of observed weight loss of attractant from a standard sachet containing 12:1:6 blend of 4-methylphenol, 3-*n*-propylphenol and octenol and the predicted loss using an hour:degree or day:degree model of sachet performance. Plots show predicted losses based on observed temperatures (●) or with temperature correction (○) of –0.2°C or –0.8°C for hour–degree and day–degreee models respectively. The insolation model shows the observed weight loss for sachets in full sun and the predicted rates based on a day–degree model with +7.6°C correction to the daily mean temperature. Solid line indicates the perfect fit between the observed and predicted weight losses.

The release rate can also be controlled by varying the concentration of attractant in the sachet using an involatile diluent, the release rate being generally less at lower concentrations. However this method is complicated by the decline in release rate with time, as the concentration of attractant declines. Moreover, the increase in release rate with concentration was either linear, exponential or monotonic according to the constituents. Despite these complications, using a diluent to control the rate is useful for dispensing very low doses of attractant for research purposes. For instance, oxen naturally produce *c.* 0.01 mg/h of octenol (Torr *et al.*, 1995). To simulate this dose using 0.15 mm thick polyethylene, at a temperature of say 27°C, would require using a sachet of 0.56 cm^2. It would be more practicable to use a 50 cm^2 sachet containing 5 g of a 0.5% mixture of octenol with dioctylphthalate.

In the absence of other factors, the release rate of a substance from the dispensers described here is determined by the rate of diffusion of the substance across the polyethylene membrane which is governed by Fick's Law., being the product of the concentration gradient across the membrane and the diffusivity of the substance in the membrane material (e.g. O'Neill, 1980). Presumably for the dispensers described here the concentration at the outer surface is zero as the material is volatilized as fast as it diffuses through, and so the concentration gradient is determined by the concentration of material at the inner surface of the membrane. This in turn is the product of the concentration in the dispenser and the partition coefficient for the material between the dispenser contents and the polyethylene. If this partition coefficient is constant over the range of concentrations evaluated, the rate of diffusion of a substance through the membrane and hence its release rate from the dispenser is directly proportional to the concentration of material in the dispenser. However, in this study the effect of concentration on release rate of a substance was studied for the whole range of concentrations of the substances from 0–100% (figs 3, 4), and it is reasonable to suppose that the partition coefficient will vary over this range, e.g. the partition coefficient for octenol between 1% octenol in dioctylphthalate and polyethylene will be different from that between 99% octenol in dioctylphthalate and polyethylene. This will give rise to curvilinear relationships between release rate and internal concentration in some cases as seen in figs 3 and 4. Thus the data in fig. 3 indicate that the partition coefficients for octenol between mixtures with the phenols and polyethylene are higher than that between octenol and polyethylene so release rates are lower than expected from a linear model. From the data in fig. 4, the opposite would seem to be the case for octenol in dioctylphthalate, and release rates are greater than expected from the linear model and limited by the solubility of octenol in the polyethylene.

In practice it should be noted that if polyethylene sachets containing volatile materials are sealed in impermeable containers for transport to field sites, material will continue to diffuse through the polyethylene until the concentration is the same inside and outside the sachet. The sachet will then be covered with a thin film of the contents when the container is opened.

Laboratory and field studies of the effect of temperature on release rate both showed that there was an exponential increase in release rate with temperature. In most cases, release rates measured in laboratory and field were similar, although the field data were more variable than those from the laboratory. This is presumably due, at least in part, to the nominal temperature in the field being the mean measured over a 2 h period when the temperature was in fact varying, whereas in the laboratory the temperature was held constant.

The results for the 8:1:4 blend of 4-methylphenol, 3-*n*-propylphenol and octenol (figs 5 and 6) showed that the release rate in the field was 20–50% greater than in the laboratory. Moreover, in the field the release rate was not a simple exponential function of temperature, the release rates at higher temperatures being consistently less than predicted. This may be an experimental artefact. The high rates of release at high temperatures in the field were obtained from sachets in direct sun. These sachets were temporarily removed to the laboratory for weighing where they cooled to at least shade temperature. The few minutes of cooling, especially at higher temperatures, would have been a significant fraction of the 2 h interval between weighing. In the laboratory studies on the other hand, the sampling intervals were 24 h or more and thus the brief period of cooling produced when the sachets were being weighed were less significant.

The good fit between the hour-degee model and the observed loss of attractants from sachets indicates that temperature is the main environmental determinant of release rate, with other environmental variables such as wind speed and humidity having little or no effect. A simple day-degree model to predict the loss of attractants from sachets in the shade also fitted the observed losses reasonably well. To estimate the loss of attractants from sachets containing other blends, rate constants (*a* and *b*) from formulae relating temperature and release rate (e.g. figs 2 and 5) can presumably be substituted into formula (2). Predicting the release rates from dispensers exposed to the sun is complicated by variations in incident solar radiation associated with latitude, elevation, season, site, and pocket colour. Thus the general formula (2) should be used cautiously in predicting the loss of attractants from sachets in the sun.

Practical use of odour dispensers

In control campaigns against *G. pallidipes* in Zimbabwe and Somalia between 1988 and 1993, traps and targets were baited with standard sachets containing *c.* 10 g of an 8:1:4 blend of 4-methylphenol, 3-*n*-propylphenol and octenol. In the laboratory at 27°C such dispensers initially released the chemicals at 0.38, 0.02 and 0.13 mg/h respectively declining to 0.27, 0.02 and 0.10 mg/h after 218 days. Such doses increase the catch of tsetse significantly (Vale & Hall, 1985b; Vale *et al.*, 1988) and the present studies showed that the catch from traps baited with new and 12-month-old sachets were not significantly different with both types increasing the catch *c.* 2.5 times.

Sachets have been used by Torr *et al.* (1995) to dispense low doses of acetone and butanone but these dispensers are not practicable for use in routine control and survey operations. The polyethylene bottles used in the present study are robust and they release butanone at effective doses (Torr, 1990; Torr *et al.*, 1995) and were used successfully in tsetse control operations in Somalia between 1987 and 1990.

The release rates of all chemicals were greatly affected by temperature. Field temperatures at Rekomitjie can range from 10°C for a sachet at night during the cold season and

50°C for one in full sun placed in a black pocket in the hot season. This temperature range can result in >100-fold differences in release rate (fig. 5).

An efficient dispenser should release chemicals only when the target species is active. Most tsetse flies, including *G. pallidipes* and *G. m. morsitans* in Zimbabwe, show one or two peaks of activity a day, in the early and/or late photophase (Brady & Crump, 1979; Hargrove & Brady, 1992). To illustrate the effect of temperature on dispenser performance, consider a standard sachet containing 4-methylphenol, 3-*n*-propylphenol and octenol in the ratio 12:1:6. In Zimbabwe, *G. pallidipes* and *G. m. morsitans* show little activity below 20°C or above 38°C (Hargrove & Brady, 1992). At the lower temperature the sachet would release 4-methylphenol, 3-*n*-propylphenol and octenol at 0.13, 0.01 and 0.06 mg/h respectively compared to 1.8, 0.15 and 0.87 mg/h at 38°C (estimated using data from figs 3 and equation 2). The low temperature rates would increase the catch significantly (Vale & Hall, 1985a,b; Vale *et al.*, 1988) but there would be a significantly greater effect at the high-temperature doses. Thus the sachets currently in use in Zimbabwe are likely to be effective at all temperatures when tsetse are active.

Butanone increases the catch significantly when dispensed at >50 mg/h (Torr, 1990; Torr *et al.*, 1995) and the polyethylene dispensers used here released <50 mg/h at <39°C. In field studies in Zimbabwe (Hall *et al.*, 1990) and Somalia (Torr, unpublished data), the catch from traps baited with butanone dispensed from polyethylene dispensers was not significantly different from that baited with acetone (500 mg/h) or butanone (500 mg/h). The better-than-expected performance of the butanone dispensers may be because they were placed in sites where they were insolated. Nonetheless, the present results suggest that there is a risk that these dispensers may not be effective at lower temperatures and it may be better to bait traps and targets with two such dispensers.

During the night, when tsetse are inactive the temperature is relatively cool and thus the release rate is much reduced for a large part of the time when tsetse are inactive. However, the sachets did release much attractant during the hot midday when tsetse are also inactive. Sachets in black pockets and exposed to the sun lost 17.4 g over 12 months which is greater than the maximum amount (10–12 g) of attractant that can be readily placed in 50 cm^2 sachets. To minimize the cost of frequent visits to replenish the attractants, sachets should be protected from insolation by placing them in the shade or in light-coloured pockets.

The blend of attractants in a sachet can be adjusted to suit other species of tsetse. For *G. m. submorsitans* Newstead for instance, a 3:1 blend of 3-methylphenol and octenol is used (Mérot & Filledier, 1991); initial studies showed that sealed polythene tubes as described above were effective dispensers (Filledier & Mérot, 1989), but more recently sachets (50 cm^2, 0.15 mm thick) containing 10 g of this blend have been found to be more effective and longer-lasting (Mérot & Torr, unpublished data). Polyethylene sachets have also been used to dispense attractants for screwworm fly, *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae). (Green *et al.*, 1993), stable fly, *Stomoxys calcitrans* (Linnaeus) (Diptera: Muscidae) (Holloway & Phelps, 1991) and repellents for tsetse (Torr *et al.*, 1996).

The sachets can also be easily adapted to suit operational demands. In carrying out tsetse surveys, the control entomologist needs to detect the presence of tsetse in a short period. It might therefore be better to dispense a high dose of attractant by using a large sachet of say 500 cm^2 in surface area and 0.15 mm thick containing an 8:1:4 blend of 4-methylphenol, 3-*n*-propylphenol and octenol. The catch of tsetse from a trap baited with such a dose of attractant would be significantly greater (Vale & Hall, 1985b; Vale *et al.*, 1988) than that produced by the standard 50 cm^2 sachet typically used with targets.

The blend ratio can also be adjusted slightly to increase the cost effectiveness of a sachet. In Zimbabwe for instance, the Tsetse Control Branch changed from using standard sachets containing an 8:1:4 blend of 4-methylphenol, 3-*n*-propylphenol and octenol to one containing a 12:1:6 blend. The present results show that the slight difference in the blend did not significantly affect the catch. Assuming that 4-methylphenol, octenol and 3-*n*-propylphenol cost £12.00, £81.58 and £913.00 per kg respectively (Barrett, 1994), then a 10 g sachet of 8:1:4 blend costs £1.02 compared to £0.82 for the 12:1:6 blend. Given that there are currently *c.* 50,000 traps and targets in Zimbabwe, each requiring say two sachets a year, changing to a 12:1:6 blend of attractant from an 8:1:4 blend produced an annual saving of £30,800 in the foreign exchange expenditure of the Department.

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References

Barrett, J.C. (1994) Economic issues in trypanosomiasis control: case studies from Southern Africa. PhD Thesis, University of Reading, 485 pp.

Brady, J. & Crump, A.J. (1979) The control of circadian activity rhythms in tsetse flies: environment or physiological clock? *Physiological Entomology* **4**, 311–318.

Bursell, E., Gough, A.J.E., Beevor, P.S., Cork, A., Hall, D.R. & Vale, G.A. (1988) Identification of components of cattle urine attractive to tsetse flies, *Glossina* spp. (Diptera: Glossinidae) *Bulletin of Entomological Research* **78**, 281–291.

Crawley, M.J. (1993) *GLIM for ecologists*. Blackwell Scientific Publications, Oxford, UK. 379 pp.

Dransfield, R.D., Brightwell, R., Kyorku, C. & Williams, B. (1990) Control of tsetse fly populations (Diptera: Glossinidae) using traps at Nguruman, south-west Kenya. *Bulletin of Entomological Research* **80**, 265–276.

Filledier, J. & Mérot, P. (1989) Pouvoir attractif de l'association M-cresol 1-octen-3-ol dans un type de diffuseur pratique pour *Glossina tachinoides* au Burkina Faso. *Revue d'Elevage et Médicine Vèterinaire des Pays Tropicaux* **42**, 541–544.

Flint, S. (1985) A comparison of various traps for *Glossina* spp. (Glossinidae) and other Diptera. *Bulletin of Entomological Research* **75**, 529–534.

Green, C.H. (1994) Bait methods for tsetse control. *Advances in Parasitology* **34**, 229–291.

Green, C.H., Hall, M.J.R., Fergiani, M., Chirico, J. & Husni, M. (1993) Attracting adult New World screwworm, *Cochliomyia hominivorax*, to odour-baited targets in the field. *Medical and Veterinary Entomology* **7**, 59–65.

Hall, D.R., Gough, A.J.E., Adams, P.H., Beevor, P.S., Cork, A., Green, C.H., Smith, J.L., Taylor, J.H.L. & Warnes, M.L. (1990) Identification of host odour attractants for tsetse flies: final report 1986–1990. Natural Resources Institute, Chatham, UK. 130 pp.

Hargrove, J.W. & Brady, J. (1992) Activity rhythms of tsetse flies (*Glossina* spp.) Diptera: Glossinidae) at low and high temperatures in nature. *Bulletin of Entomological Research* **82**, 321–326.

Hargrove, J.W & Langley, P.A. (1990) Sterilizing tsetse in the field – a successful trial. *Bulletin of Entomological Research* **80**, 397–403.

Holloway, M.T.P. & Phelps, R.J. (1991) The responses of *Stomoxys* spp. (Diptera: Muscidae) to traps and artificial host odours in the field. *Bulletin of Entomological Research* **81**, 51–55.

Mérot, P. & Filledier, J. (1991) Resultats obtenus au Burkina Faso sur la recherche d'attractifs olfactifs pour *Glossina tachinoides*. In "Twentieth Meeting of the International Scientific Council for Trypanosomiasis Research and Control, Mombasa, Kenya, 1989". pp. 423–424. OAU/STRC, Nairobi.

O'Neill, W.P. (1980) Membrane systems." pp. 129–182 *in* Kydonieus, A.F. (*Ed.*) *Controlled release technology: methods, theory and applications*. Boca Raton, Florida, CRC Press.

Owaga, M.L.A., Hassanali, A. & McDowell, P.G. (1988) The role of 4-cresol and 3-n-propylphenol in the attraction of tsetse flies to buffalo urine. *Insect Science and its Application* **9**, 95–100.

Torr, S.J. (1990) Dose responses of tsetse flies (*Glossina*) to carbon dioxide, acetone and octenol in the field. *Physiological Entomology* **15**, 93–103.

Torr, S.J., Hall, D.R. & Smith, J.L. (1995) Responses of tsetse flies (Diptera: Glossinidae) to natural and synthetic ox odours. *Bulletin of Entomological Research* **85**, 157–166.

Torr, S.J., Mangwiro, T.N.C. & Hall, D.R. (1996) Responses of tsetse flies (Diptera: Glossinidae) to synthetic repellents in the field. *Bulletin of Entomological Research* **86**. 609–616.

Vale, G.A. (1991) Responses of tsetse flies (Diptera: Glossinidae) to odour-baited trees. *Bulletin of Entomological Research* **81**, 323–331.

Vale, G.A. (1993a) Development of baits for tsetse flies (Diptera: Glossinidae) in Zimbabwe. *Journal of Medical Entomology* **30**, 831–842.

Vale, G.A. (1993b) Visual responses of tsetse (Diptera: Glossinidae) to odour-baited targets. *Bulletin of Entomological Research* **83**, 277–289.

Vale, G.A. & Hall, D.R. (1985a) The role of 1-octen-3-ol, acetone and carbon dioxide in the attraction of tsetse flies, *Glossina* spp. (Diptera: Glossinidae), to ox odour. *Bulletin of Entomological Research* **75**, 209–217.

Vale, G.A. & Hall, D.R. (1985b) The use of 1-octen-3-ol, acetone and carbon dioxide to improve baits for tsetse flies, *Glossina* spp. (Diptera: Glossinidae) *Bulletin of Entomological Research* **75**. 219–231.

Vale, G.A. & Hargrove, J.W. (1979) A method for studying the efficiency of traps for tsetse flies (Diptera: Glossinidae) and other insects. *Bulletin of Entomological Research* **69**, 183–193.

Vale, G.A., Hargrove, J.W., Cockbill, G.F. & Phelps, R.J. (1986) Field trials of baits to control populations of *G. morsitans morsitans* Westwood and *G. pallidipes* Austen (Diptera: Glossinidae) *Bulletin of Entomological Research* **76**, 179–193.

Vale, G.A., Hall, D.R. & Gough, A.J.E. (1988) The olfactory responses of tsetse flies, *Glossina* spp. (Diptera: Glossinidae), to phenols and urine in the field. *Bulletin of Entomological Research* **78**, 293–300.

Willemse, L. (1991) A trial of odour baited targets to control the tsetse fly, *Glossina. morsitans centralis* (Diptera: Glossinidae) in west Zambia. *Bulletin of Entomological Research* **81**, 351–357.

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