

FINAL TECHNICAL REPORT

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IDENTIFICATION OF HOST ODOUR ATTRACTANTS FOR TSETSE FLIES

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EXECUTIVE SUMMARY

Tsetse flies, *Glossina* spp. (Diptera: Glossinidae), inhabit over 11 million square kilometres of Africa and are vectors of trypanosomes which cause sleeping sickness in man and a similar disease, nagana, in domestic livestock. The presence of trypanosomiasis is a major constraint on agricultural production.

Trypanosomiasis is controlled by drugs or by breaking the transmission cycle by control of the vector tsetse fly. During the last twentyfive years, considerable progress has been made in developing methods for control of tsetse based on attracting the flies to devices which trap or kill them, and NRI scientists have played a major part in identifying the chemicals produced by host animals that attract tsetse flies and in devising slow-release dispensers for these attractants.

This project aimed to capitalise on this earlier work and to provide significant improvements in this method of tsetse control by identifying new attractants and improving dispensing systems, contributing to the Purpose of developing and promoting sustainable and cost-effective strategies for the control of trypanosomiasis in Zimbabwe and tsetse-infested countries of sub-Saharan Africa.

The project was carried out in close collaboration with scientists of the Zimbabwe Department of Veterinary Services (DVS), the EU Regional Tsetse and Trypanosomiasis Control Programme for Malawi, Mozambique, Zambia and Zimbabwe (RTTCP) and the ODA TC-funded Insect Pest Management Initiative (IPMI) in Zimbabwe which were the main uptake pathways for the project outputs.

Research activities on identification of attractants included development of new methods for collection and assay of volatiles from host animals, analysis of volatile collections by gas chromatography linked to electroantennography to detect and identify olfactory stimulants and assistance with field trials of natural and synthetic attractants in Zimbabwe. Optimisation of dispensing systems involved laboratory studies under controlled conditions of sealed polythene bottles and sachets for dispensing attractants.

No significant new attractants for tsetse were identified during this project in collections of volatiles from host animals or in carbolineum which was reported to be attractive to tsetse by collaborators in Zimbabwe. However, methods for collecting and assaying known and candidate attractants in the field were developed and experiments were carried out that conclusively demonstrated the existence of unidentified attractant(s) in ox odour and characterised this as very volatile, trapped but not very effectively on activated charcoal and not affected by sodalime.

Work on previously identified repellents for tsetse was completed, and, although they were shown to be genuine repellents effective in the absence of an attractive odour and to cause a dramatic reduction in catches of tsetse in traps, it was concluded that they are not sufficiently potent to reduce infection of animals with trypanosomiasis in practice.

Laboratory work on sealed polythene dispensers for tsetse attractants was completed and a definitive publication describing their characteristics and use was produced with RTTCP and IPMI collaborators.

As a result of this project, work on new attractants for tsetse carried out under RTTCP and IPMI projects has focussed on the very volatile components of ox odour. Similarly, work is in progress to identify the tsetse repellents previously shown to be present in human odour as these should be effective in reducing the tsetse challenge to animals in conditions of low fly density and/or infection rate. The outputs of this project also provide a basis for studying the effects of host physiology on attractiveness to tsetse which will contribute towards understanding of the epidemiology of trypanosomiasis and rationalisation of the use of insecticide-impregnated cattle for tsetse control. Sealed polythene dispensers are now used for tsetse attractants in many African countries.

BACKGROUND

Tsetse flies, *Glossina* spp. (Diptera: Glossinidae), inhabit over 11 million square kilometres of Africa and are vectors of trypanosomes which cause sleeping sickness in man and a similar disease, nagana, in domestic livestock. The presence of trypanosomiasis is a major constraint on agricultural production.

Trypanosomiasis is controlled by drugs or by breaking the transmission cycle by control of the vector tsetse fly. Drugs are expensive and their effective administration requires an infrastructure which is often not present in Africa. No vaccine is yet available, and its use would presumably suffer the same problems as drug administration in Africa.

Many methods have been used to control or eradicate tsetse fly. In the past, these have included clearance of host game animals and/or bush habitat, but these methods are now environmentally unacceptable and unworkable. Control of tsetse by release of sterile males has been attempted, but this technique requires sophisticated equipment which is difficult to maintain in most African countries. This technique is also not particularly appropriate for tsetse because the slow rate of reproduction of the flies makes it difficult to rear the vast numbers required.

Control of tsetse with insecticides has involved ground spraying of tsetse resting sites with persistent insecticides or aerial spraying by the sequential aerosol technique (Allsopp, 1984). The former requires highly-developed logistical organisation and has generally used persistent insecticides which are now environmentally unacceptable. The latter technique uses non-persistent insecticides which have been shown to be highly selective for tsetse. It is extremely effective in flat terrain, but requires sophisticated equipment and is less effective in rugged terrain.

During the last twentyfive years, considerable progress has been made in developing methods for control of tsetse based on attracting the flies to devices which trap or kill them (Green, 1994). This approach is particularly suitable for tsetse because of their very slow rate of reproduction compared with those of most insect species, and because of their dependence on finding host animals for regular blood meals. Furthermore, the technology for manufacture

and deployment of simple trapping devices is appropriate for African countries, and any insecticides are used in very small quantities localised on man-made devices.

Riverine species of tsetse can be effectively trapped with devices that employ only visual cues. However, savannah species rely more on olfactory cues, and use of trapping techniques with these species has required development of effective odour attractants.

NRI scientists have played a major part in identifying the chemicals produced by host animals that attract tsetse flies and devising slow-release dispensers for these attractants, particularly for the species present in Zimbabwe, *Glossina pallidipes* and *G. m. morsitans* (Hall *et al.*, 1984; Bursell *et al.*, 1988; Vale *et al.*, 1988; Hall, 1990a; Hall, 1990b; Hargrove *et al.*, 1995). Chemicals identified in this work have been used by other workers to develop attractive blends for other species of tsetse, e.g. *G. longipalpis* (Jaenson *et al.*, 1991) and *G. tachinoides* (Filleard & Mérot, 1989). The chemicals and dispensers developed so far are used to bait traps and cloth targets impregnated with insecticide and these now provide the basis for the preferred methods of tsetse control in several African countries - e.g. Zimbabwe, Malawi, Zambia, Kenya - with over 75,000 devices currently deployed in Zimbabwe (e.g. Vale *et al.*, 1988b; Dransfield *et al.*, 1990; Willemsse, 1991).

This project aimed to capitalise on this earlier work and to contribute significant improvements in this method of tsetse control by identifying new attractants and improving dispensing systems. Such developments would increase the cost-effectiveness of the method by reducing the number of devices required to be set up and maintained, increasing the rate at which control is achieved and/or increasing the service intervals for the devices (Barrett, 1994).

The project was carried out in close collaboration with scientists of the Zimbabwe Department of Veterinary Services (DVS), the EU Regional Tsetse and Trypanosomiasis Control Programme for Malawi, Mozambique, Zambia and Zimbabwe (RTTCP) and the ODA TC-funded Insect Pest Management Initiative (IPMI) in Zimbabwe which were the main uptake pathways for the project outputs.

PROJECT PURPOSE

The Purpose of this project was to develop and promote sustainable and cost-effective strategies for the control of trypanosomiasis in Zimbabwe and tsetse-infested countries of sub-Saharan Africa by improving the cost-effectiveness of traps and targets used for monitoring and control of tsetse.

RESEARCH ACTIVITIES

Project activities were carried out in the laboratory at NRI and in the field in Zimbabwe. Laboratory work involved development of methods for trapping volatiles from host animals, analysis of these by gas chromatography linked to electroantennography (GC-EAG) to detect and identify compounds that stimulate olfactory receptors on the tsetse antennae and are thus potential attractants or repellents, and studies of dispensing systems under controlled conditions. Some laboratory work on tsetse attractants was funded by the RTTCP, and this covered most of the GC-EAG work. Field work was carried out in collaboration with

scientists of the Zimbabwe DVS, the RTTCP and the IPMI project in Zimbabwe, and involved provision of chemical inputs for collection of host odours and testing of behavioural responses to natural and synthetic odours.

Most of the work carried out under this project is described in three papers co-authored with collaborating scientists (Torr *et al.*, 1995; Torr *et al.*, 1996a; Torr *et al.*, 1996b), and these are included as Appendices 1-3.

Identification of new attractants

Collection and assay of volatiles

Host animals were maintained in underground pits in Zimbabwe and volatiles were exhausted from the pit with a fan *via* a ventilation shaft to the base of a device for catching tsetse flies, either a trap or an electrified grid. Methods were developed for removing organic volatile component from the pit effluent with an activated charcoal filter and for removing carbon dioxide and other acidic compounds with a sodalime filter (Torr *et al.*, 1995).

Methods were developed for sampling the volatiles such that sampling could be carried out at the same time as the odours were assayed for attractiveness to tsetse (Torr *et al.*, 1995). Volatiles were also collected from a calf at the School of Veterinary Medicine, University of Bristol, and analysed but this was not found to be satisfactory as amounts of volatiles were much lower due to the lower temperatures, and it was not possible to measure the attractiveness of these volatiles to tsetse.

- (a) Carbon dioxide production was measured with an infra-red gas analyser attached to a logger (Torr *et al.*, 1995).
- (b) Phenols, 1-octen-3-ol (octenol) and compounds of similar volatility were trapped on Porapak filters. These were returned to NRI, extracted with dichloromethane and the volatiles analysed quantitatively by GC, GC-EAG and/or GC linked to mass spectrometry (GC-MS) (Torr *et al.*, 1995).
- (c) More volatile compounds, particularly acetone and butanone were trapped on filters packed with carbonised molecular sieves, extracted with propyl acetate containing 1% N,N'-dimethylformamide and analysed quantitatively by GC (Torr *et al.*, 1995).
- (d) Towards the end of this project, a specific method for trapping and analysing carbonyl compounds, e.g. acetone and butanone, was developed involving trapping on commercially-available SepPak filters containing silica gel impregnated with 2,4-dinitrophenylhydrazine, eluting dinitrophenylhydrazones with acetonitrile and assaying these quantitatively by high performance liquid chromatography with diode array detection.
- (e) Also towards the end of this project, equipment was commissioned for collection of highly volatile materials on Tenax or activated charcoal and analysis of these by GC-MS after thermal desorption.

Gas chromatography linked to electroantennography (GC-EAG)

Collections of volatiles or other attractant sources were analysed by capillary GC-EAG in which the effluent from the GC is split so that half passes through the normal flame ionisation detector and half passes over a tsetse fly antenna set up for electroantennographic recording of the depolarisation that occurs across the antenna when olfactory receptors on the antenna are stimulated, as described by Cork *et al.* (1990). Tsetse flies, *G. morsitans morsitans*, from a colony maintained at the Tsetse Research Laboratory, Bristol, were used throughout.

Field evaluation of natural and synthetic attractants and repellents

The behavioural activity of natural and synthetic odours were assayed in Zimbabwe with traps and/or electrified grids as described in Torr *et al.* (1995) and Torr *et al.* (1996a)

Optimisation of dispensing systems

Dispensing systems for tsetse attractants based on sealed polythene bottles or sachets were developed and characterised. The particular contributions of this project involved studies of release rates under controlled conditions in a laboratory windtunnel, and the determination of release rates for the individual components of blends using methods for trapping volatiles described above (Torr *et al.*, 1996b).

OUTPUTS

Identification of new attractants

GC-EAG analyses of cattle volatiles collected on Porapak

Detailed GC-EAG analyses of ox volatiles collected on Porapak failed to reveal any consistent, significant olfactory stimulants for *G. m. morsitans* other than those identified previously, 1-octen-3-ol (octenol) (Hall *et al.*, 1984) and eight phenols found in cattle urine (Hassanali *et al.*, 1986; Bursell *et al.*, 1988).

In some analyses, responses were detected to components subsequently identified as pentadecane and naphthalene by GC-MS and comparison of GC retention times with those of authentic standards. These were tested in the field in Zimbabwe at various doses but failed to show any behavioural effects on *G. m. morsitans* or *G. pallidipes*. Subsequently it has been found that pentadecane is an impurity from the Porapak and naphthalene is probably an impurity in the dichloromethane. However, pentadecane has recently been reported to be an oviposition attractant for *G. m. morsitans* (Saini, ICIPE, *pers comm*).

GC-EAG analyses of carbolineum

The paraffin fraction of carbolineum was reported to be attractive to tsetse by collaborators in Zimbabwe. This was analysed by GC-EAG, but no strong olfactory stimulants were found. In subsequent experiments, the attractiveness of this fraction could not be confirmed and laboratory work was discontinued.

Rates of production of known attractants by cattle

Rates of production of tsetse attractants by cattle were measured in Zimbabwe. Initially these measurements concentrated on the compounds known to be attractive - carbon dioxide, acetone, butanone, octenol, phenol, 3- and 4-methylphenol, 3- and 4-ethylphenol and 3- and 4 propylphenol (Torr *et al.*, 1995).

Subsequently measurements were made for 41 compounds, including the above 11, that have been implicated in attraction of tsetse for various reasons over the years. These are shown in Table 1.

Field evaluation of natural and synthetic attractants

Comparisons were made of the attractiveness to tsetse of synthetic blends of known attractants dispensed at the natural release rates from an ox, and natural ox odour. The effects of removing certain known attractants from the natural odour were also evaluated. Results described in Torr *et al.* (1995) are summarised as follows:

- (a) Natural ox odour caught twice as many *G. pallidipes* and 1.5 times as many *G. m. morsitans* as a synthetic blend of carbon dioxide, acetone, butanone, octenol, 4 methylphenol and 3-propylphenol released at the same rates as naturally produced by an ox.
- (b) Addition of high doses of the phenols to either natural ox odour or the synthetic blend did not significantly affect catches of tsetse by either; addition of high doses of octenol to either natural ox odour or the synthetic blend significantly increased catches by both but natural odour still caught significantly more than the synthetic; addition of high doses of acetone and butanone had no effect when added to natural ox odour but significantly increased the catch by the synthetic.
- (c) When ox odour was passed through filters of fresh activated charcoal and sodalime, catches of tsetse were not significantly different from catches with no odour; catches with ox odour passed through the charcoal filter only were similar to those with carbon dioxide alone; catches with ox odour passed through a fresh charcoal filter were consistently lower than catches with a charcoal filter used for greater than six hours.
- (d) The above filtering experiments were repeated with high doses of synthetic acetone, octenol and the phenols added after the filters. The charcoal filter still reduced catches of tsetse by the natural ox odour and the passing the natural odour through a sodalime filter only reduced catches to a level that was still significantly higher than that with the known synthetic attractants alone.

These results showed that the synthetic blend of known attractants is significantly less attractive to tsetse than natural ox odour, indicating that ox odour contains additional, unidentified attractant(s). Removal of organic volatiles and carbon dioxide from natural ox odour with fresh charcoal and sodalime filters respectively removes all attractiveness to tsetse,

both known and unknown attractants. However, when acetone, octenol and the phenols are added to ox odour passed through the sodalime, the catch is still significantly higher than with acetone, octenol and phenols alone, indicating that the unknown attractant(s) pass through the sodalime filter. Furthermore, a charcoal filter used for more than six hours is less effective at trapping the unknown attractant(s) even though it still traps octenol and the phenols, suggesting that the unidentified attractant(s) is highly volatile, at least as volatile as acetone which was measured to break through the charcoal filter in 25-30 hours.

One anomalous result in (b) above is that addition of high levels of acetone and butanone gives a greater increase in attractiveness with the synthetic blend than with natural ox odour, suggesting that the levels of these ketones in the synthetic blend were too low compared with those in the natural odour. This led to development of an alternative method for assaying levels of acetone and butanone by trapping as the 2,4-dinitrophenylhydrazone derivatives. Measurements with this method confirmed previous figures of approximately 10 mg/hr/ox for acetone and < 1 mg/hr/ox for butanone, confirming that the unidentified attractant(s) is not acetone and that acetone must be able to mimic its effect to a certain extent.

In the above experiments, the synthetic blend contained only the compounds found in ox odour known to be attractive to tsetse - carbon dioxide, acetone, butanone, octenol, 4 methylphenol and 3 propylphenol. Subsequently the comparison was repeated using a blend of 41 compounds all of which have been implicated in attraction of tsetse for some reason in the past, released at levels comparable to those observed in natural ox odour (Table 1). Most of these compounds could not be shown to have any behavioural effect in the field, but it was thought that synergistic interactions might occur when they were all blended together. The results (Fig 1) confirmed that even this blend ("total") was still significantly less attractive than natural ox odour, and little different from the attractiveness of the blend of known attractants dispensed at rates found in natural ox odour ("standard"). Release rates of the components were confirmed to be as predicted by measurements made during these experiments.

Table 1. Composition and release rates in “total” and “standard” blends

Compound	Rel amount at source		release rate (mg/hr/ox)		
	Total	Standard	Total	Standard	ox
carbon dioxide (l/min)			2.0	2.0	2.1
acetone			5.3	13.6	17.5
butanone			15.8	6.7	0.4
octenol	1	1	0.03	0.03	0.02
phenol	10		0.03		0.03
4-methylphenol	100	100	0.43	0.37	0.34
3-methylphenol	10		0.04		0.08
4-ethylphenol	5		0.04		0.02
3-ethylphenol	2.5		0.01		0.002
4-propylphenol	1		0.01		0.02
3-propylphenol	3	3	0.02	0.02	0.03
2-methoxyphenol	0.2		<0.002		<0.002
indole	0.2		0.002		0.002
3-methylindole	0.04		<0.002		<0.002
vanillin	1		0.003		0.003
dimethylsulphone	1		0.01		0.02
naphthalene	1		0.12		0.04
2-phenylethanol	1		<0.002		<0.002
phytol	1		<0.002		<0.002
Peaks6/7	1		<0.002		<0.002
carboxylic acids					
(approx 0.002 mg/hr)					
acetic	3-methylbutyric	nonanoic	benzoic		
propionic	hexanoic	decanoic	phenylacetic		
butyric	heptanoic	dodecanoic	3-phenylpropanoic		
pentanoic	octanoic	tetradecanoic	lactic		

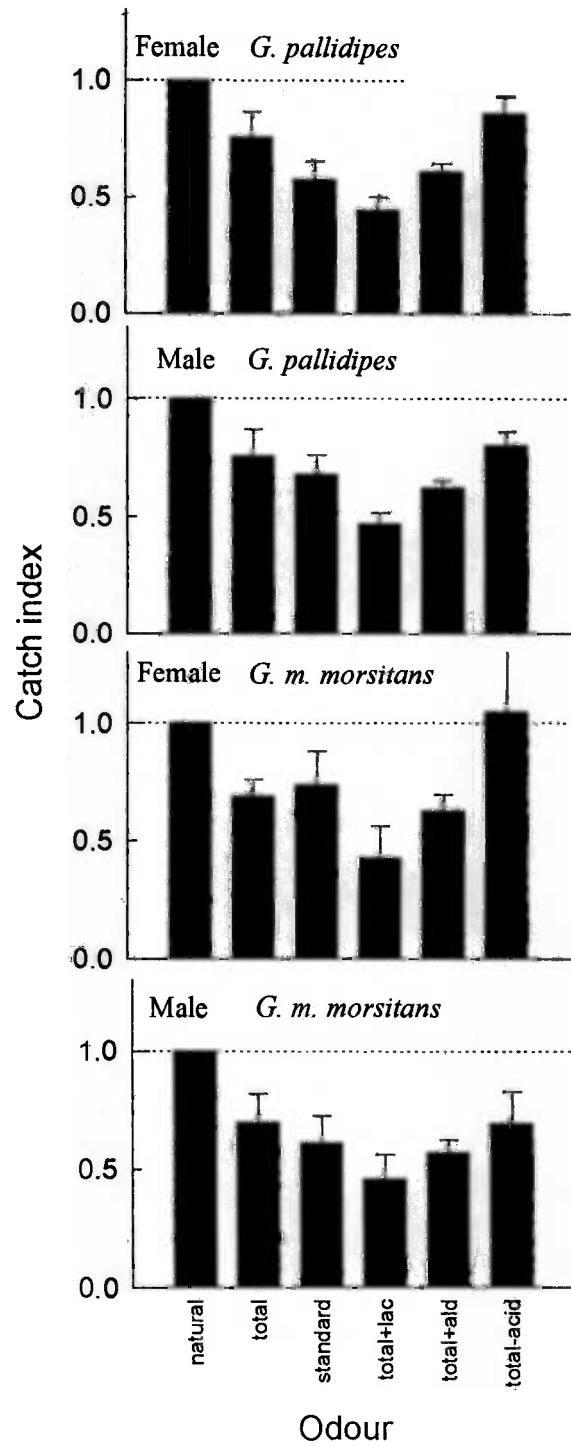


Fig 1. Catch indices for *G. pallidipes* and *G. m. morsitans* with natural and synthetic odours, Rekomitjie April-May 1994.

Repellents for tsetse

During previous work on tsetse attractants, acetophenone, 2-methoxyphenol and various carboxylic acids were found to reduce the catches of tsetse in odour-baited traps. It was thought that these cheap, readily available compounds might be used for reducing the tsetse challenge on animals as one component of an integrated strategy for controlling tsetse and trypanosomiasis rather than eradicating the vector and disease. During this project, support was given to Dr Torr and Mr Mangwiro of the ODA IPMI project to complete this work, and the work was written up (Torr *et al.*, 1996a).

The main findings were as follows.

- (a) Acetophenone, 2-methoxyphenol and hexanoic acid reduced catches of tsetse in traps baited with acetone, octenol and phenols by up to 86%. Use of blends of all three chemicals did not reduce the catch further than with 2-methoxyphenol alone.
- (b) These compounds reduced the catches of tsetse in unbaited traps by up to 75%.
- (c) Acetophenone and pentanoic acid halved the efficiency of traps, but 2-methoxyphenol had no significant effect.
- (d) Acetophenone and 2-methoxyphenol halved the numbers of tsetse attracted to an odour-baited target, but none of the compounds tested had a significant effect on the numbers landing.
- (e) 2-Methoxyphenol halved the numbers of tsetse attracted to an ox, but acetophenone and pentanoic acid had no significant effect. Of these compounds only pentanoic acid significantly affected the rate of feeding of attracted flies.

It was concluded that these repellents are not sufficiently effective to give significant protection to animals against infection with trypanosomiasis. A model was constructed relating density and infection levels of the flies to the probability of a host animal being infected. This predicted that at low densities and/or infection rates of flies, as easily obtained by target control operations, more powerful repellents could greatly reduce the risk of infection.

It should be noted that, to the best of our knowledge, the compounds studied here are the first examples of true insect repellents ever reported. Most other related work has been on "repellents" for mosquitoes where the effect is to reduce the attractiveness of a source of attractive odour - i.e. a human host. These tsetse repellents are true repellents active in the absence of attractive odours.

Optimisation of dispensing systems

Previous work by RTTCP and NRI scientists established sealed polythene bottles and sachets as cost-effective, practical slow-release dispensers for tsetse attractants in the field. In this project, laboratory work on characterisation of these dispensers was completed and combined with field data collected by RTTCP and IPMI collaborators in a comprehensive publication describing the use of these dispensers (Torr *et al.*, 1996b).

The main findings of the laboratory work were as follows.

- (a) Release rates of butanone, octenol and phenols from these sealed polythene dispensers were independent of the amount present, giving uniform release rates until the contents are exhausted.
- (b) Release rates were related directly to the surface area, inversely to the thickness of polythene and increase exponentially with temperature, although for blends of components these factors did not significantly effect the relative amounts of the different components released.
- (c) In blends of octenol, 4-methylphenol and 3-propylphenol, release rates of the two phenols present were linearly dependant upon the concentration present, but octenol showed an exponential dependence upon concentration.
- (d) Actual release rates of the individual components from eight blends of octenol, 4-methylphenol and 3-propylphenol were measured and followed for 218 days.
- (e) Mixture of volatile components with an involatile diluent was shown to be an effective alternative method for controlling release rate of the volatile component, although the relationship between release rate and concentration was not linear.

These data were combined with those from RTTCP and IPMI collaborators on performance of these dispensers in the field to produce a definitive publication on these dispensers which included a model for predicting the persistence of dispensers under different temperature regimes in shade or sunlight.

CONTRIBUTION OF OUTPUTS

Identification of new attractants

Identification of attractants

Although no significant new attractants were identified during this project, the presence of unidentified attractant(s) in natural ox odour that could double the catches of tsetse was established. Although the attractiveness of natural ox odour can be matched by increasing the release rates of the known attractants over those naturally produced, the scope for this is limited whereas with natural ox odour the attractiveness to tsetse increases with dose to very

high levels (Hargrove *et al.*, 1995). Although the cost of these unidentified attractant(s) is obviously not known, in principle their identification could increase the effectiveness of the method by reducing the number of devices required to be set up and maintained, increasing the rate at which control is achieved and/or increasing the service intervals for the devices (Barrett, 1994).

Although not identified, the unidentified attractant(s) was characterised as being very volatile, trapped but not very effectively on a charcoal filter and not affected by a sodalime filter (Torr *et al.*, 1995). These results have focussed efforts on collection, analysis and identification of the more volatile components of ox odour in continuation of this work funded by the RTTCP and the ODA IPMI project. Five candidate chemicals are being evaluated.

Identification of repellents

Work on previously identified repellents for tsetse was completed and written up (Torr *et al.*, 1996a). Although it was concluded that these particular compounds would not in fact provide significant protection to animals against infection with trypanosomiasis, the principle of using repellents was established, and these results have focussed subsequent work funded by the RTTCP and ODA IPMI on the powerful repellents for *G. pallidipes* and *G. m. morsitans* shown to be present in human odour.

Effects of host physiology on attractiveness to tsetse

During this project, methods have been developed for measuring rates of production of tsetse attractants and repellents by host animals, and baseline data has been obtained for experimental animals. Resulting from this, a proposal for a new project has been submitted to the ODA Livestock Production and Animal Health Programmes which will investigate the effects of host physiology - age, sex, condition, infection with trypanosomiasis, etc. - on attractiveness to tsetse, and attempt to correlate the results with changes in production of attractants and/or repellents and in behaviour. Results from these studies will be of value in modelling the epidemiology of trypanosomiasis and in rationalising the use of insecticide-treated cattle for tsetse control. This approach is becoming increasingly popular in many countries of Africa, but at present is applied in a purely empirical manner with little knowledge of factors which influence its success or failure. This project will complement work being carried out by RTTCP scientists and contribute towards developing models for this approach.

Optimisation of dispensing systems

Sealed polythene dispensers for butanone, octenol and phenols were developed previously by RTTCP and NRI scientists, and this project has contributed towards a definitive publication describing their characteristics and use.

The polythene sachets are widely used as dispensing systems for tsetse attractants and are simple to make and use in the field. Sachets for use in Zimbabwe and Zambia Western Province are prepared at the Zimbabwe DVS. They are commercially available from Appropriate Applications in the UK, and NRI has also provided sachets and/or information on their production for research and operational use against a variety of tsetse species in Kenya (ICIPE, KETRI), Tanzania (MOA), the Gambia (ITC), Ethiopia (ILRI).

Polythene sachets have also been used to dispense attractants for New World screwworm fly, *Cochliomyia hominivorax* (Green *et al.*, 1993) and stable flies (Holloway & Phelps, 1991).

Dissemination

Published papers

This project contributed to four published papers:

TORR, S.J., HALL, D.R. AND SMITH, J.L. (1995). Responses of tsetse flies (Diptera: Glossinidae) to natural and synthetic ox odours. *Bulletin of Entomological Research*, **85**, 157-166.

HARGROVE, J.W., HOLLOWAY, M.T.P., VALE, G.A., GOUGH, A.J.E. AND HALL, D.R. (1995). Catches of tsetse (*Glossina* spp.) (Diptera: Glossinidae) from traps and targets baited with large doses of natural and synthetic host odour. *Bulletin of Entomological Research* **85**, 215-227.

TORR, S.J., MANGWIRO, T.N.C., & HALL, D.R. (1996a). Responses of tsetse flies (Diptera: Glossinidae) to synthetic repellents in the field. *Bulletin of Entomological Research*. in press.

TORR, S.J., HALL, D.R., PHELPS, R.J. AND VALE, G.A. (1996b) Methods for dispensing attractants for tsetse flies (Diptera; Glossinidae). *Bulletin of Entomological Research*. in press.

Other dissemination

CORK, A., BEEVOR, P.S., AND HALL, D.R. (1992) Identification of host odour attractants for tsetse fly and New World Screwworm. Proceedings of XIX International Congress of Entomology, 500e.

HALL, D.R. AND TORR, S.J. (1993) Tsetse Control with Traps and Targets: Recent Research Results. FAO/IAEA International Seminar "Research and Development of Controlled Release Formulations of Pesticides", Vienna, Austria, September 1993.

CORK, A, HALL, D. R., AND TORR, S. J. (1994). Current status of host odour attractants for tsetse. Abstracts of Third International Conference on Tropical Entomology, Satellite meeting on Tsetse Behaviour and Ecology, Mombasa,. Contributory paper CM16.

HALL, D.R. (1995) "Semiochemicals from Animal Hosts", Rank Prize Symposium "New Directions in the Study of Insect Semiochemicals" Grasmere, 15-8 May 1995.

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- 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'Élevage et Médecine vétérinaire des Pays tropicaux*, **42**, 541-544.
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APPENDIX 1

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

Running header: Responses of tsetse to 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.) 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; Willems, 1991).

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 synergises 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 x 1.5 m, was placed 1 m downwind of the odour source. The net was mounted on a corrugated tray coated with polybutene. Flies 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 x 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 dia.) fitted with a 12 V co-axial fan (Vale, 1974a). The pit was cleaned daily to minimise 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 X 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 organised 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 cm²) 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 dia) 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, U.S.A.; 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 carbonised 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 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 x 0.32 mm id) with helium carrier gas (inlet pressure 1 kg/cm²), split injection (20 ml/min split) at 200° C, flame ionisation 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 x 20 mm) containing acetone or butanone and fitted with caps with 1-6mm 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 X sites X treatments. Only one site was used in studies of filtered odour and so treatments were compared using a randomised 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 normalised 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 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 $\pm 10\%$ 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 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 or phenols (table 2). Acetone added to SO increased the catch significantly but had no significant effect when added to ox odour (table 2), and the difference between SO and ox odour was apparently reduced in the presence of high doses of acetone or butanone (fig. 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 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 artificial blend 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-propylphenol (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 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 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 charcoal filter only. The dose of carbon dioxide used was comparable to that produced by the four oxen, which also implies that the sodalime is 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 than 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 old 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 were 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 have 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 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, 1988ab).

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 synergised 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* 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 Einar Bursell whose work contributed to its origins and whose inspiration and enthusiasm provided an example to us all.

Acknowledgements

We thank the staff of Rekomitjie Research Station, in particular Messrs. C. Chipere and P. Mugwamadzi, for field assistance in Zimbabwe, Dr R. D. White and Mr. D. Farman for assistance with analyses carried out at NRI, Drs J. Brady, C. H. Green, J. W. Hargrove, G. A. Vale and M. L. Warnes and Miss Sheila Green for their encouragement and advice, and the Directors of the Tsetse and Trypanosomiasis Control Branch and the Natural Resources Institute for permission to publish. Financial support for the work was provided by the Overseas Development Administration of the United Kingdom.

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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	<i>n</i>	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		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	6	8	1.0	0-8.4	0.18

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	<i>n</i>	<i>P</i>	S.E.	Ox	SO	<i>P</i>	S.E.
<i>G. pallidipes</i>										
	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
<i>G. m. morsitans</i>										
	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/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). See text and Table 1 for composition of SO and ox odour, respectively.

LIST OF FIGURES

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

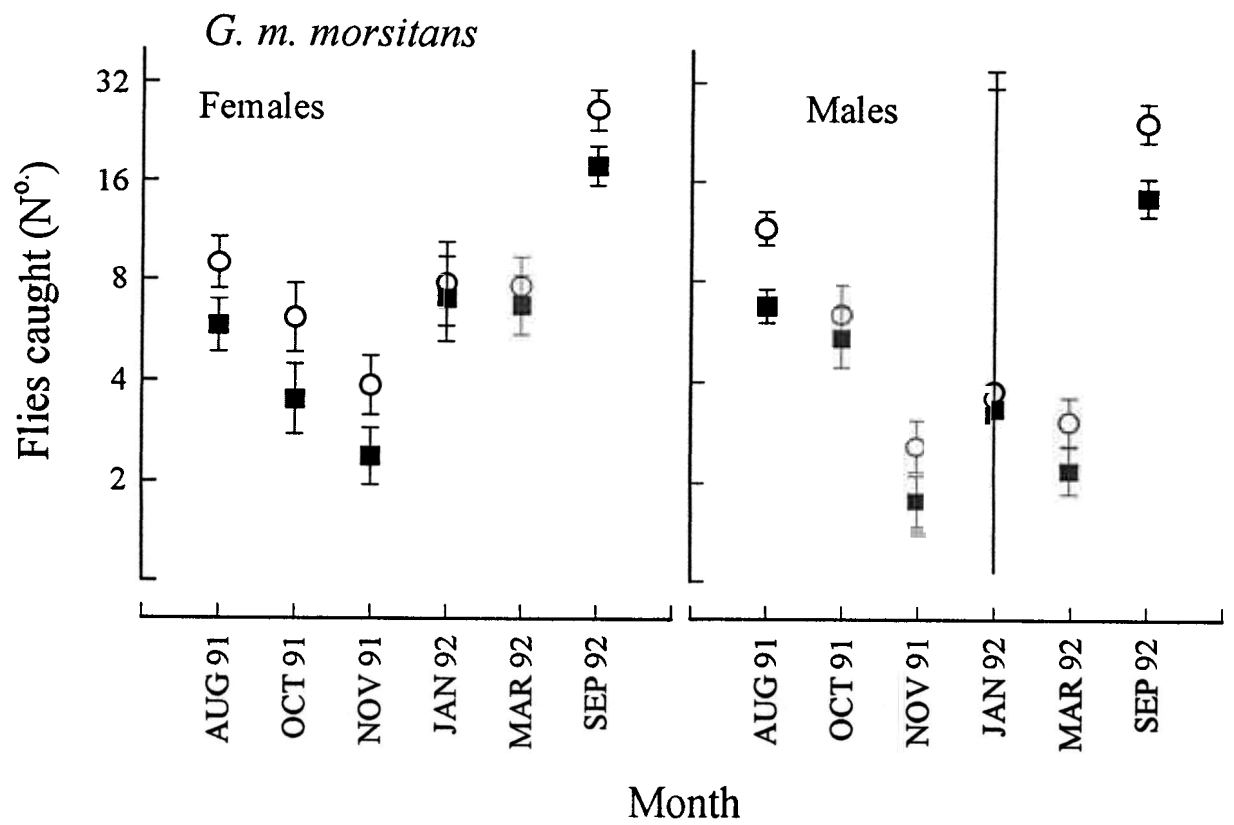
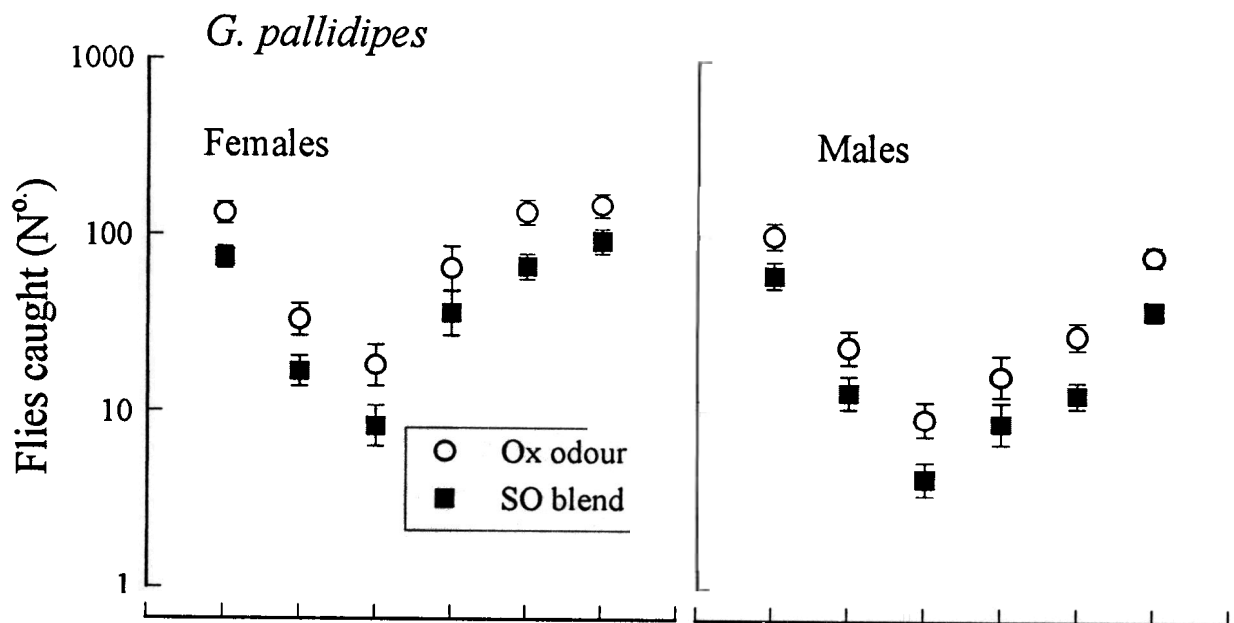
Fig. 2. Catch (\pm S.E.) of male tsetse (solid bars) or female tsetse (open bars) attracted to either ox odour or SO \pm various. For attractants added to ox odour (A.) or SO (B.), the catches are expressed as a proportion of a standard consisting of the catch from a target baited with a single ox or 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).

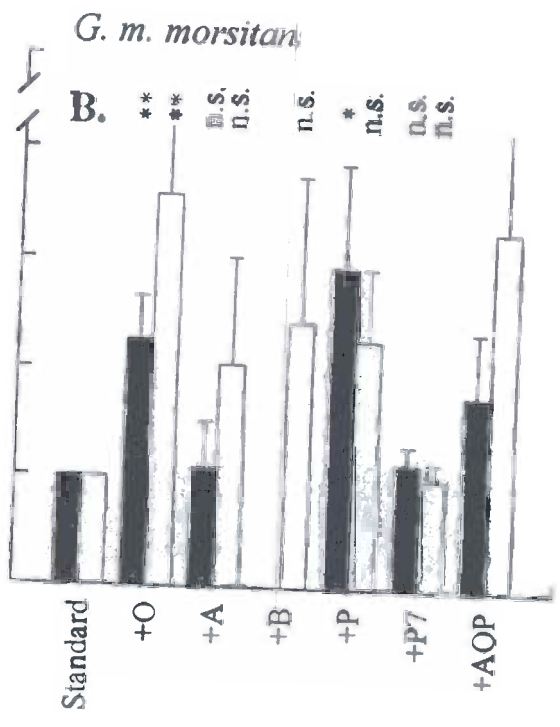
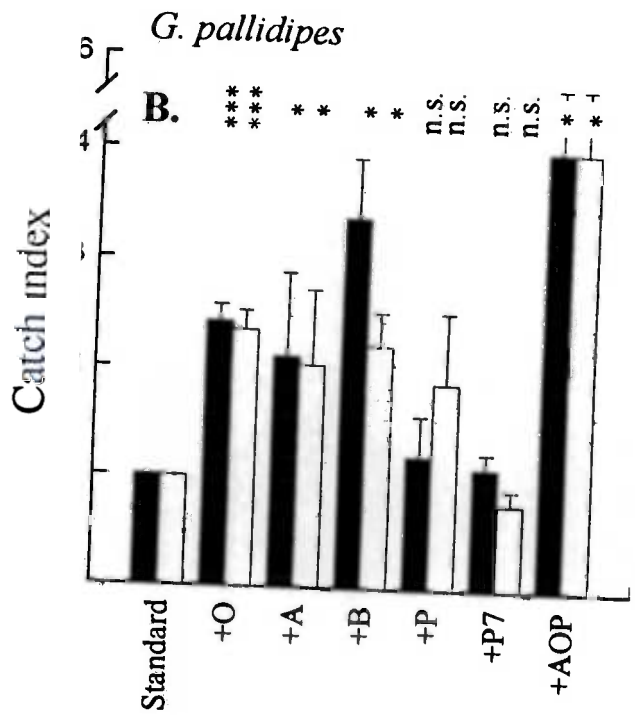
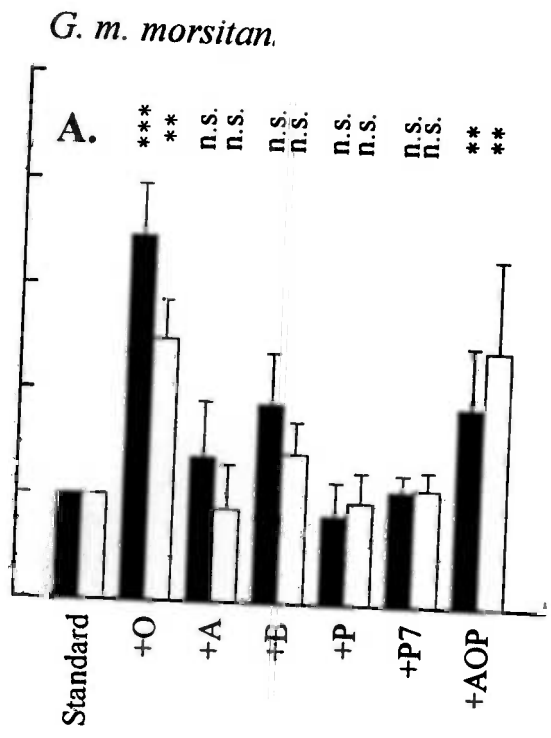
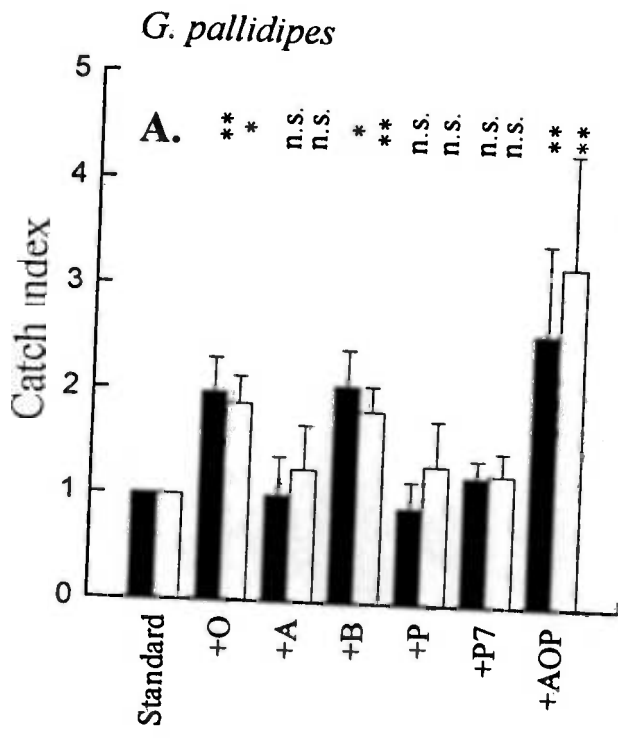
Fig. 3. Mean daily catch of tsetse (\pm S.E.) from a target baited with acetone (500 mg/h), octenol (2 mg/h), 4-methylphenol (4 mg/h), 3-n-propylphenol (0.5 mg/h) plus either natural ox odour or SO. Means are based on six to eight replicates of each treatment.

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.

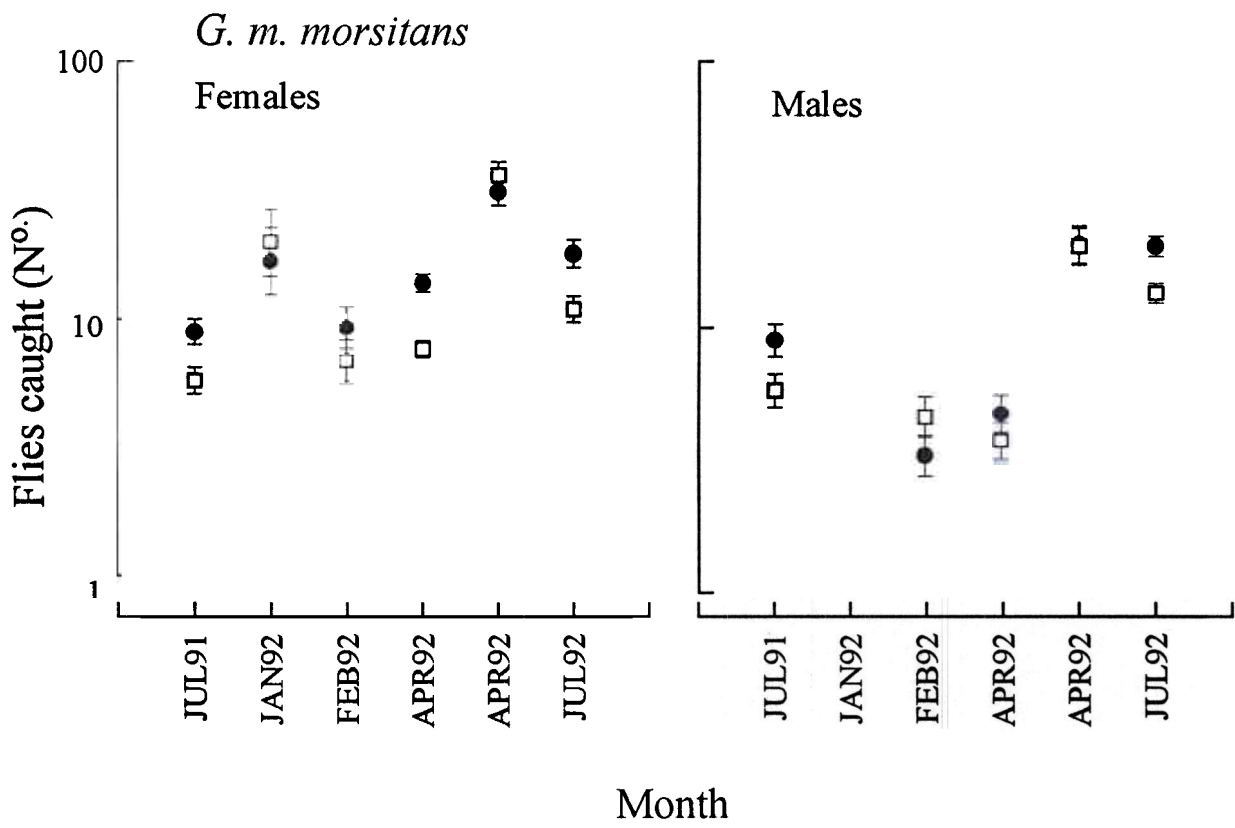
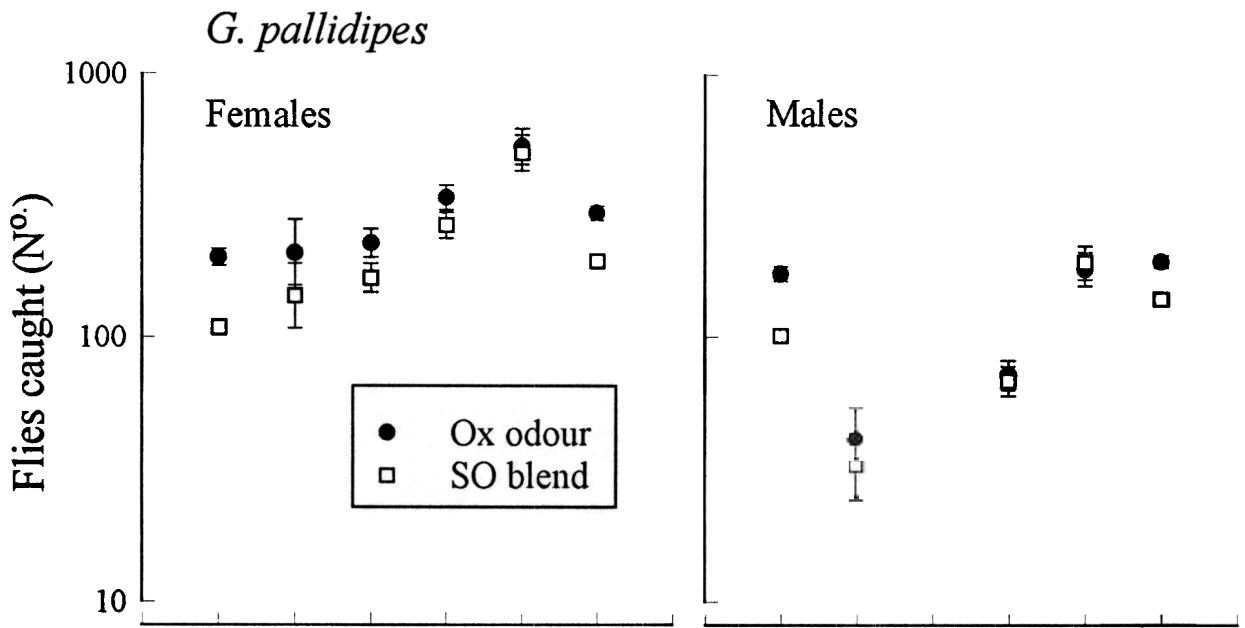
Fig. 5. Mean daily catch (\pm S.E.) of male tsetse (solid bars) or female tsetse (open bars) attracted to a source of odour from a single ox passed through filters of new charcoal (CHAR), old charcoal (CHAR^Ω) or sodalime (SODA) with or without a blend of octenol and phenols (OP) added after the filters. 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 fig. 2.

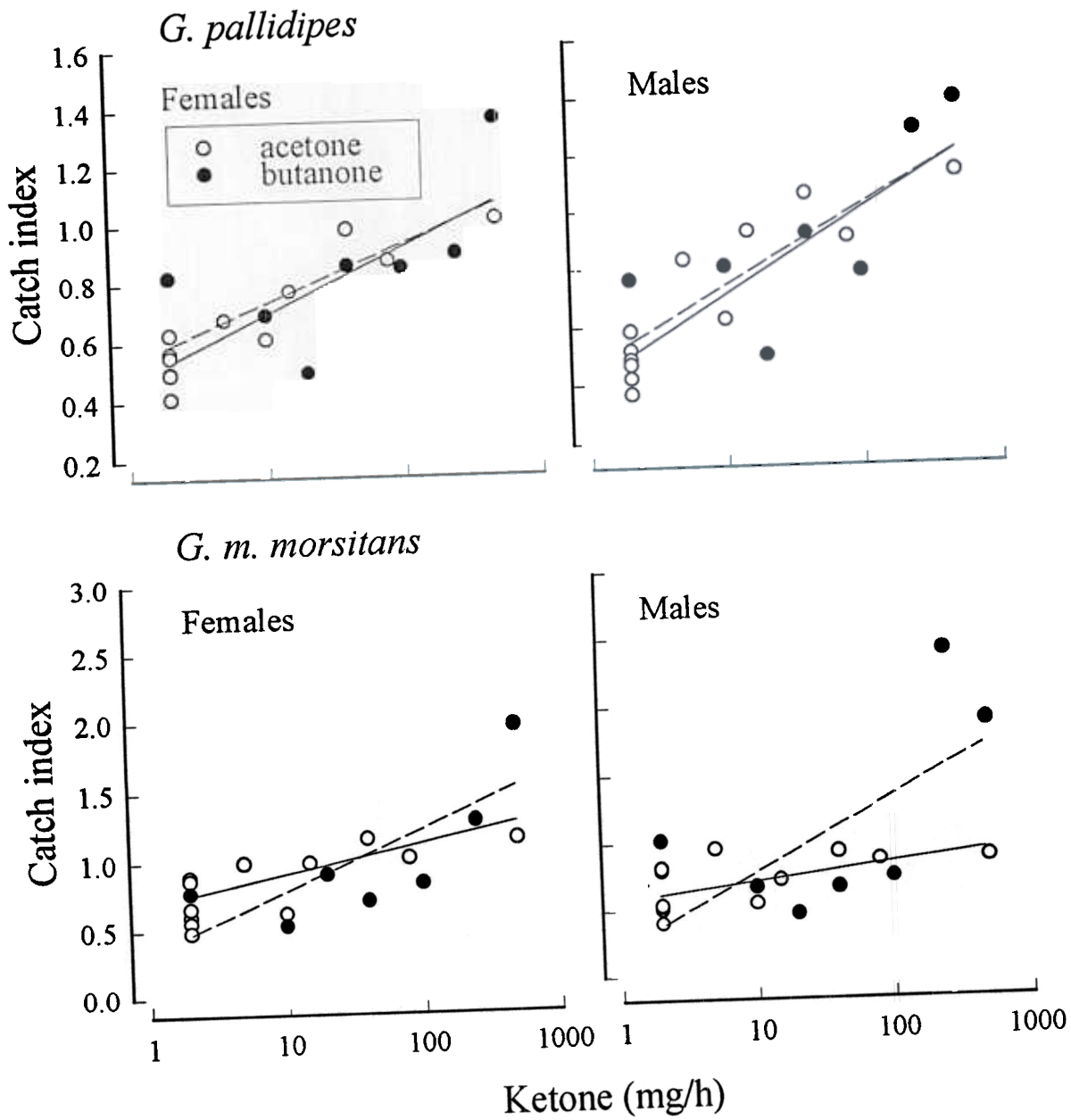
Fig. 6. Mean daily catch (\pm S.E.) of male tsetse (solid bars) or female tsetse (open bars) attracted to a source of acetone (500 mg/h), octenol (4 mg/h), 4-methylphenol (4 mg/h) and 3-n-propylphenol (0.5 mg/h) and the odour from four oxen passed through charcoal (CHAR) or sodalime (SODA) filters with 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 fig. 2.

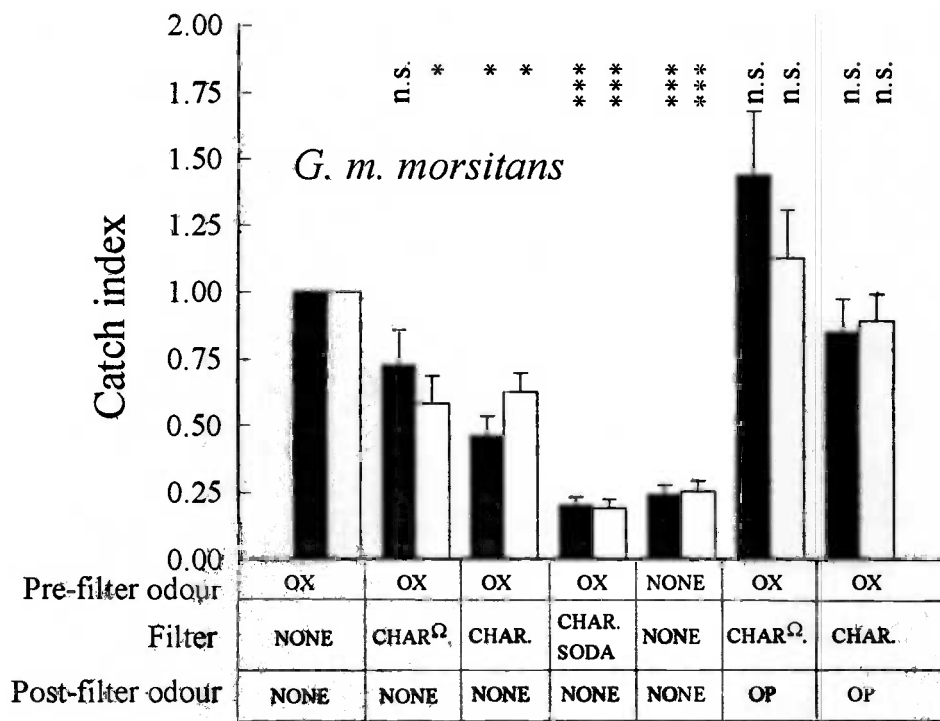
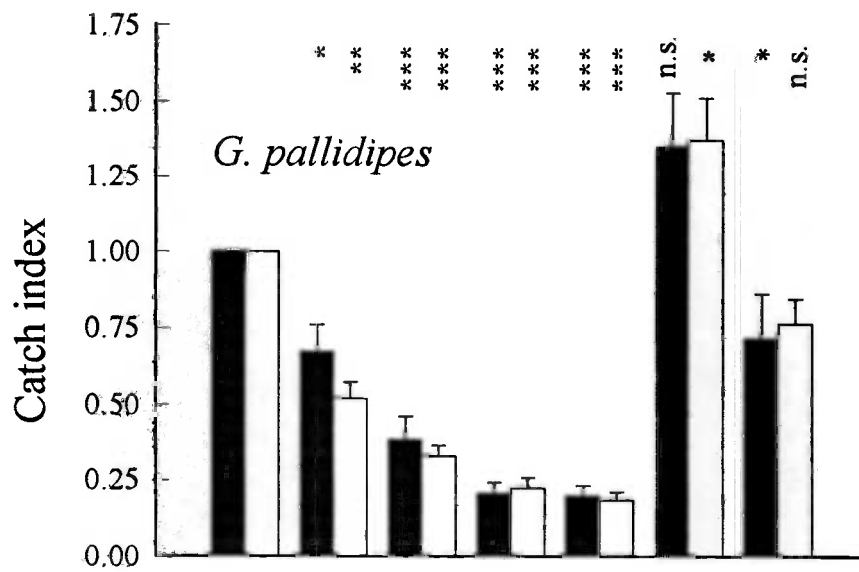




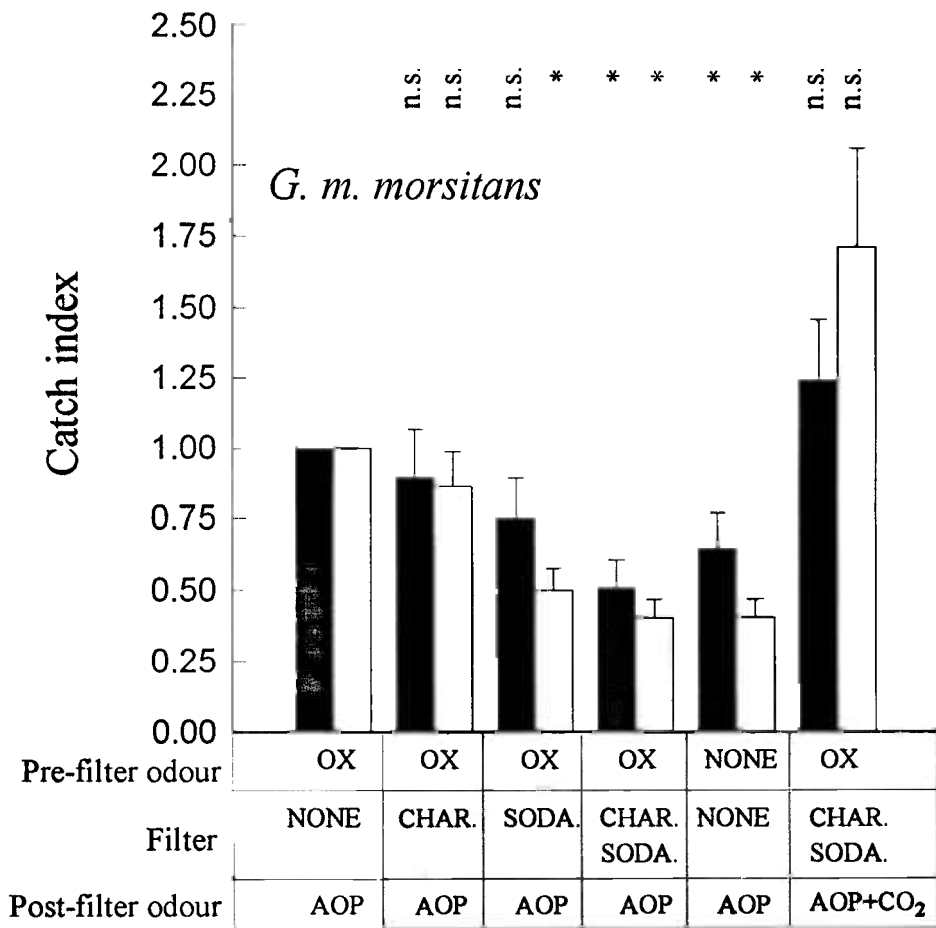
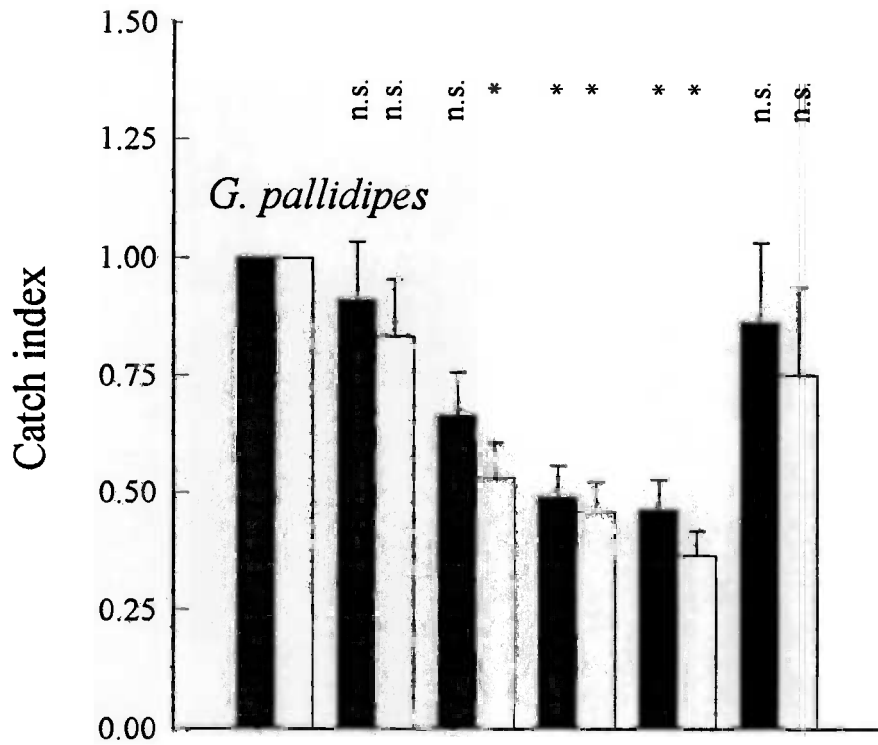
Odour







Odour & filters



Odours & filters

APPENDIX 2

Responses of *Glossina pallidipes* Austen (Diptera: Glossinidae) to synthetic repellents in the field.

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**Responses of *Glossina pallidipes* Austen (Diptera: Glossinidae)
to synthetic repellents in the field.**

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Abstract

In Zimbabwe, studies were made of the responses of *Glossina pallidipes* Austen to known and candidate repellents. Various chemicals, dispensed at ca. 5-10 mg/h, were placed beside Epsilon traps already baited with a blend of acetone, octenol, 4-methylphenol and 3-n-propylphenol. Pentanoic or hexanoic acids or acetophenone halved the catch and 2-methoxyphenol reduced the catch by 90%. There were no consistent differences in the responses of males and females. Pentanoic acid or acetophenone or 2-methoxyphenol at an unbaited trap reduced the catch by 40%, 75% and 60%, respectively. Baiting traps with a combination of pentanoic acid, acetophenone and 2-methoxyphenol did not reduce the catch below that produced by 2-methoxyphenol alone. Pentanoic acid and 2-methoxyphenol reduced the efficiency of traps from ca. 40% to 20% but acetophenone had no significant effect. Acetophenone and 2-methoxyphenol halved the numbers of tsetse attracted to a target baited with acetone, octenol and phenols but none had a significant effect on the proportion that landed. 2-Methoxyphenol significantly reduced the numbers of tsetse attracted to a source of natural ox odour but only pentanoic acid had a significant effect on feeding responses, reducing the proportion that fed on an ox from 59 to 45%. It is concluded that these repellents do not provide any useful degree of protection against trypanosomiasis. In areas where tsetse are abundant (500 bites/ox/day) and infection rates in tsetse are high (>5%) it is highly unlikely that any repellents would be useful. However, in areas where tsetse are less abundant (1 bite/ox/day) and/or infection rates are low (ca. 0.5%) the potent, unidentified repellents present in human odour might effectively complement the control of disease using trypanocidal drugs.

Introduction

Currently, the most important methods of combatting human and animal trypanosomiasis rely on insecticide-treated baits to control tsetse (Green, 1994), or trypanocidal drugs to treat or prevent the disease (Jordan, 1986). The baits consist of either traps or insecticide-impregnated targets, sometimes baited with synthetic attractants (Vale *et al.*, 1986), or cattle treated with an insecticidal dip or 'pour-on' insecticide (Thomson *et al.*, 1991).

Early studies by Vale (1974a) showed that chemicals present in human odour reduce the numbers of tsetse attracted to a host and also the proportion that subsequently feed. Such chemicals could provide a useful tool in the integrated management of trypanosomiasis. For instance, in areas where cattle are protected from trypanosomiasis by the use of prophylactic drugs, disease challenge might be reduced by treating cattle with repellents. A large reduction in challenge would reduce drug costs and increase productivity (Barrett, 1994; Holmes & Torr, 1988). The same approach might be applied in areas where trypanotolerant breeds of cattle are used (Jordan, 1986). Repellents may also be useful in protecting people against sleeping sickness.

Although the identities of the repellents present in human odour remain unknown, a number of other potent repellents have been discovered in the course of identifying attractants. For instance, high doses of lactic acid (Vale, 1977b), acetophenone (Vale, 1980) and 2-methoxyphenol (Vale *et al.*, 1988) have been shown to reduce the catch of traps by 50-90%.

We report here investigations of the responses of tsetse to various known and candidate repellents dispensed at doses that might be practicably released from a dispenser attached to a host. The study assessed whether repellency could be enhanced by combining several repellents and estimated the level of protection that these chemicals might provide against trypanosomiasis. In addition to the chemicals previously reported to be repellent, studies were also made of N,N- diethyl-m-toluamide (DEET), a repellent for mosquitoes (Davis & Bowen, 1994), naphthalene, a commonly used repellent for insects (Den Ouden *et al.*, 1984) that is also known to be detected by tsetse (P Beever, pers. comm.), and various volatile carboxylic acids known to be present in human sweat (A Cork, unpublished results).

Materials and Methods

All experiments were carried out at Rekomitjie Research Station in the Zambesi Valley of Zimbabwe where *Glossina pallidipes* Austen and *G. morsitans morsitans* Westwood (Diptera: Glossinidae) are present.

Catching methods

Traps.- All catches were made by Epsilon traps (Hargrove & Langley, 1990) supplied by Bonar Industries, Harare, Zimbabwe.

Targets.- Targets consisted of a panel of black cloth (1 x 1 m) flanked with 0.5 x 1-m high panels of black mosquito netting. To estimate the number of tsetse that contacted the target, an electrocuting grid (Vale, 1974b) covered all its surfaces. The target was mounted on a corrugated tray covered with polybutene. Tsetse that contacted the grid were killed or stunned and fell onto the tray where they became stuck. Tsetse generally fall vertically (Vale, 1974b) and so, by recording the numbers of tsetse caught directly below the netting and cloth panels, it was possible to gauge the proportion that was caught next to the netting or the cloth.

Odours

Synthetic attractants.- Traps and targets were baited with a blend of acetone (500 mg/h), 1-octen-3-ol (0.4 mg/h), 4-methylphenol (0.8 mg/h) and 3-*n*-propylphenol (0.1 mg/h) at rates known to increase the catch of tsetse in Zimbabwe (Vale & Hall, 1985; Vale *et al.*, 1988) and henceforth this blend of odours is termed AOP. Acetone was dispensed from an open bottle and octenol and the phenols were dispensed from sealed sachets of low density polyethylene, 0.15 mm thick with a surface area of 50 cm² (Laveissière *et al.*, 1990).

Natural attractants.- In some experiments, studies were made of the numbers of tsetse attracted to a source of natural ox odour with or without various repellents. Natural odour was obtained by placing an ox (ca. 400 kg) in a roofed pit and exhausting the air from the pit at 2000 l/min via a ventilation shaft (25 cm dia.) fitted

with a 12 V co-axial fan (Vale, 1974a). To gauge the numbers of tsetse attracted to the odour, an electric net (Vale, 1974b), 1.5 x 1.5 m, was placed 1 m downwind of the odour source. Tsetse orientate imprecisely to an odour source unless it is marked by a visual stimulus (Vale, 1974a) and so a target, consisting of a panel of black cloth (75 x 75 cm), was sewn on to the centre of the electric net.

Repellents.- Repellents were dispensed undiluted from either glass vials (1.7 cm wide and 4.3 cm deep) or from sealed polythene sachets. Unless reported otherwise, the odours were placed on the ground 30 cm downwind of the trap, target or electric net.

Effect of odours on trap and feeding efficiency

To estimate the effect of repellents on trap efficiency, a trap baited with various odours was placed at the centre of an incomplete ring (8 m dia.) of six electric nets (1.5 x 1.5 m). The nets covered about 36% of the circumference of the ring and so the number of tsetse that approached the vicinity of the trap was taken to be the catch of the trap itself plus 2.78 times the catch from the inside surface of the ring of nets. Following Vale & Hargrove (1979), trap efficiency is defined as the catch of the trap expressed as the percentage of flies that approach the vicinity of a trap.

To assess the effects of repellents on feeding efficiency, an ox with or without various repellents was placed at the centre of the ring of nets. Flies were separated according to the side of the net on which they were caught and classed as fed or unfed based on the presence or absence of red blood being visible through the abdominal wall. Following Vale (1977a), feeding efficiency was estimated as the number of tsetse containing red blood caught on the inside of the ring nets expressed as a percentage of the total catch from the inside of the ring.

Experimental design and analysis

All field experiments were carried out in the 3 h before sunset when tsetse are most active (Hargrove & Brady, 1992). The various repellents were incorporated into a series of replicated Latin squares consisting of days X sites X treatments. For studies

using the ring of nets, treatments were compared using a randomised 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 normalised using a $\log_{10}(n+1)$ transformation and subjected to analysis of variance. The detransformed means are reported, accompanied by their transformed means and standard errors so that more detailed comparisons can be made. In studies of feeding and trap efficiency the results were analysed using GLIM4 (Francis *et al.*, 1993) which fits models using a maximum likelihood method. A binomial model with a logit link was used to control for non-normal and non-constant variance. The significance of changes in deviance were assessed by χ^2 . The catches of *G. m. morsitans* were generally too low to analyse their responses to the various repellents. Consequently only the results for *G. pallidipes* are reported.

Experiments and results

Effects of repellents on trap catch

Traps with odour.- Initial screening of various known and putative repellents were carried out using traps. Studies were made of the effect of adding candidate repellents to traps baited with AOP. The results (table 1) show that 2-methoxyphenol, acetophenone, pentanoic or hexanoic acid reduced the catches significantly. Lactic acid also produced a significant reduction in catch at 100 mg/h but not at 10 mg/h. Neither DEET nor naphthalene had a significant effect.

There was no significant difference in the responses of males and females. For instance, a trap baited with AOP had a detransformed mean catch of 42 male and 80 female *G. pallidipes*. Adding acetophenone reduced the catch of males and females by 71% and 68% respectively and adding 2-methoxyphenol reduced the catch by 84% and 87% respectively. The data for the sexes are therefore pooled henceforth.

The effects of adding several repellents to traps baited with AOP are shown in table 2. Adding pentanoic acid and/or acetophenone to 2-methoxyphenol did not reduce the catch further than the reduction produced by 2-methoxyphenol alone (Experiment 1). However, adding 2-methoxyphenol to either pentanoic acid (Experiment 2) or to acetophenone (Experiment 3) did reduce the catch further, suggesting that 2-methoxyphenol is a more potent repellent than either acetophenone or pentanoic acid. It is noteworthy that the repellents completely negated the effect of attractants. For instance, adding AOP increased the catch as expected but the catch with a combination of AOP and 2-methoxyphenol was either not significantly different (Experiment 1) or even significantly less (Experiment 3) than that with no odour.

Traps without odour.- Adding repellents to otherwise unbaited traps also reduced the catch (table 3). The catches from traps baited with acetophenone or 2-methoxyphenol were significantly less than that from an unbaited trap (Experiments 1 and 2) but for pentanoic acid the effect was not significant (Experiment 2).

After this preliminary screening, more detailed studies were made of responses to 2-methoxyphenol, acetophenone and pentanoic acid to assess whether they might provide a useful degree of protection against trypanosomiasis.

Effects of repellents on attraction and landing

The effects of the various repellent blends on attraction were assessed by the reduction in total catch observed when they were added to an AOP-baited target, with effects on landing measured from the proportion of tsetse caught on the cloth section of the target. The results (table 4) show that 2-methoxyphenol and acetophenone reduced the total catch of tsetse but there was no significant effect produced by pentanoic acid. None of the treatments had a significant effect on the landing response.

To assess attraction to a natural host, targets were baited with natural ox odour. The results (table 5) were broadly in accord with those from the AOP-baited targets. Addition of 2-methoxyphenol had a clear and significant effect, reducing the

catch by ca. 50%, while acetophenone also reduced the catch of tsetse but the effect was smaller and only significant in one experiment. Pentanoic acid had no significant effect.

Effect of repellents on trap efficiency

The results from the traps and targets indicated that pentanoic acid reduced the catch in traps but not at targets, whereas 2-methoxyphenol and acetophenone reduced the catch by both. This might be due to differential effects on trap efficiency, so studies were made of the effect of repellents on this variable. The results (table 6) show that 2-methoxyphenol and pentanoic acid reduced the efficiency of Epsilon traps significantly from 64 to 41-48% but acetophenone had no significant effect. Pentanoic acid appears to exert its repellent effect solely by reducing the tendency for tsetse to enter a trap. 2-Methoxyphenol, on the other hand, has this effect and also reduces the numbers of tsetse that are attracted to the vicinity of the bait.

Effect of repellents on feeding responses

The studies of feeding response (table 7) showed that neither 2-methoxyphenol nor acetophenone had a significant effect on the proportion of tsetse that fed on an ox, whereas pentanoic acid had a slight but significant effect, reducing the percentage feeding from 59 to 44%.

Discussion

The present results show that low doses (ca. 10 mg/h) of 2-methoxyphenol, acetophenone, pentanoic and hexanoic acid reduce the catch of baited traps by 45-85%. These results confirm previous findings (Vale, 1980; Vale *et al.*, 1988) that baiting traps or targets with acetophenone (2000 mg/h) or hexanoic acid (100 mg/h) or 2-methoxyphenol (ca. 2 mg/h) reduced the catch of tsetse significantly. Lactic acid was also repellent when dispensed at 100 mg/h but not at 10 mg/h, and was a potent repellent when dispensed by saturating a cloth (0.18 m²) with 10% lactic acid or by treating an ox with 4.5 l of a 1% solution (Vale, 1977b). These latter treatments represent doses of 11 g/day and 49 g/day applied to the cloth or ox respectively for a

3-h experiment, so the release rates used by Vale were probably much greater than the 10 mg/h used here.

The results show that 2-methoxyphenol is the most potent repellent of those tested, reducing trap catches by ca. 85%. However, its repellent effect was not enhanced by adding either pentanoic acid or acetophenone. The behavioural basis of this difference between 2-methoxyphenol and the other two seems to be due to pentanoic acid reducing trap entry but having no effect on attraction to an odour source, and to acetophenone having no effect on trap entry but reducing attraction. 2-Methoxyphenol, on the other hand, reduces both efficiency and attraction, hence its greater potency as a repellent and the absence of any enhanced effect when either acetophenone or pentanoic acid were added to it. Thus, from a practical viewpoint, combinations of these odours are no more effective than 2-methoxyphenol alone.

No behavioural activity was demonstrated for DEET which is a repellent for many insect species (Rutledge *et al.*, 1978), particularly mosquitoes (Davis & Bowen, 1994), or for naphthalene which has been used as a repellent against the cabbage root fly, *Delia radicum* Linnaeus (Diptera: Anthomyidae) (Den Ouden *et al.*, 1984). Vale (1977b) was also unable to show a response of tsetse to DEET.

It should be noted that adding 2-methoxyphenol, acetophenone and pentanoic acid to unbaited traps reduced the catch of tsetse, and thus these compounds qualify as true repellents as defined by Dethier *et al.* (1960). This is in contrast to most work on insect repellents which involves chemicals that reduce attraction to sources of attractive odours. As observed by Davis (1985), the term “repellent” is more widely used for chemicals which elicit a combination of behavioural responses resulting in prevention of biting by an insect. Invariably, the specific behavioural patterns by which this result is attained are not determined, and there are even less data on the sensory mechanisms involved. Davis (1985) suggested at least five potential sensory mechanisms by which these “attraction inhibitors” might act: (a) they may interact with and inhibit the response of a sensory neuron to a normally attractive signal; (b) they may interact with their own specific receptors and be attractants at low intensities

but become repellent at (c) they may activate a receptor system that media compete with each other they may activate specific noxious repellent receptors, (d) they may simultaneously activate several different types that mediate various behaviour patterns that any sensory signal specifies host finding lost the incoming barrage of sensory input.

In the case of host setse repellent mediated mechanism (c) be excluded because they are absent of attractant although interestingly this is the only host kairomone which has been demonstrated DEET reduced response of tsetse to the feeding attractant acetate (D & Ven, 1981) and the repellent attractant ethyl propionat (Kuljala & Vittregan mechanisms (b), (c) and (d) should be noted that both *o*-methyl oxyphenol and pentyl acetate are produced by host Mal. oxyphenol is very common (Bursell *al.* 1981), acetate is 4-5% the major kairomone of the tsetse. It is noted that who odour thus typically than 100 mg/hr (cf Tölg compared with the tsetse used in the studies. However addition of *o*-methyl oxyphenol to these low rates of release to AOP blends caused only small, insignificant increases in host attraction (Tölg, Mangwani & Hall, unpublished). Pentyl acetate is present in odour of tsetse at 100 mg/hr addition of a mixture of the two compounds to the blends of AOPs caused slight increase in host attraction. Mechanism (b) above thus seems unlikely at least for host kairomones and pentyl acetate but mechanisms (c) and (d) not be ruled out. The fact that repellents produced by host animal might suggest key plant kairomone interactions perhaps acting through a specific receptor system. The behavioural response mechanism (c) or (d) could be produced either directly by unsuitable hosts activating specific noxious repellent receptors as mechanism (d). (Wynnerto 1936) reported that tsetse and key kairomones of tsetse that attempt to feed them more recently Randolph *al.* (1991) drew attention to the skin mortality associated with feeding may therefore be adapted for tsetse to be able to

recognise, and thus avoid, those hosts that have a high probability of killing tsetse attempting to feed.

Preliminary electrophysiological evidence (C. den Otter & K. Voskamp, *pers. comm.*) indicates that 2-methoxyphenol and acetophenone activate several receptor types that also respond to attractive compounds. This suggests that these repellents might act by mechanism (e), causing a barrage of sensory input that jams any signal specific to host finding whether olfactory, visual or any other type. 2-Methoxyphenol obviously has some structural similarities to the attractant phenols, 4-methylphenol and 3-*n*-propylphenol, and acetophenone was originally tested by Vale (1980) as an analogue of the attractant ketone, acetone.

Practical implications

The present study underscores a problem recently highlighted by Vale (1993) concerning the use of traps to make deductions about tsetse behaviour - or indeed the behaviour of any insect. The initial studies using traps indicated that the repellents studied were potent and might therefore be effective in protecting animals against trypanosomiasis. However, analysis of the responses of tsetse attracted to and then feeding on an ox revealed that the effect was relatively slight. Adding 2-methoxyphenol and pentanoic acid to a trap resulted in a pronounced decrease in trap efficiency but there did not appear to be an equivalent and large close-range effect with a host. Clearly, making deductions regarding the behaviour of tsetse, based solely on their responses to traps, must be done very cautiously.

The repellents studied here halved the number of tsetse attracted to a host, but none had an effect on landing, and only pentanoic acid had a slight and significant effect on feeding. These data suggest that baiting an ox with these chemicals would only halve the number that are attracted to the ox and, at best, reduce the proportion that subsequently feed by ca. 25%. So the net reduction in biting rate afforded would then be ca. 60%.

The probability of a tsetse bite resulting in an infection is the product of the prevalence of infection in the tsetse population (p) and the probability of a bite by an infected fly resulting in an infection in a host which Rogers (1988) termed b_1 . At Rekomitjie, the prevalence of *T. vivax* is ca. 3% (Ford & Leggate, 1961; Woolhouse et al., 1993) and Rogers (1988) estimated b_1 as being 0.29. Thus the overall probability of infection per bite would be 0.0087 and the probability of not being infected therefore 0.9913. If a host is bitten by n flies then the probability of an ox not being infected is 0.9913^n and hence the chance that they are infected is $1-0.9913^n$. At Rekomitjie a stationary ox might be typically visited by 600 flies per afternoon (Vale, 1977a, table 1) of which 45% feed successfully. Such an animal might be conservatively estimated to receive 270 bites a day. Baiting the animal with a repellent that reduced the bites/day by 60% would reduce the daily probability of infection with *T. vivax* from 0.91 to 0.61. The known repellents are clearly useless in these circumstances.

The efficacy of a repellent will vary according to tsetse challenge. To illustrate this, it is pertinent to consider three epidemiological scenarios based on a “high” infection rate of 7.6% (*T. congolense* at Muhaka, Kenya; Tarimo *et al.*, 1985), a “medium rate” of 3.1% (*T. vivax* at Rekomitjie, Zimbabwe; Woolhouse *et al.*, 1993) and a “low” rate of 0.16% (*T. brucei* at Rekomitjie, Zimbabwe; Woolhouse *et al.*, 1993). The transmission co-efficients (b_1) for these species of *Trypanosoma* are 0.46, 0.29 and 0.62 respectively (Rogers, 1988).

Considering a range of fly densities (fig. 1) shows that at high infection rates a repellent exerts a proportionally greater reduction in disease risk at lower densities of tsetse. For instance, with daily biting rates of 500 bites/day a repellent that reduced the biting rate by 90% would reduce the risk of contracting *T. congolense* by 17% to 0.83. At densities of 10 bites/day the same repellent would reduce the risk by 88% to 0.03. At low infection rates the non-linear response between tsetse density and disease risk is not so apparent. Using the same repellent with a daily biting rates of 500/day or 10/day would reduce the risk of infection with *T. brucei* by 82 and 90% respectively. The medium infection rate (*T. vivax* at Rekomitjie) lies between these two extremes

with a clear curvilinear relationship between bites/day and infection probability. Thus repellents might be useful in situations with low fly densities and/or low infection rates.

Where cattle are kept in tsetse infected areas of Zimbabwe the tsetse density is low with mean daily catches of <1 tsetse/trap/month and cattle are treated with diminazene if they become infected with trypanosomiasis (Barrett, 1994). In such areas, a repellent that reduced the biting rate by 95% could significantly reduce disease incidence and hence drug costs. Low infection rates are typical for *T. brucei* (Rogers, 1988) and thus a repellent might also be particularly useful in protecting people against sleeping sickness.

Vale (1977a) showed that placing men adjacent to an ox halved the numbers of tsetse attracted and reduced the proportion that fed by 75% giving an overall reduction in biting rate of ca. 90%. This reduction is largely a response to human body odour (Vale, 1979) and thus significant reductions in challenge are apparently achievable through the use of such repellents and work is currently underway to identify these chemicals

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Table 1. The catch index of *Glossina pallidipes* from traps baited with AOP plus various putative repellents. The catch index is the detransformed mean catch of tsetse expressed as a proportion of that from a standard AOP-baited trap. *n*=number of replicates; *P*=probability that the catch index is significantly different from unity (F-test) at the *P*<0.05 (*), *P*<0.05 (**) or *P*<0.001 (***) levels of probability.

Chemical	Release rate (mg/h)	n	Standard catch		Catch index	<i>P</i>
			detrans- formed	transformed ±SE		
2-Methoxyphenol	10	12	123	2.093±0.0648	0.14	***
Acetophenone	5	12	123	2.093±0.0648	0.31	***
Hexanoic acid	5	12	33	1.530±0.0689	0.36	**
Pentanoic acid	10	12	61	1.792±0.0553	0.55	*
Lactic acid	100	8	107	2.034±0.053	0.56	***
	10	12	5	0.778±0.0738	0.70	ns
Acetic acid	10	12	5	0.778±0.0738	0.61	ns
	10	12	16	1.227±0.065	0.72	ns
Butanoic acid	5	12	21	1.342±0.0720	0.87	ns
	20	12	134	2.130±0.0807	0.80	ns
Propionic acid	5	16	15	1.208±0.0386	0.83	ns
Formic acid	10	8	107	2.034±0.053	0.91	ns
3-methyl butanoic acid	5	12	33	1.530±0.0689	0.98	ns
DEET	10	12	17	1.255±0.0833	1.29	ns
treated cloth (20 x 30 cm)		12	17	1.255±0.0833	0.91	ns
Naphthalene	10	6	128	2.111±0.0728	1.04	ns
	trace	6	43	1.643±0.0797	1.13	ns

A=acetone (500 mg/h), O=1-octen-3-ol (0.4 mg/h), P=4-methylphenol (0.8 mg/h) + 3-n-propylphenol (0.1 mg/h).

Table 2. The detransformed mean catch of *Glossina pallidipes* from traps baited with combinations of 2-methoxyphenol (2-ME), pentanoic acid (PEN) and acetophenone (ACETO). For each experiment, transformed means followed by a different letter are different at the $P < 0.05$ level of probability (LSD-test).

Bait	Mean catch	
	Detransformed	Transformed
Experiment 1. (12 reps)		
AOP only	37.4	1.584a
AOP+2-ME	6.3	0.863b
AOP +2-ME+PEN	5.4	0.806b
AOP +2-ME+ACETO	8.5	0.978b
AOP +2-ME+PEN+ACETO	7.9	0.949b
None	5.2	0.792b
Transformed SE		0.0874
Experiment 2. (12 reps)		
AOP only	61.2	1.794a
AOP+PEN	33.6	1.539b
AOP+PEN+2-ME	6.5	0.875c
Transformed SE		0.0552
Experiment 3. (12 reps)		
AOP only	123.1	2.094a
AOP+ACETO	38.2	1.593b
AOP+ACETO+2-ME	19.6	1.314c
None	33.6	1.539b
Transformed SE		0.0622

A=acetone (500 mg/h), O=1-octen-3-ol (0.4 mg/h), P=4-methylphenol (0.8 mg/h) + 3-n-propylphenol (0.1 mg/h). For release rates of repellents see table 1.

Table 3. The detransformed mean catch of *Glossina pallidipes* from traps baited with acetophenone, 2-methoxyphenol or pentanoic acid. Asterisks indicate that the means are significantly different from no odour at the $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***) level of probability.

Bait	Mean catch	
	Detransformed	Transformed
Experiment 1. (14 reps)		
No odour	30.1	1.493
Acetophenone	4.0***	0.695
2-Methoxyphenol	12.4**	1.126
Transformed SE		0.0734
Experiment 2. (8 reps)		
No odour	9.7	1.029
Acetophenone	2.5*	0.544
2-Methoxyphenol	3.6*	0.667
Pentanoic acid	5.7	0.829
Transformed SE		0.0920

For release rates of repellents see table

Table 4. The detransformed mean catch (transformed \pm SE in brackets) of *Glossina pallidipes*, and mean percentage (\pm SE) alighting on the cloth panel, from targets baited with AOP with or without various repellents. Each treatment was repeated for 12 replicates. Asterisks indicate that the means are significantly different from AOP ($P < 0.05$, F-test).

Bait	Mean catch	Percent landing
AOP	82.9 (1.924 \pm 0.0312)	30 (\pm 6.0)
AOP+ 2-methoxyphenol	28.9 (1.476 \pm 0.0312)*	27 (\pm 4.6)ns
AOP+ pentanoic acid	67.9 (1.838 \pm 0.0312)ns	28 (\pm 3.6)ns
AOP+ acetophenone	40.7 (1.620 \pm 0.0312)*	25 (\pm 3.6)ns

A=acetone (500 mg/h), O=1-octen-3-ol (0.4 mg/h), P=4-methylphenol (0.8 mg/h) + 3-n-propylphenol (0.1 mg/h). For release rates of repellents see table 1.

Table 5. The detransformed mean catch (transformed mean in brackets) of *Glossina pallidipes* from a target at a source of natural ox odour with or without various repellents from *n* replicates and the probability (*P*) that the means are different (*F*-test).

Repellent	<i>n</i>	Ox odour only	Ox odour + repellent	Trans-formed SE	<i>P</i>
2-Methoxyphenol	12	63.4 (1.809)	37.1 (1.581)	0.0265	***
	6	53.6 (1.737)	15.9 (1.228)	0.0387	***
Acetophenone	12	107.9 (2.037)	87.6 (1.947)	0.0283	*(<i>P</i> =0.0488)
	10	8.3 (0.968)	7.3 (0.919)	0.0694	ns
Pentanoic acid	12	79.3 (1.905)	68.4 (1.841)	0.0317	ns

For release rates of repellents see table 1

Table 6. Trap efficiency, sample size (*n*) and the efficiency index (\pm SE) for *G. pallidipes* from an Epsilon trap baited with AOP \pm various repellents from 6 replicates. Asterisks indicate that the addition of a repellent to AOP has reduced efficiency index at the $P < 0.001$ level of probability (F-test). Efficiency index is the catch from the trap expressed as a proportion of the total (trap+inside of the ring of nets) catch.

Odour	Percent efficiency	n	Efficiency index
AOP	64.0	366	0.75 (± 0.033)
AOP+2-ME	41.1	232	0.55 (± 0.043)***
AOP+ACETO	66.6	223	0.77 (± 0.029)
AOP+VAL	48.3	277	0.58 (± 0.042)***

For release rates of repellents see table

Table 7. Percent feeding efficiency for *G. pallidipes* feeding on an ox ± various repellents. Feeding efficiency is the total catch of fed flies from the inside of the ring of nets expressed as a proportion of the total (fed+unfed) catch (*n*). Asterisks indicate that the means are significantly different from AOP ($P < 0.05$, F-test).

Odour	Feeding efficiency		Repli- cates	<i>P</i>
	Ox	+repellent		
2-Methoxyphenol	46.8 (664)	40.8 (409)	24	ns
Acetophenone	45.3 (547)	45.1 (563)	13	ns
Pentanoic acid	59.2 (299)	43.8 (194)	29	*

For release rates of repellents see table 1

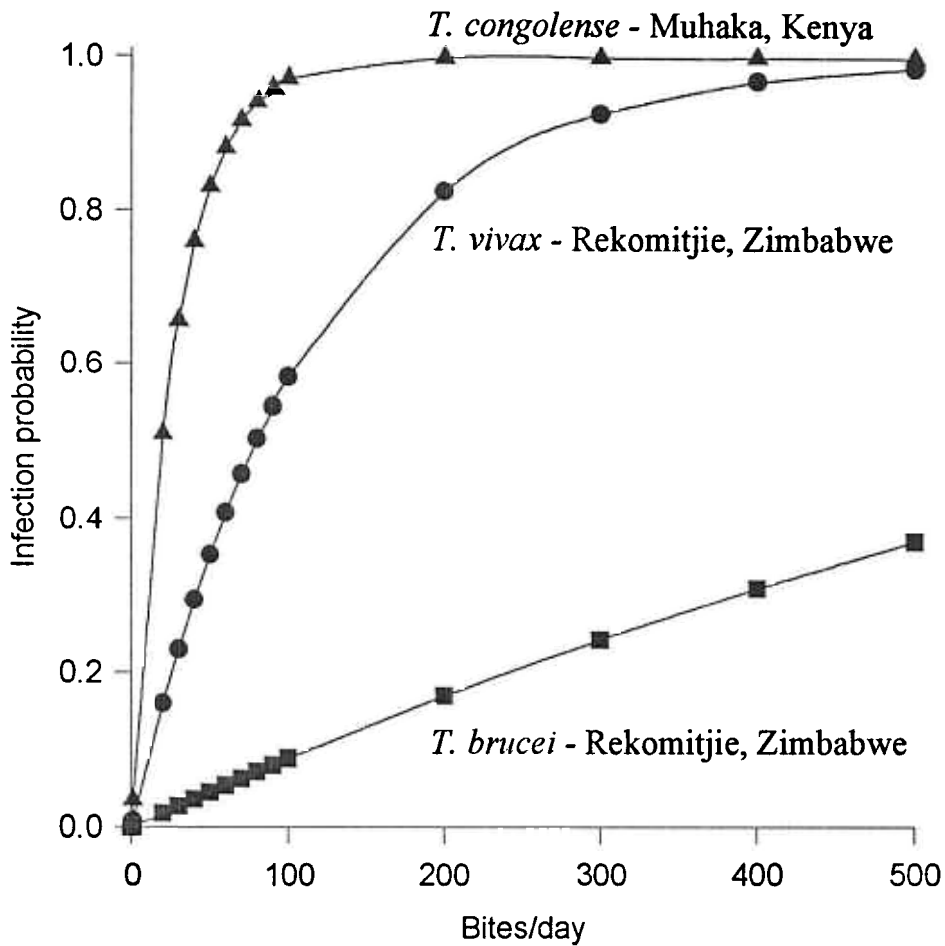
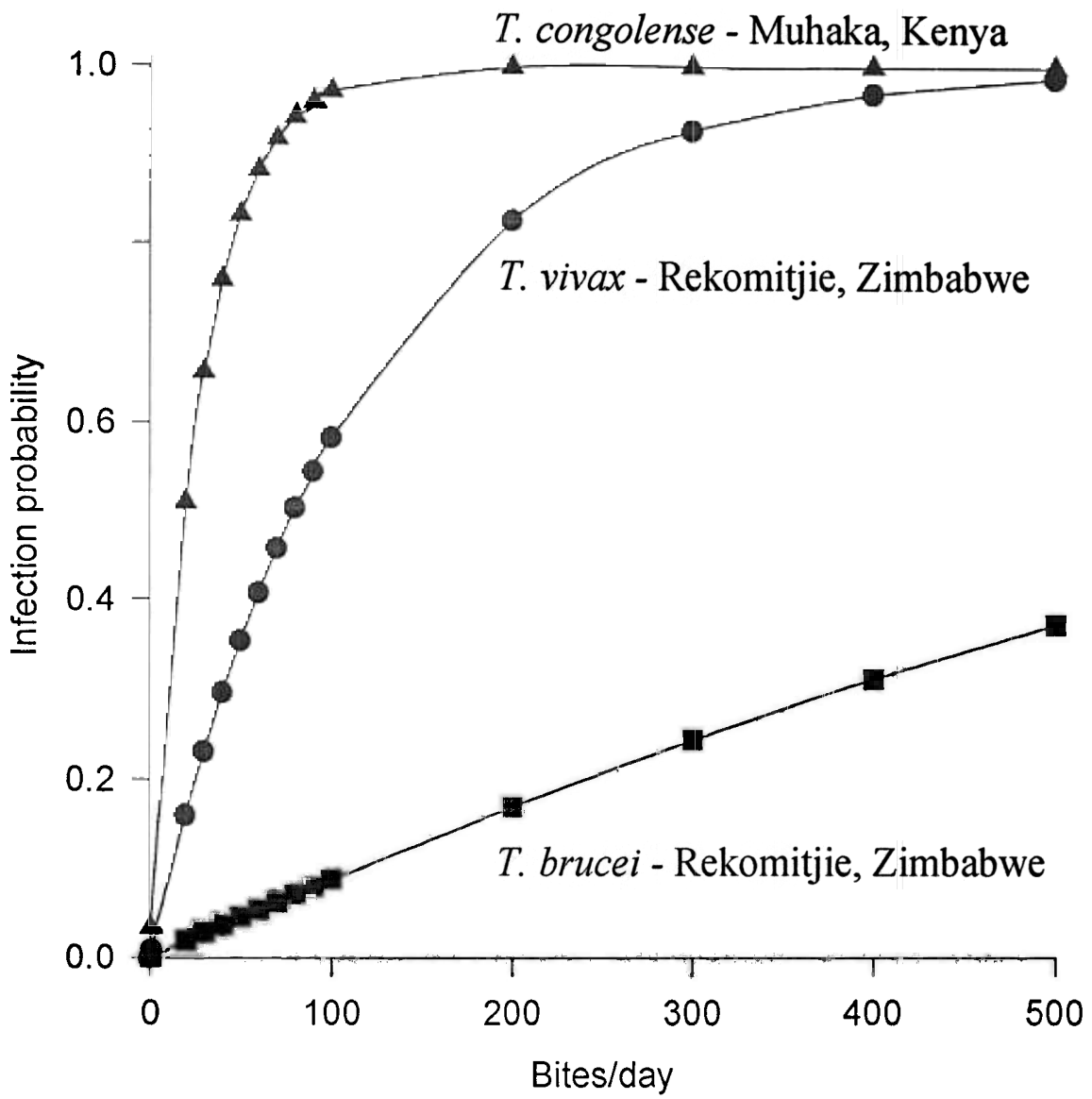


Fig.1 The daily probability of an ox contracting trypanosomiasis with given infection rates in tsetse (p), transmission coefficients (b_1) and fly densities. Given that proportion p of tsetse are infected, that the probability of a bite from an infected fly producing an infection is b_1 , and an ox is bitten by n flies, then the daily probability of an ox contracting trypanosomiasis is $1-(1-p.b_1)^n$. Illustrative examples of p and b_1 for *T. brucei* ($p=0.0015$, $b_1=0.62$) and *T. vivax* ($p=0.03$, $b_1=0.29$) are from Woolhouse *et al.* (1993) and Rogers (1988) and from Tarimo *et al.* (1985) and Rogers (1988) for *T. congolense* ($p=0.07$, $b_1=0.46$).

FIGURES

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APPENDIX 3

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

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Abstract.

Laboratory studies were made of methods for dispensing tsetse attractants using sealed polyethylene sachets and bottles. 1-Octen-3-ol (octenol), 4-methylphenol and 3-*n*-propylphenol were dispensed singly or as blends from sachets 25-200 cm² 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, probably because of a plasticising effect of this substance. A similar effect was seen with blends of the attractants and an involatile plasticiser. 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 10-1000-fold increase in the cost:effectiveness of traps and targets (Vale, 1993a), mainly by improving the efficiency of traps and targets and by identifying potent attractants. The most important attractants for practical purposes are acetone, butanone, 1-octen-3-ol (Vale and Hall, 1985a, 1985b) and various phenols (Owaga *et al.*, 1988; Bursell *et al.*, 1988), and combinations of these have been used to control tsetse in Zimbabwe (Vale *et al.*, 1986), Zambia (Willemsse, 1991) and Kenya (Dransfield, 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 derived by developing cheap and robust systems to dispense attractants over long periods, thereby reducing the need for expensive maintenance visits to replenish the odour baits (Barrett, 1994). Such a system should be capable of dispensing various 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 polyethylene sachets and bottles.

The responses of *Glossina pallidipes* Austen and *G. morsitans morsitans* Westwood to octenol, phenols, acetone and butanone are well established (Vale & Hall, 1985; 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.

General 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, U.K.), 4-methylphenol (Aldrich Chemical Company Ltd., Gillingham, Dorset, U.K.), 3-*n*-propylphenol (Palmer Research, Clwyd, U.K.), 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 ($\pm 1^\circ\text{C}$) 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, MA 01757, U.S.A.; 50-80 mesh). In early studies, the dispenser was held in a silanised, 2-litre, round-bottomed flask, and air was drawn in through an activated charcoal filter (15 cm x 2 cm; 6-8 mesh) and out through a filter containing Porapak Q (5 cm x 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 x 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, U.K.) 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 minute 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 x sites x days.

Statistics.- The significance of differences in the release rates of attractants from different types of dispensers were 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 normalised and the variances homogenised by transformation 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 x 8 x 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 plasticiser 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.

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. x 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 and neither of these effects were prevented by adding the plasticiser 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 cm² 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 cm² 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 diffuses more readily than acetone through polyethylene and consequently studies concentrated on dispensers for butanone since 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 cm² in surface area and had walls 0.15 mm thick.

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 cotton drill bag.

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 by measuring release rates from sachets containing 22 different blends maintained in the laboratory wind tunnel. The data (fig. 3) show 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 a 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) show that there was a curvilinear relationship between concentration and release rate which is fitted by an inverse linear curve.

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 blends (4 ml) of 4-methylphenol, 3-*n*-propylphenol and octenol 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 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 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

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 initially filled with *c.* 10 g of an 8:1:4 blend of 4-methylphenol, 3-*n*-propylphenol and octenol. The results (table 2) show that that there was no significant difference in the catch of traps baited with old or 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) show 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 increases exponentially with temperature and a simple exponential model is a good fit ($r^2=0.96$), the measured release rates at high temperatures ($>45^\circ\text{C}$) are systematically less than the fitted line. Fitting a further polynomial term ($y=-4.289+0.1774.x-0.001016x^2$) 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).

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 25-36°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) show 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 insulated 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 while 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 x 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 second five sachets containing of 6 end of 4-methylphenyl-propylphenol and placed in black bag 10 cm were suspended in the shade full beneath small pitched roof of waxed cardboard. The sachet weighed monthly intervals to measure monthly rates of release per year. Sachet fully depleted in less than year and therefore enclosed near also. The results (fig 7) show that the rate of release recorded under full sun and shade. The cumulative loss of drug from the sachet full sun was compared to from the sachet shade. The largest amount of drug from the sachet full sun per month (November-December) compared to per month for the sachet shade. The release rate of full sun was higher than the blue bag comparable attributes, probably because blue bag reflects more radiation than the black thus subject less isolated.

The release rate of full sun dispenser also increased full. The release rate from sachet (Bertix 100 ml) placed in black bag full was 0.54 mg/h (range 0.4-0.6). This occurred when the shade temperature was 30°C. The release rate of sachet dispenser in the shade was 0.3 mg/h.

The surface temperature of sachet in black pocket full was recorded continuously for period of 10 days from full sun to July 1995. The results (fig 8A) show that the black pocket full sun was 30°C hotter than the day and 10°C cooler than the shade temperature. The results (fig 8B) show that the temperature of black and blue sachet full sun was higher during July-September 1995. The results (fig 8B) show there was significant difference in the temperature at each day. The black pocket was hotter than the blue. This is in accord with the fact that full sun sachet black pockets have higher release rate than sachet blue pocket. Looking at the data for blue bags, the black sachet was 10°C warmer than the 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) show that 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 - 12.6). 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 - 8.6) in June and 1.7°C (-2.1 - 9.7) 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

cat) of *G. pallipes* from ap ed with acetone plus chet 66
 26 +0.066 ran: formed mean F) are d (72 066 !) for the
 sachet

Rel for the sachet were red at h intervals for fig. 5, and
 the equation relating tempera C) and rele rate mg/h (y) for the sachet
 was found

$$y = 0.0001x^2 - 0.0004x + 0.0001 \quad (r^2 = 0.99)$$

T model: based ly lai measu its temperatu
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 cal ed by ng equati ane substitui ng the hourly est mates of shade
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 $r^2=0.99$) 8°C $r^2=0.84$ for the tempe () Th ss plicat of
 ay ree mod red effect redicti :curacy of the model
 The adjusti he sachet mod pr :bably el: ted to the
 rati: that ler t temperature ng g.

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. 5 & 6) 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:-

$$Y = \sum_{t=1}^n \exp(a + b(c + x_t)) \cdot 24 \quad (2)$$

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).

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 remain constant until the contents are exhausted, whereas with thicker polythene tubing the release rate declined with age and this was associated with a hardening of the polythene. 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 cm² in surface area and with walls 0.15-0.3 mm thick.

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 ca. 0.01 mg/h of octenol (Torr *et al.*, 1995). To simulate this dose using 0.15mm thick polyethylene, at a temperature of say 27° C, would require using a sachet of 0.56cm². It would be more practicable to use a 50 cm² sachet containing 5 g of a 0.5% mixture of octenol with dioctylphthalate.

In the absence of other factors, it seems that the release rate of a substance is governed by a permeability factor, dependent upon the nature of the substance, and the concentration gradient across the polyethylene. Presumably, the concentration at the outer surface is zero as the material is volatilised as fast as it diffuses through, and so the gradient is proportional to the internal concentration. However, in mixtures with the phenols, the release rate of octenol is second order (fig. 3), suggesting that the octenol has some concentration-dependent plasticising effect increasing the release rate.

Dioctylphthalate is a known plasticiser, and a similar effect might be occurring in mixtures where this was used as an involatile diluent (fig. 4). Thus the release rates at low concentrations of the attractants, i.e. high concentrations of dioctylphthalate, are higher than expected from a linear model. However, in these cases, the monotonic increase in release rate with concentration was described by an inverse linear curve similar in form to the Michaelis equation used to describe the relationship between concentration and the rate of enzyme-catalysed reactions (Morris, 1974). This equation describes two-stage reactions

in which the first step is reversible and the second is irreversible. The enzyme forms an enzyme-substrate complex and the reversible step is the rate-determining step to produce the product with regeneration of the enzyme. In an experiment using an attractant diluted in dioctylphthalate, there was a significant difference in the rate of release from the dioctylphthalate and the polyethylene, and it was concluded that polyethylene can diffuse irreversibly through it and into the atmosphere. The form of the equation indicates that the release rate is directly proportional to the attractant concentration, but at higher concentrations, it is limited by the rate of diffusion through the polyethylene.

It is suggested that practical sachets should be made of polyethylene sachets containing volatile material sealed in a polyethylene bag for sport field material. It will continue to diffuse through the polyethylene until the concentration inside and outside the sachet is equal. The sachet will be used to determine the amount of the contents when contained.

Laboratory analysis of the effect of temperature on the release rate both showed that there is an optimum temperature for the release rate. In most cases, the release rate in the laboratory and field were similar, although the field data were more variable. However, from laboratory trials, it is assumed that at least part of the field data may be due to the effect of temperature on the release rate. The release rate in the laboratory was held constant.

The results for the release of 4-methylphenol, *n*-propylphenol and octenol are shown in Figure 1. It is noted that the release rate in the field is 20-30% greater than the laboratory. Moreover, the release rate in the field is a function of temperature, being consistently less than predicted. This may be a field artefact due to high temperatures, the results obtained from sachets in the field. The sachets were stored in the laboratory for a short time before they were used at field temperature. The few minutes of storage, especially at high temperatures, may 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.

A simple day:degree model to predict the loss of attractants from sachets in the shade 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. 5 & 6) 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 ca. 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, 1985; 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 ca. 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 was 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. 1 and 10). The low temperature rates would increase the catch significantly (Vale & Hall, 1985; 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 were not significantly different from those 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 insulated. 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 cm² sachets. To minimise 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* for instance, a 3:1 blend of 3-methylphenol and octenol is used (Mérot & Filledier, 1991); initial studies showed that sealed polythene tubes were effective dispensers (Filledier & Mérot, 1989), but more recently sachets (50 cm², 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* L. (Green *et al.*, 1993), stable fly, *Stomoxys calcitrans* L. (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 cm² 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, 1985, Vale *et al.*, 1988) than that produced by the standard 50 cm² 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 effect 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 8:4:1 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 2 sachets a year, changing to a 12:1:6 blend of attractant from an 8:1:4 blend produced an annual saving of £30,800.00 in the foreign exchange expenditure of the Department.

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Table 1. Release rates 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 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	0.26	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	0.01	0.23	0.17	29:1:24	20:1:15
16:1:8	0.38	0.25	0.010	0.01	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	0.1	0.09	9:1:3	5:1:2

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

Table 2. Detransformed mean catch (transformed mean in brackets) of *G. 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	<i>G. m. morsitans</i>	<i>G. pallidipes</i>
	26 (1.438)a	467 (2.670)a
	28 (1.465)a	493 (2.694)a
	21 (1.352)a	193 (2.287)b
	12 (1.275)b	106 (2.031)c
	0.0621	0.0438

Fig. 1. Effect of surface area (A) and sachet thickness (B) on the release rates of attractant from 50 cm² sachets. Attractant consisted of an 8:1:4 blend of 4-methylphenol, 3-*n*-propylphenol and octenol. Open and solid 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.

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 $y=x/(a+b.x)$. Standard errors of a and b for octenol were 1.631 and 0.04033, and for 4-methylphenol 8.550 and 0.1982, respectively.

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 cm², 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.

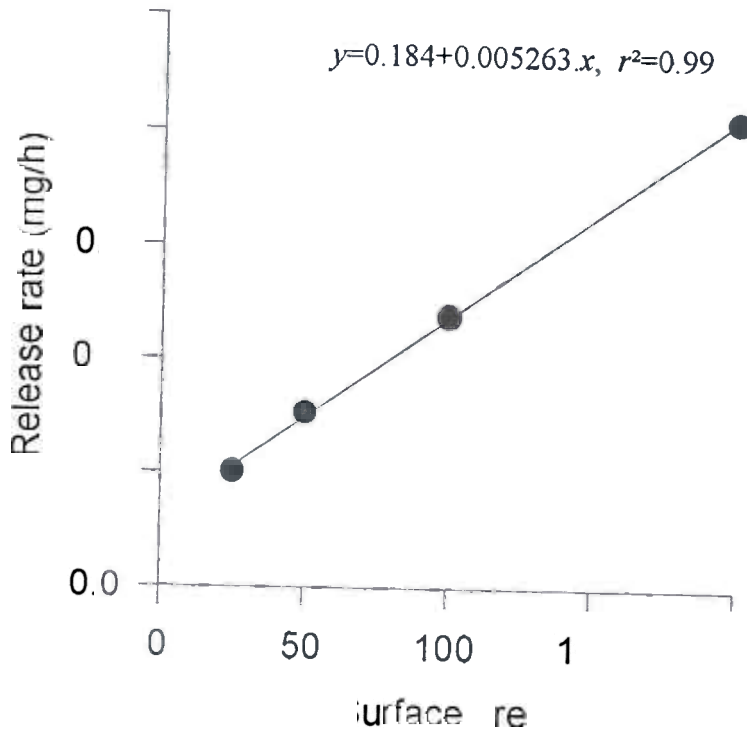
Fig. 7. Mean release rate of attractants from standard sachets in blue or black pockets and placed in the shade (solid bars), exposed to direct sunlight (open bars) or protected by cardboard shields (hatched bars, black pockets only) and the mean temperature (circles) for each month. All release rates were estimated gravimetrically. Blue and black pockets contained sachets of 4-methylphenol, 3-*n*-propylphenol and octenol in the ratio 8:1:4 or 12:1:6 respectively.

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 black bulb temperature (D).

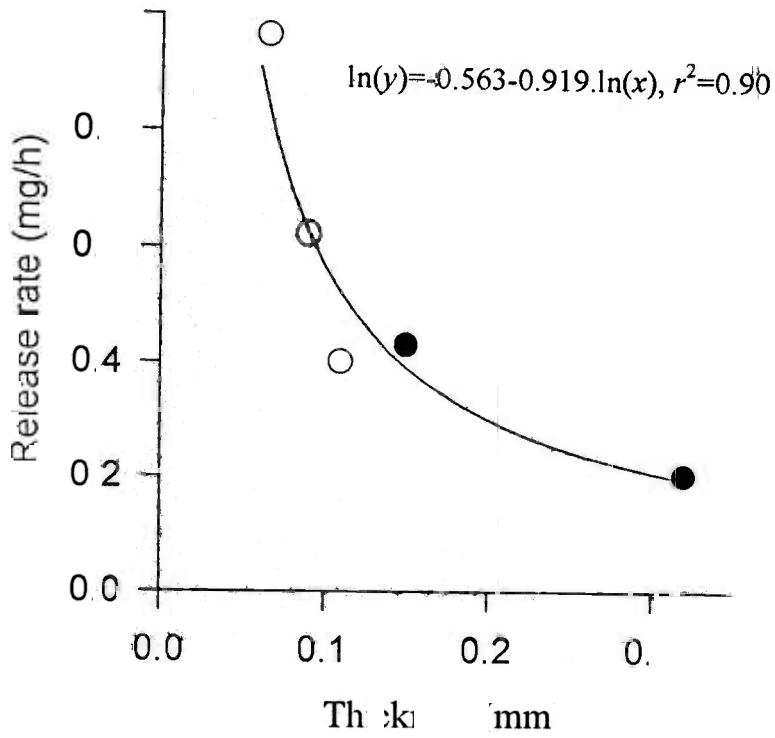
Fig. 9. Scatterplot of observed weight loss of attractant from a Standard sachet containing 12:1:6 blend of 4-methylphenol, 3-*n*-propylphenol and octenol and the predicted loss using an hour:degree or day:degree model of sachet performance. Plots show predicted losses based on observed temperatures (●) or with temperature correction (○) of -0.2° C or -0.8°C for hour-degree and day-degree models respectively. The insolation

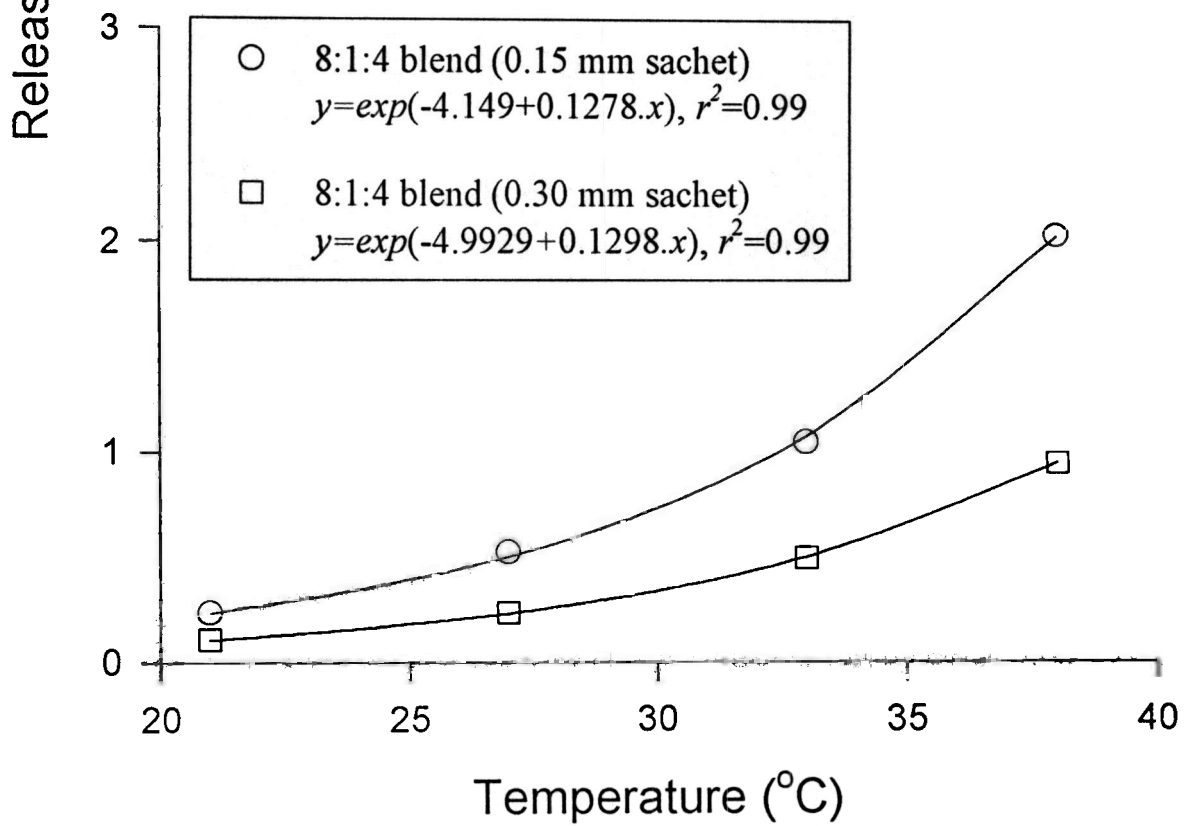
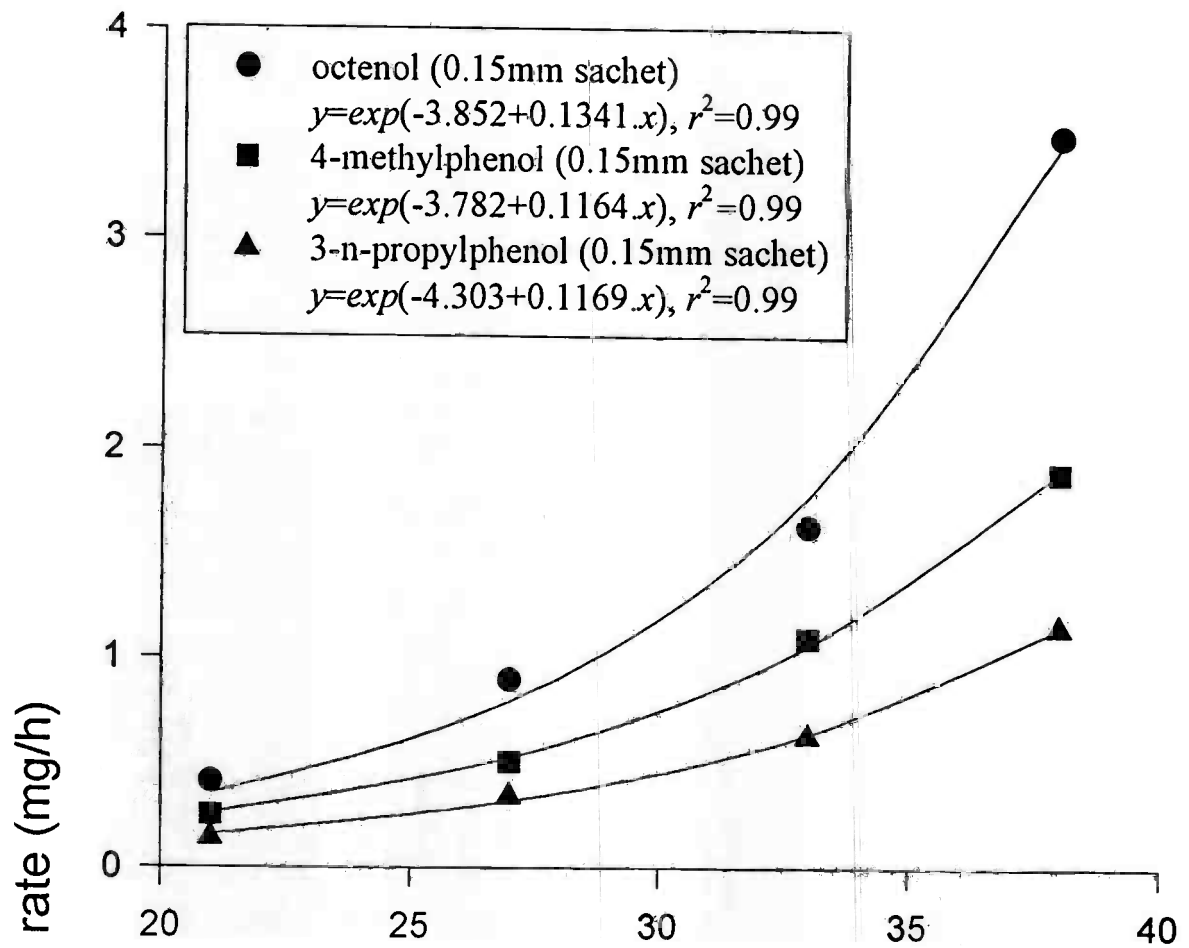
model shows the observed weight loss for sachets in full sun and the predicted rates based on a day-degree model with $+7.6^{\circ}\text{C}$ correction to the daily mean temperature. Solid line indicates the perfect fit between the observed and predicted weight losses.

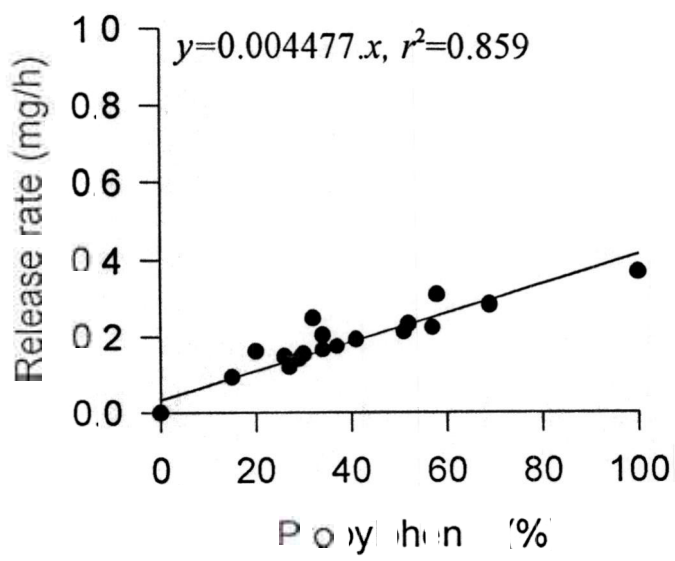
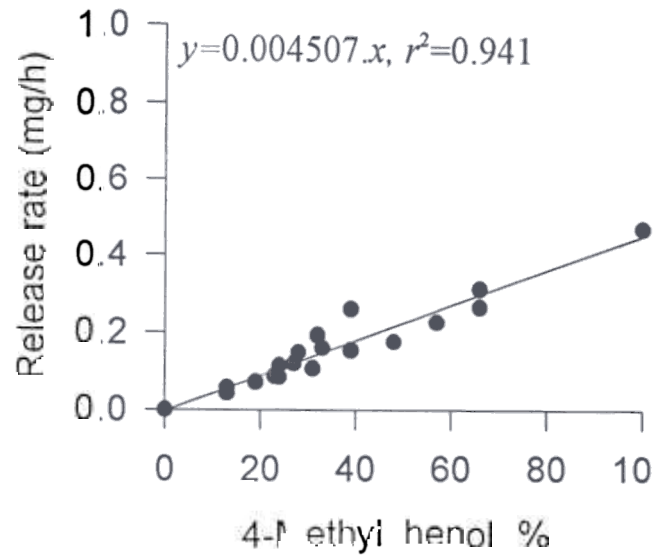
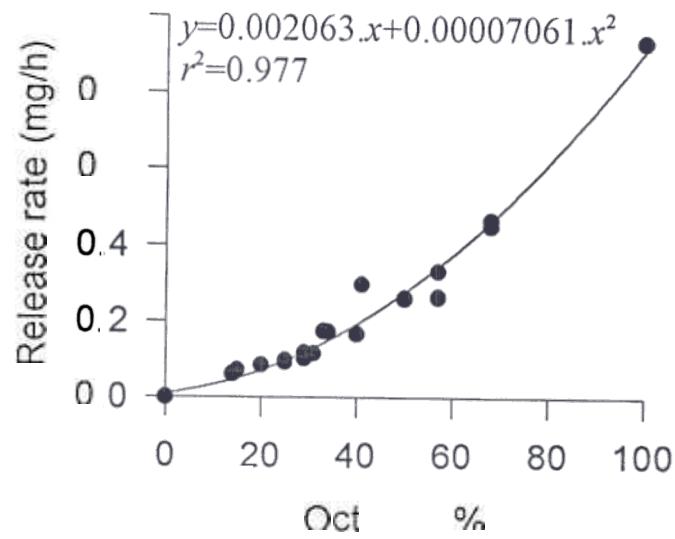
A. Effect of surface area on release rate

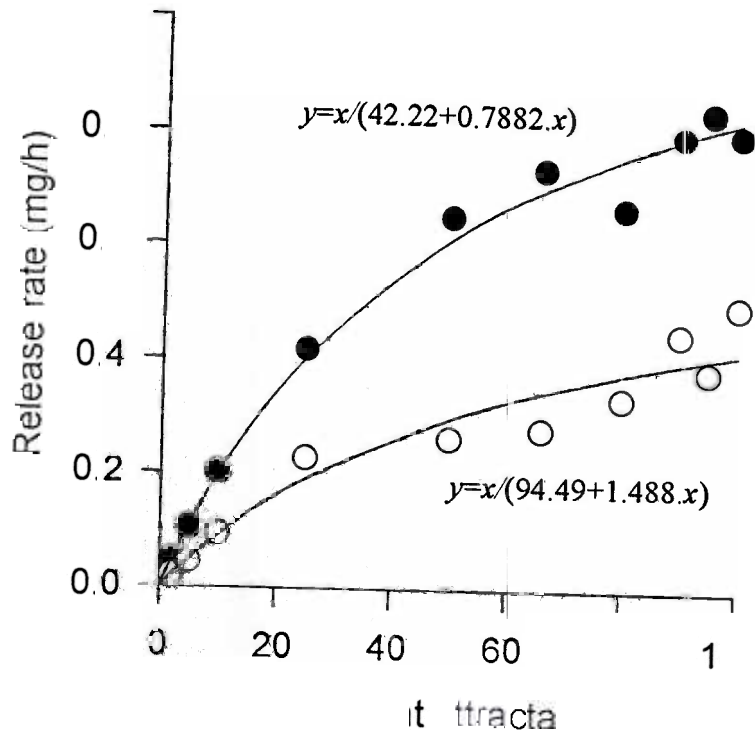


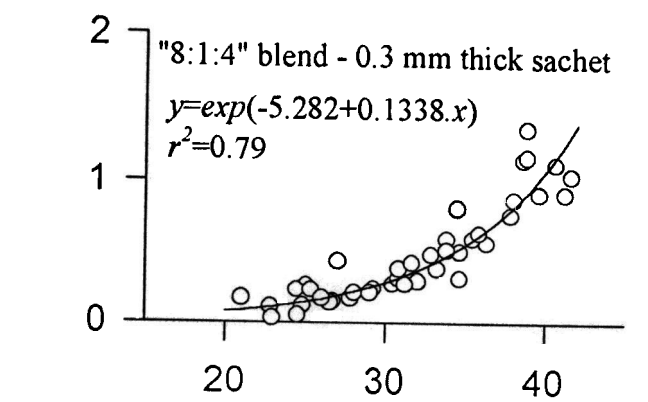
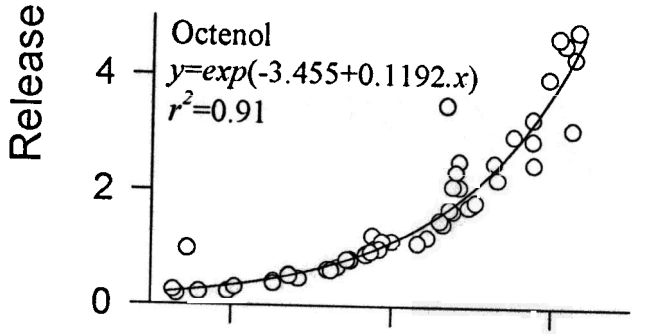
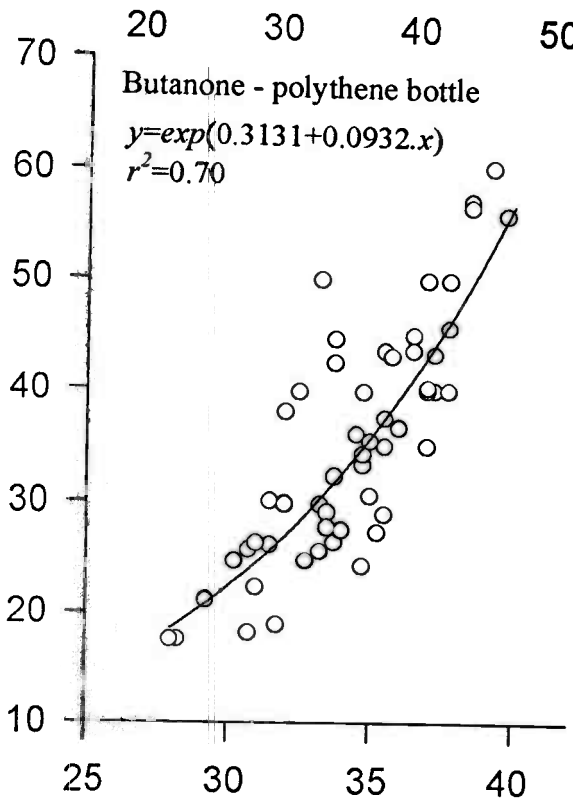
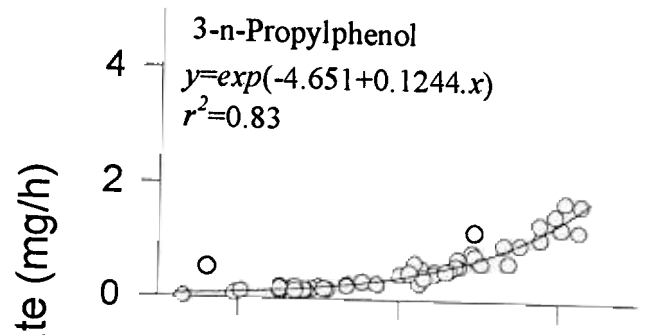
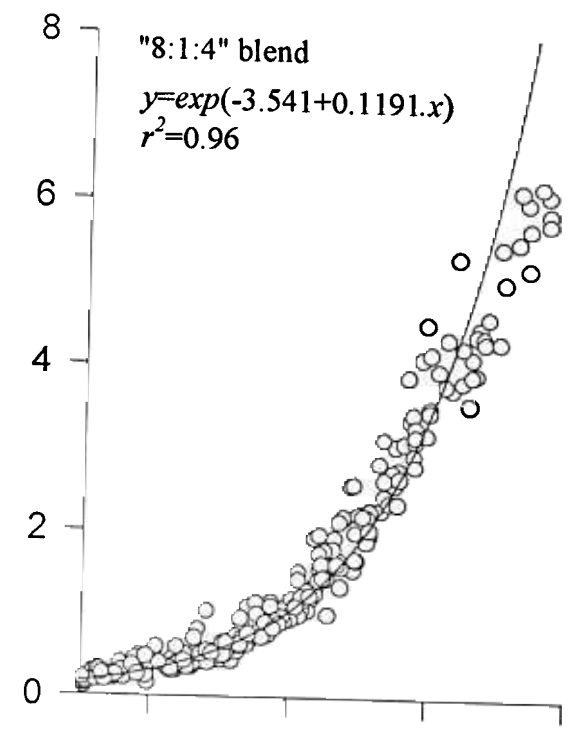
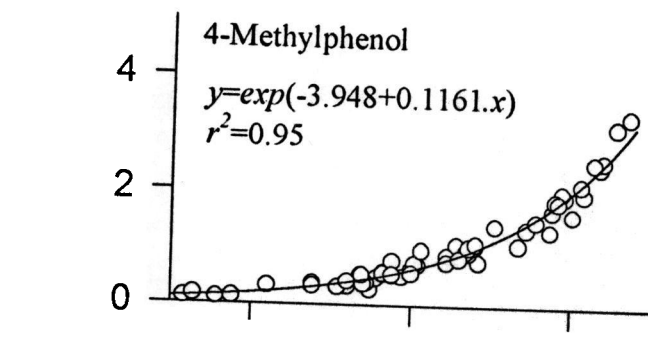
B. Effect of thickness on release rate





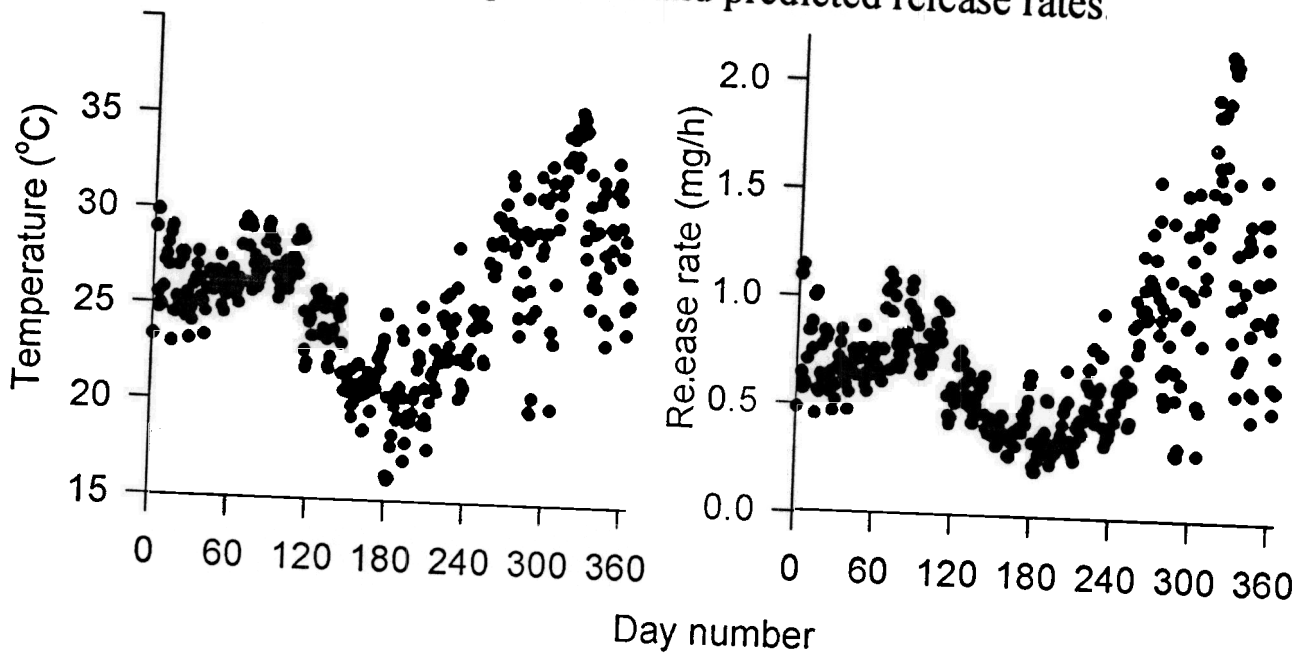




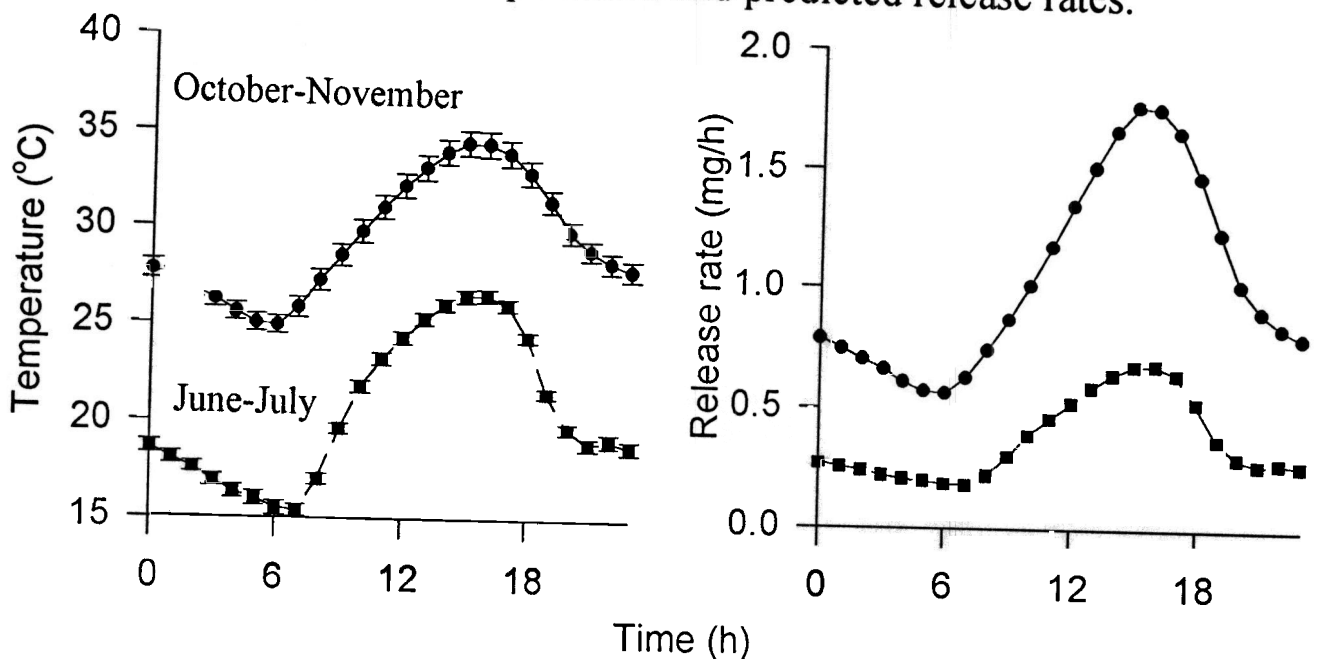


Temperature (°C)

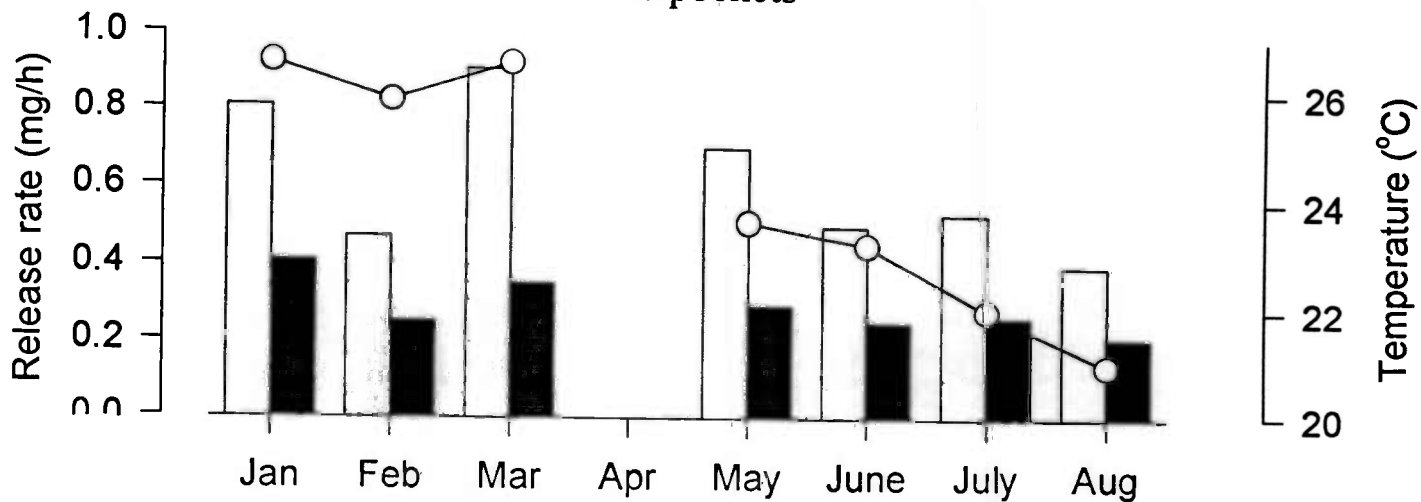
A. Daily mean temperatures and predicted release rates



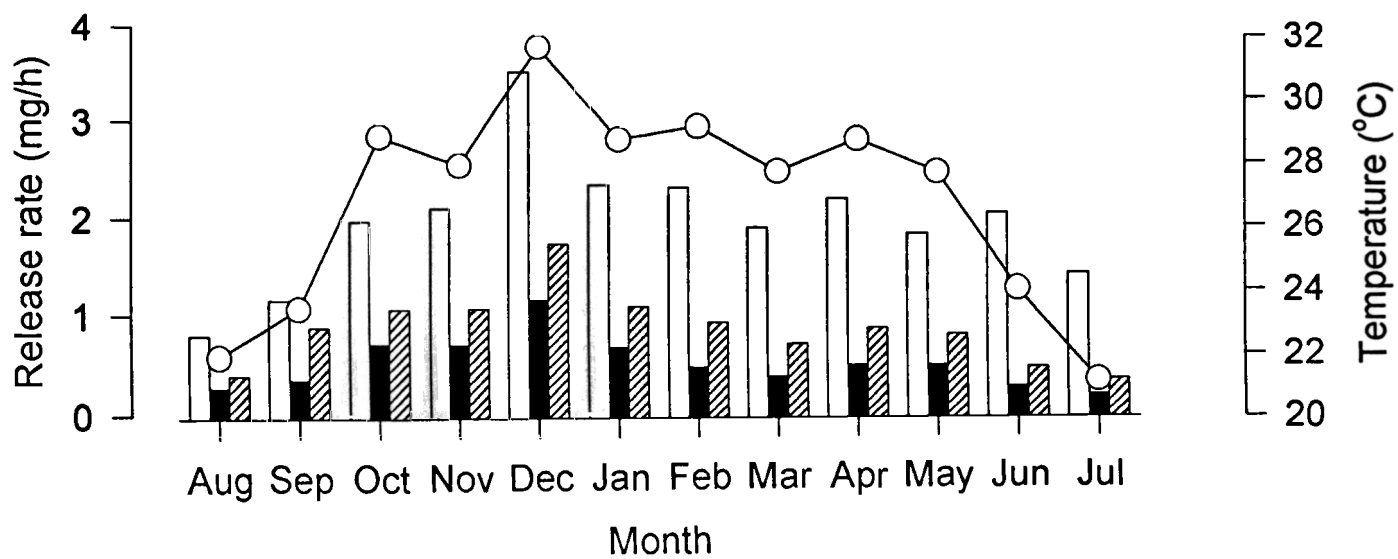
B. Hourly mean temperatures and predicted release rates.



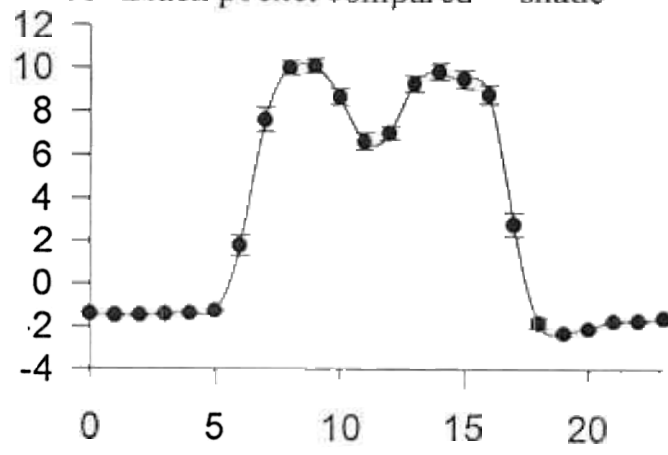
Blue pockets



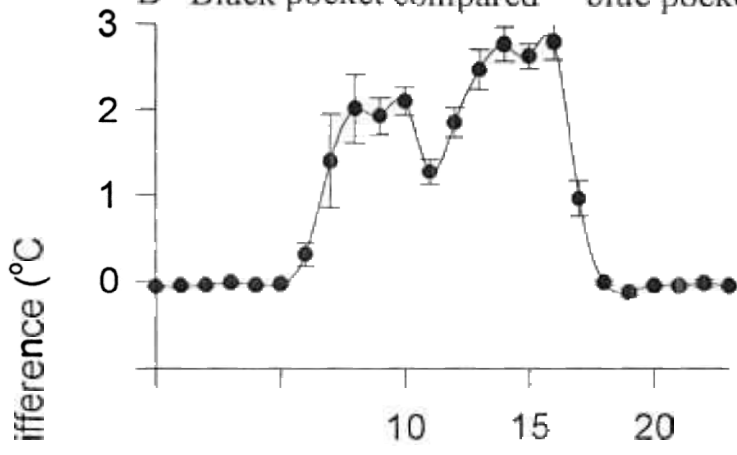
Black pockets



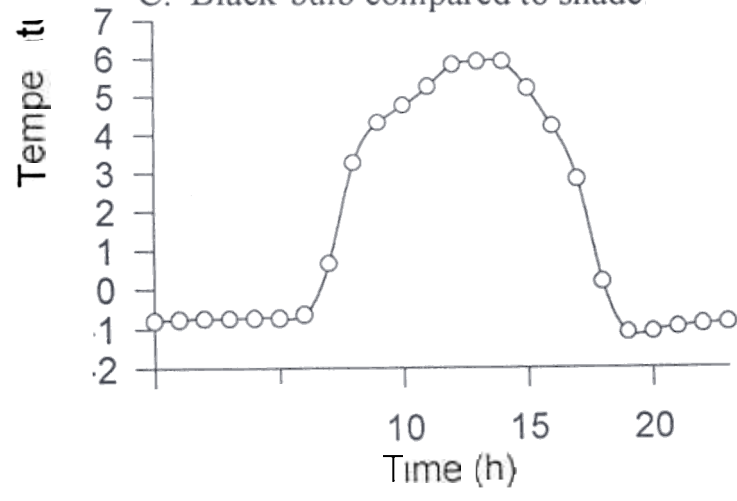
A Black pocket compared shade



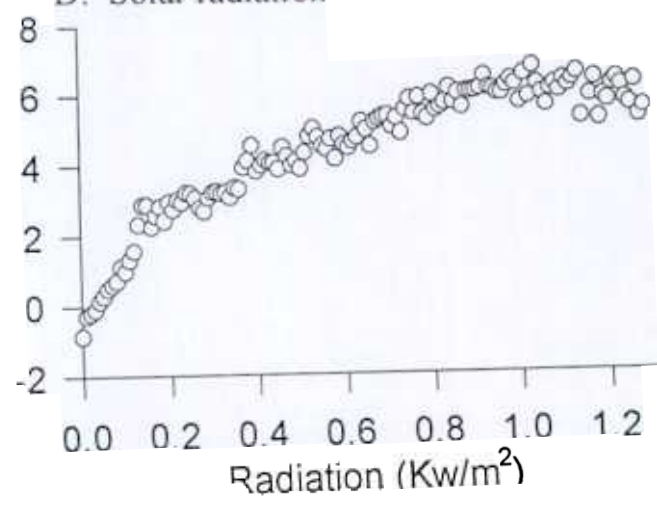
B Black pocket compared blue pocket



C. Black-bulb compared to shade



D. Solar radiation



A. Hour-degree model

