Natural Resources Institute

IDENTIFICATION OF HOST ODOUR ATTRACTANTS FOR TSETSE FLIES

FINAL REPORT 1986-1990

Regional Tsetse and Trypanosomiasis Control Programme
Malawi, Mozambique, Zambia and Zimbabwe

FIFTH EUROPEAN DEVELOPMENT FUND
REGIONAL TSETSE AND TRYPANOSOMIASIS CONTROL PROGRAMME

NATURAL RESOURCES INSTITUTE

TSETSE RESEARCH LABORATORY

IDENTIFICATION OF HOST ODOUR ATTRACTANTS FOR TSETSE FLIES

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1. SUMMARY AND RECOMMENDATIONS

1.1. INTRODUCTION

Tsetse flies, *Glossina* spp., are blood-feeding insects and vectors of trypanosomes, microorganisms which cause sleeping sickness in man and a similar disease, "nagana" in domestic animals. The economic importance of trypanosomiasis is the constraint it imposes on orderly rural development in Africa, leading to under-exploitation of infested land and over-exploitation and degradation of trypanosomiasis-free areas.

Traps and targets which attract tsetse flies and kill them could provide environmentally-acceptable, appropriate technology for monitoring and control of tsetse in Africa. Unbaited devices providing only visual attraction have proved effective in monitoring and control of riverine species of tsetse, but not the savannah species found in the flybelt of Malawi, Mozambique, Zambia and Zimbabwe covered by the EDF Regional Tsetse and Trypanosomiasis Control Project (RTTCP).

Previously, collaborative was begun between glossinologists of the Zimbabwe Department of Veterinary Services (DVS) and UK Tsetse Research Laboratory (TRL) and chemists at NRI. This brought together the experience of the DVS in the field, the experience of TRL in laboratory bioassay work, and the experience of NRI in using gas chromatography linked to electroantennography (GC-EAG) and chemical techniques to detect and identify insect behaviour-modifying chemicals.

Tsetse attractants produced by host animals were identified and synthesised, and dispensing systems for these compounds devised. Traps and targets impregnated with insecticide, baited with these lures were shown to provide effective control of the savannah tsetse species, *G. pallidipes* and *G. m. morsitans*.

1.2. OBJECTIVES

This Project aimed to speed up the ongoing work at NRI and TRL to increase the attractiveness, longevity and practicality of odour baits used with traps and targets for monitoring and control of savannah species of tsetse.

RTTCP funding provided an additional post-doctoral fellow for three years 1986-1989, and additional funding for NRI and TRL staff in a fourth year, 1989-1990. Funds were also provided for field and liaison visits by Project staff, and for setting up and maintenance of a calf box at TRL.

The cost of the Project to the RTTCP over the four years 1986-1990 was £120,135.

1.3. ACHIEVEMENTS

1.3.1. Isolation, identification and synthesis of compounds eliciting EAG responses from tsetse flies.

(a) Most of the attractiveness of cattle urine to tsetse flies was shown to be due to 4-methylphenol and 3-propylphenol. A mixture of these two compounds can increase catches of *G. pallidipes* in traps already baited with acetone and 1-octen-3-ol (octenol) by up to six times (Section 3.2.5.).

(b) 3-Propylphenol was previously unavailable commercially. Palmer Research Ltd. were assisted in producing this material, and 300 kg have been supplied to date to the RTTCP and other tsetse control organisations (Section 3.2.9.).

(c) 2-Methoxyphenol, a minor component of cattle urine, was shown to be highly repellent to *G. pallidipes* and *G. m. morsitans*. Acetophenone was found to be similarly repellent, and these compounds could have use in reducing the tsetse challenge to cattle (Section 3.2.6.).

(d) Indole and 3-methylindole were identified as minor components of cattle urine eliciting strong EAG responses from tsetse flies, and field experiments suggested they may have some attractiveness to tsetse in the field (Section 3.3.).

(e) Two other minor components of cattle urine eliciting strong EAG responses from tsetse, Peak 6 and Peak 7, were identified as the carotenoid metabolites, *cis* and *trans* 3,3,5-trimethyl-4-hydroxy-4-(3-oxobutyl)-cyclohexanone. The synthetic compounds showed behavioural activity in the laboratory bioassay but not in the field. These compounds have not been isolated previously from animal sources (Section 3.4.).

(f) Bushpig bedding sacks were reported to be attractive to tsetse flies in the field. Extensive analytical work identified vanillin, acetovanillone and 1-methylhydantoin as new compounds present. Vanillin and acetovanillone showed behavioural activity towards tsetse in the laboratory bioassay, but no effects could be measured in the field (Section 3.5.).

(g) Although previous field and laboratory experiments suggested that ox odour contained component(s) - "Omega" - that passed through a charcoal filter and greatly increased the attractiveness of the already-identified tsetse attractants, more definitive field experiments indicated that this effect may have been due to carbon dioxide, and that no major tsetse attractants remain to be identified in ox odour (Section 3.6.).

(h) During laboratory work on Omega, water vapour was shown to be the only compound causing an EAG response from tsetse in unconcentrated human or cattle breath. Addition of water vapour did not increase catches of tsetse in the field (Section 3.6.).

(i) 2,6-Di-*tert*-butyl-1,4-benzoquinone was identified as the compound responsible for EAG response elicited by extracts of charcoal used to filter ox odour. This compound did not attract tsetse flies in the field (Section 3.7.).

(j) Dr. Warnes of TRL was assisted in his investigations of the effect of cattle sebum on tsetse behaviour. (*E*)-Phytol was identified as a major component of the sebum (Section 3.8.).

(k) In all, 21 compounds were investigated during this project (Section 3.9.).

1.3.2. Development of dispensing systems for use with tsetse attractants in the field

(a) Standard methods were developed for measuring release rates of tsetse attractants in the laboratory (Section 4.1.1.).

(b) Polythene vials, tubes and sachets were investigated as sealed dispensers for mixtures of octenol and the phenolic attractants in the laboratory and field (Section 4.1.).

(c) Release rates from polythene tubes declined after about 10 weeks in the field, but they have been recommended for use with *G. tachinoides* in West Africa (Section 4.1.5.).

(d) Polythene sachets containing a 8:1:4 mixture of 4-methylphenol, 3-propylphenol and octenol were shown to give highly linear release of the contents and to remain attractive to tsetse for at least three months under field conditions. These have been adopted as standard dispensers in the Region and elsewhere (Section 4.1.9.).

(e) Sealed polythene and polypropylene dispensers for butanone were developed that give linear release and are at least as attractive to tsetse as the open bottle dispensers for acetone currently used in the field (Section 4.2.).

1.3.3. Collaboration with the Tsetse Research Laboratory.

(a) The post-doctoral fellow working on this Project was employed by TRL and Bristol University, and close collaboration was maintained with TRL during this project,

particularly in the bioassay work of Prof. Bursell and the work on ox sebum by Dr. Warnes (Section 5).

(b) All tsetse flies used during laboratory work were supplied from colonies maintained at TRL with funding from ODA (Section 5.2.).

1.3.4. Liaison with tsetse control operations in West Africa.

(a) Close liaison was maintained throughout the Project by exchange of materials and results with tsetse workers at the CRTA in Burkina Faso and at GTZ and the OCCGE in Côte d'Ivoire. Assistance was also given to Swedish workers in Guinea-Bissau (Section 6).

(b) Project staff visited Burkina Faso and Côte d'Ivoire on consultancy visits to ensure that results on tsetse attractants obtained in West Africa were comparable with those obtained in the Region (Section 6).

(c) *G. tachinoides* was shown to be attracted by host odours in Burkina Faso, and most of this attractiveness could be accounted for by carbon dioxide, 3-methylphenol, 4-methylphenol and octenol. Acetone was not attractive (Section 6.2.).

(d) Polythene tubes developed during this Project containing a 3:1 blend of 3-methylphenol and octenol were recommended as lures for *G. tachinoides* in Burkina Faso (Section 6.2.8.).

(e) In Côte d'Ivoire and Guinea-Bissau, *G. longipalpis* was attracted by the urine phenols and acetone but not octenol (Sections 6.3., 6.5.).

(f) No reliable attractants were found for *G. palpalis*, although some attraction by acetone and octenol was claimed (Section 6.4.).

1.3.5. Field and liaison visits by Project personnel.

(a) Twelve field and/or liaison visits between Europe and Africa by Project staff were funded by the Project (Section 7.1.1.).

(b) In addition, two visits to West Africa were funded by the EC DGXII and GTZ respectively (Section 7.2.).

1.3.6. Dissemination of results

(a) Six refereed papers were published by Project staff (Section 8.1.).

(b) Seven presentations were given by Project staff at Conferences or meetings (Section 8.2.).

(c) Assistance by Project staff was acknowledged in 10 other papers (Section 8.3.).

1.4. RECOMMENDATIONS

1.4.1. Isolation, identification and synthesis of compounds eliciting EAG responses from tsetse flies.

(a) The 3- and 4-*iso*propylphenols elicited strong EAG responses from tsetse and are readily available. Although they were reported to have no field activity as replacements for 3-propylphenol, this should be checked (Section 3.2.).

(b) Indole and 3-methylindole elicited strong EAG responses from tsetse, and there was some indication of attractiveness in the field: this should be tested further (Section 3.3.).

(c) The carotenoid metabolites (6), (7), (6K) and (7K) were active in both EAG and windtunnel bioassays but not in the field. It is unlikely that it would be economically feasible to use these unusual compounds in tsetse control, but it is recommended that their behavioural activity be investigated further (Section 3.4.).

(d) Variable results on the attractiveness of vanillin and acetovanillone in the field should be checked definitively (Section 3.5.).

(e) Field experiments indicating that ox odour does not contain any major unidentified attractants for tsetse should be completed definitively. These experiments will also confirm whether there are still some minor unidentified attractants which are adsorbed on a charcoal filter (Section 3.6.).

(f) The lack of field activity of 2,6-di-*tert*-butyl-1,4-benzoquinone should be confirmed (Section 3.7.).

(g) Chemical support of Dr. Warnes work on compounds in ox sebum affecting tsetse behaviour should continue (Section 3.8.).

(h) The possibilities of making electrophysiological recordings from single receptors on tsetse antennae should be investigated in order to examine further the specificity of compounds detected in linked GC-EAG analyses (Section 3.9.).

1.4.2. Development of dispensing systems for use with tsetse attractants in the field.

The effects of temperature on release rates from sealed dispensers for the phenols/octenol and for the acetone/butanone components of tsetse baits should be determined in controlled laboratory experiments (Section 4.3.).

1.4.3. Liaison with tsetse control operations in West Africa

Exchange of results and materials should be continued with organisation in West Africa in order to confirm the results so far on attractants for West African species of tsetse (Section 6.6.).

1.4.4. Collaboration in Europe

As a result of visits to three other laboratories in Europe, it was recommended that collaboration should be started with Prof. Boeck of Regensburg University in Germany to investigate the possibilities of making electrophysiological recordings from single olfactory receptors on tsetse antennae.

2. INTRODUCTION AND OBJECTIVES

2.1. INTRODUCTION

2.1.1. Tsetse and trypanosomiasis

Tsetse flies, *Glossina* spp (Diptera: Glossinidae), are blood-feeding insects and vectors of trypanosomes, microorganisms which cause sleeping sickness in man and a similar disease, "nagana", in domestic animals. The flies have been reported to infest around 11 million square kilometres of Africa (Hagan and Wilmshurst, 1975), and human and animal trypanosomiases causes much mortality and morbidity. The economic importance of tsetse and trypanosomiasis is as a constraint on orderly rural development, discouraging human settlement because of the presence of human trypanosomiasis and/or animal trypanosomiasis which prevents the keeping of animals, especially cattle, for meat, milk, manure and draught power over extensive areas. The persistence of tsetse flies in Africa thus leads to under-exploitation of infested land and over-exploitation and degradation of fly-free areas.

Trypanosomiasis is most commonly controlled with drugs, but these are relatively expensive and their effective administration requires an infrastructure which is often not present in Africa. Extensive research is being carried out to develop a vaccine, but this is hampered by the ability of trypanosomes to alter the antigenic nature of their cell wall, and administration of a vaccine would suffer from the same problems as drug administration in Africa.

2.1.2. Control of tsetse fly

The alternative approach is control or eradication of the vector of the disease, the tsetse fly. In the past, this has been achieved by destruction of woody vegetation depriving tsetse of their favoured habitat, and by destruction of wild animals which act as hosts for tsetse and reservoirs for trypanosomes. With the advent of organic insecticides, ground spraying of tsetse resting sites with persistent insecticides was used to clear large areas of tsetse and to keep them tsetse-free, e.g. in Nigeria, Zimbabwe, Kenya, Uganda and Chad (Allsopp, 1984). More recently, two new, more environmentally-acceptable methods for tsetse control have been developed - the sequential aerosol technique (SAT) (Allsopp, 1984) and use of traps and targets (Vale *et al.*, 1985; Laveissière *et al.*, 1990).

In the SAT, non-persistent insecticides are applied from fixed-wing aircraft as aerosol droplets which impact on the flies, and treatments are applied sequentially to coincide

with successive pupal emergences. SAT can be applied rapidly over large areas, and utilises very low doses of non-persistent insecticides which have been proven to have minimal side effects on non-target organisms. The technique has been used widely in Zimbabwe and Botswana, but it is expensive in foreign currency, requiring aeroplanes and sophisticated application and navigation equipment as well as insecticide. Good organisation is also required to ensure the regular and complete applications which are essential for success of the technique.

2.1.3. Traps and targets

In this approach, tsetse flies are attracted to traps or screens - so-called "targets" - and are either caught or killed by insecticide on the devices. The first extensive use of traps for tsetse control was by Harris (1938) in Zululand, but this method became unfashionable with the advent of organic insecticides. More recently, this approach has been exploited by French workers in West Africa for monitoring and control of riverine species of tsetse. "Biconical" or "monoconical" traps constructed of blue, white and black cloth on a wood or metal frame are used for detecting the presence of tsetse, and similar traps or just simple blue cloth screens impregnated with insecticide are used for control. These devices simulate the visual stimuli provided by host animals to tsetse flies. They are relatively cheap and simple to construct, often with a high local content and they require only relatively simple organisation of local labour to deploy and maintain along riverine tsetse habitat

Such traps and targets have ben used to control riverine species of tsetse in Côte d'Ivoire, the Congo and Burkina Faso (Laveissière *et al.*, 1990; Cuisance *et al.*, 1984, 1990). However, they were not effective against the more wide-ranging, savannah species of the *morsitans* group. Thus in Burkina Faso, insecticide-impregnated traps and screens placed at 100 m intervals along riverine vegetation were used to reduce populations of riverine tsetse species, *G. tachinoides* and *G. palpalis gambiensis*, prior to release of sterile males, and also to provide a barrier to reinvasion (Cuisance *et al.*, 1984, Politzar and Cuisance, 1984, Cuisance. *et al.*, 1990). These unbaited traps and screens were ineffective against the savannah species, *G. m. submorsitans*, and it was calculated that densities of 33 screens per sq. km. would be required.

2.2. WORK LEADING TO THIS PROJECT

2.2.1. Early work

Working in Zimbabwe, Vale (1974) showed that the savannah tsetse species *G. pallidipes* and *G. m. morsitans* are strongly attracted to host animals by smell. By random screening of chemicals likely to be responsible for this attraction, he showed that carbon dioxide and acetone are attractants (Vale 1980). In an isolated, island situation of 4.5 sq. km., four traps baited with carbon dioxide and acetone at high dose rates of several litres per minute and several grams per hour respectively were used to reduce an introduced population of the two tsetse species (Vale *et al.*, 1986a).

2.2.2. Collaboration with NRI and TRL

Collaboration between Dr. Vale of the Zimbabwe Department of Veterinary Services (DVS), Prof. Bursell at the Tsetse Research Laboratory (TRL) and chemists at NRI began in 1982.

Vale (1982) had confirmed experimentally that tsetse detect both attractive and repellent odours by means of receptors on their antennae, and it was proposed to make use of electro-physiological techniques, particularly electro-antennography (EAG) to detect and subsequently identify the components of host odours which are responsible for attraction of tsetse flies. In EAG, microelectrodes in contact with the antennae are used to record DC potentials which occur across the antenna when antennal receptors are stimulated. NRI pioneered the use of EAG linked directly to gas chromatography (GC) in which a mixture of volatile compounds is separated into its components by the GC column, and the responses of an insect to these components are measured on-line as they elute from the column (Moorhouse *et al.*, 1969; Cork *et al.*, 1990). These techniques have been particuarly effective in identification of insect pheromones, and have been used by the NRI group to identify sex and aggregation pheromones of over 33 insect pests from developing countries.

However, EAG itself gives no information on the behavioural response of the insect to the stimulating chemical, and it was proposed that Prof. Bursell would take compounds causing an EAG response from tsetse which were identified at NRI and screen them for behavioural activity in a laboratory windtunnel before testing likely attractants in the field.

Methods for collecting ox odour were developed by NRI, and in analyses of these odours by linked GC-EAG a very minor component was detected as giving a strong EAG response from tsetse, and this was identified as 1-octen-3-ol (octenol) by chemical and spectroscopic methods (Hall *et al.*,.1984). Initial field tests with octenol failed to show any

attractiveness, but Bursell (1984) showed it was active in the laboratory. In further field work it was established that octenol increased the attractiveness of ox odour, and that mixtures of synthetic carbon dioxide, acetone and octenol could increase catches of *G. pallidipes* and *G. m. morsitans* in traps by 64 times (Vale and Hall, 1985a, 1985b).

Production rates of acetone and octenol by cattle were measured, and slow-release dispensers for octenol devised (Vale and Hall 1985a). Octenol was then incorporated into control trials in place of carbon dioxide which is not suitable for large-scale use. In the control trial referred to in para. 2.2.1., the four traps were replaced by 20 insecticide-impregnated targets baited with acetone and octenol at the economically-acceptable release rates of 100 mg/hr and 0.5 mg/hr respectively, and these rapidly eliminated all tsetse on the island (Vale *et al.*, 1986a). In a subsequent trial, similar baited targets were used at a density of four per sq. km. to reduce populations of *G. m. morsitans* and *G. pallidipes* by over 99.99% in the Rifa Triangle, an area of 600 sq. km. in north-west Zimbabwe (Vale *et al.*, 1988a).

2.3. PROJECT PURPOSE AND ORGANISATION

2.3.1. Project purpose

This project aimed to speed up the ongoing work at NRI and TRL to identify new attractants for tsetse flies and optimise the use of existing attractants. It primarily provided funding for an additional post-doctoral fellow to work with staff at NRI and TRL. Dr. Andrew Gough was recruited and worked on the Project from April 1986 to April 1989. From May 1989 to March 1990, the Project provided additional funding for NRI and TRL staff.

2.3.2. Project organisation

The Project was managed by the Scientist-in-Charge in Zimbabwe, Dr. Glyn Vale, and the Scientist-in-Charge in the UK, Dr. David Hall.

Throughout the Project, RTTCP funding was supported by an input of NRI staff time from ODA R&D funds, and NRI staff who worked with Dr. Gough included Peter Beevor, Dr. Alan Cork, Dr. Paul Adams, Mrs. Jane Smith and Jonathan Taylor. At TRL, Prof. Bursell was funded from ODA Technical Cooperation (TC) funds to Zimbabwe, and Drs. Chris Green and Martin Warnes were otherwise funded by ODA R&D. Fly production was provided from ODA funding to TRL.

2.4. OBJECTIVES

2.4.1. Wider Objectives

The wider objective of this project was to remove the constraint trypanosomiasis imposes on orderly rural development in Africa by control of tsetse flies as vectors of trypanosomiasis, using cost-effective, appropriate and environmentally-acceptable methods.

2.4.2. Immediate Objective.

The immediate objective was to increase the attractiveness, longevity and practicality of odour baits used with traps and targets for monitoring savannah species of tsetse, particularly in the flybelt of Malawi, Mozambique, Zambia and Zimbabwe covered by the EDF Regional Tsetse and Trypanosomiasis Control Project (RTTCP).

2.4.3. Outputs

The expected outputs of the Project gave rise to the headings in the Annual Work Programmes, as follows:

(a) detection, identification and synthesis of compounds causing electroantennographic responses from tsetse flies;

(b) development of dispensing systems for use with tsetse attractants in the field;

(c) collaboration with the Tsetse Research Laboratory;

(d) liaison with tsetse workers in West Africa and interchange of results on use of traps and targets to monitor and control tsetse;

(e) field and liaison visits by Project personnel;

(f) dissemination of results.

2.5. REPORT FORMAT

This Final Report is conveniently formatted according to the headings used in the Annual Work Programmes which in turn correspond to the expected outputs of the Project listed above. The work of the Project has been fully described in successive Quarterly Reports and Annual Reports, but the results in these are necessarily presented in chronological sequence. This Final Report aims to summarise the results of the Project in a coherent, logical order, and to provide a single reference document. It is intended to give sufficient detail for the Report to stand alone, but full details are available in the previous reports, and, where appropriate, these are referenced as e.g. QR 87/3 (Quarterly Report for third quarter of 1987/88), AR 89 (Annual Report for 1989/90).

3. ISOLATION, IDENTIFICATION AND SYNTHESIS OF COMPOUNDS ELICITING EAG RESPONSES FROM TSETSE FLIES

3.1. INTRODUCTION

This Project started soon after the demonstration that cattle urine was attractive to tsetse flies, and much of the work carried out in the Project on identification of potential tsetse attractants involved investigation of components of animal urines and other animal residues. A second major theme arose from prior work by Vale and Hall in Zimbabwe which indicated that ox odour contained very volatile attractants or synergists of other attractants, which were not trapped by activated charcoal. This component - or components - was christened "Omega" by Prof. Bursell.

3.2. IDENTIFICATION OF PHENOLIC ATTRACTANTS FROM CATTLE URINE

3.2.1. Introduction

Cattle urine was shown to attract tsetse flies, particularly *G. pallidipes*, by workers at ICIPE in Kenya (Owaga, 1984; 1985) and this was subsequently confirmed by Vale *et al.* (1986b) in Zimbabwe. This project started soon after the discovery by Dr. Vale, using materials supplied by NRI, that the phenolic fraction of cattle urine accounted for most of the attractiveness to tsetse of the whole urine. Initial work thus concentrated on identifying the components of this phenolic fraction, determining the relative EAG activities of the different phenols and supplying material for laboratory bioassay at TRL and for field testing in Zimbabwe.

3.2.2. Extraction and fractionation of cattle urine

Both cattle urine and chloroform or dichloromethane extracts of urine were shown to increase catches of *G. pallidipes* in traps baited with acetone and octenol (Vale *et al.*, 1986b; Bursell *et al.*, 1988). Dichloromethane extracts of active urine were analysed by linked GC-EAG. The main components were simple alkylphenols, and these elicited strong EAG responses from both *G. m. morsitans* and *G. pallidipes*. There was also a relatively much stronger EAG response to one very minor component, christened Peak 6 according to its order of elution in the chromatogram (Fig. 3.2.1.).

A standard procedure was developed for chemical fractionation of urine samples with dichloromethane into phenolic, acidic

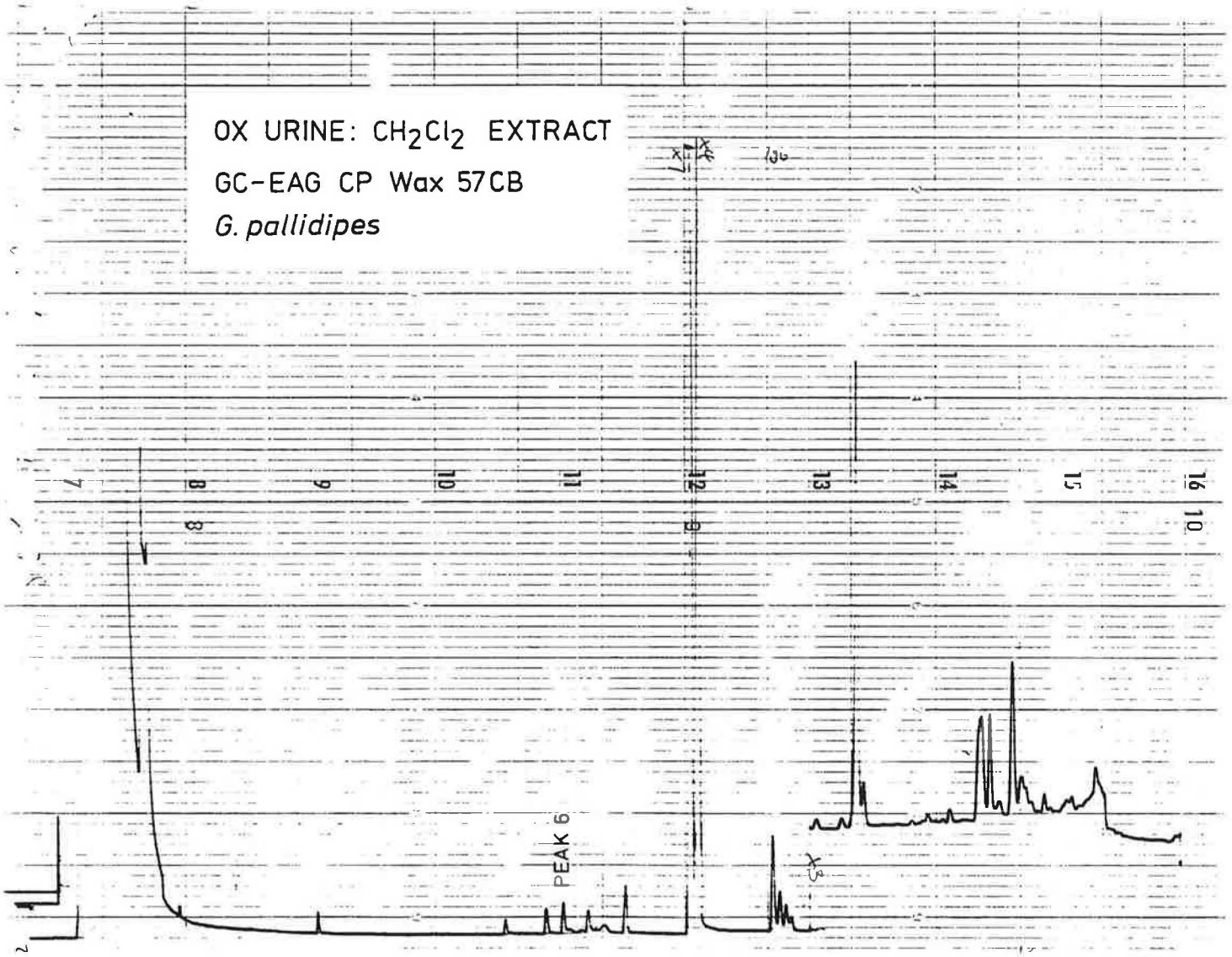


Fig. 3.2.1. Gas chromatogram of dichloromethane extract of ox urine (polar CP Wax 57CB fused silica capillary column)

and non-acidic fractions (Bursell *et al.*, 1988). The Peak 6 was a major component of the non-acidic fraction (Fig. 3.4.1.), and this was separated from the other components in this fraction by liquid chromatography (LC). The phenolic and acidic fractions were field tested in Zimbabwe along with fractions excluding those containing Peak 6 from LC of the non-acidic fraction. Results showed that the phenolic fraction was as attractive to *G. pallidipes* as the unfractionated urine (Table 3.2.1.).

Table 3.2.1. Catch indices for addition of urine and fractions to F3 traps baited with acetone and octenol, Rekomitjie 1985.

Additional odour[1]	Catch Index[2] GMM	GP
Ox urine	1.27	1.74**
Acidic fraction	1.54	1.38
Phenolic fraction	1.01	2.92***
Non-acidic fraction: non-polar	1.01	1.01
Non-acidic fraction: medium polar	1.10	0.73
Non-acidic fraction: polar	1.09	0.65*

[1] urine and fractions equivalent to 200 ml urine; all traps baited with acetone at 500 mg/hr and octenol at 0.5 mg/hr

[2] catch index relative to mean catch in traps baited with acetone and octenol only; *, **, *** indicate indices differ from unity at 5%, 1% and 0.1% levels

3.2.3. Analysis of phenolic fractions

The phenolic fraction was analysed by capillary GC with a flame ionisation detector or linked to an Ion Trap Detector giving mass spectra of the eluted components. Components were identified by their mass spectra and co-chromatography with synthetic phenols, and a typical GC trace is shown in Fig. 3.2.2.

Capillary GC did not resolve the 3- and 4-propylphenols. However, it was discovered that after simple acetylation these

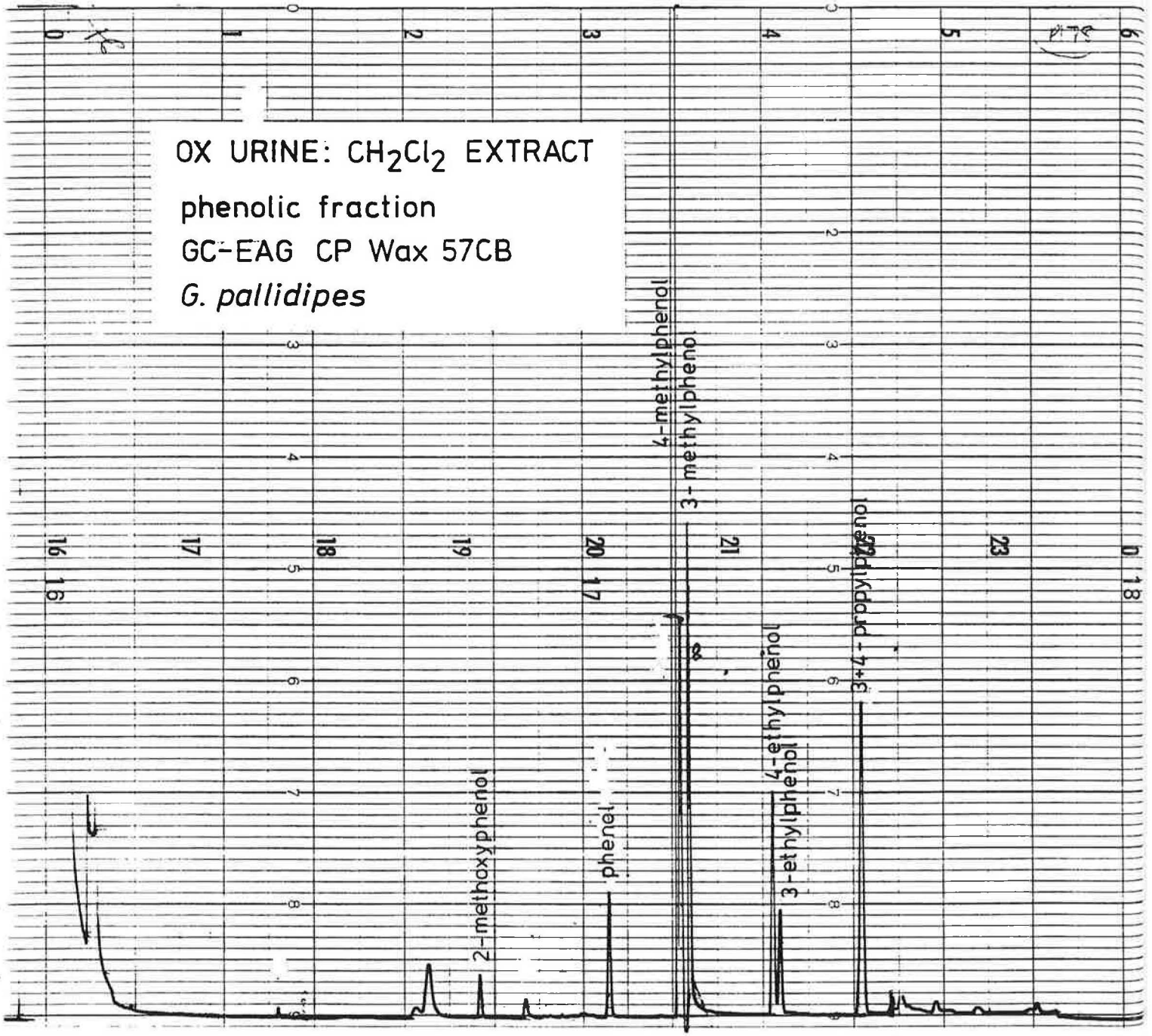


Fig. 3.2.2. Gas chromatogram of phenolic fraction from dichloromethane extract of ox urine (polar CP Wax 57CB column)

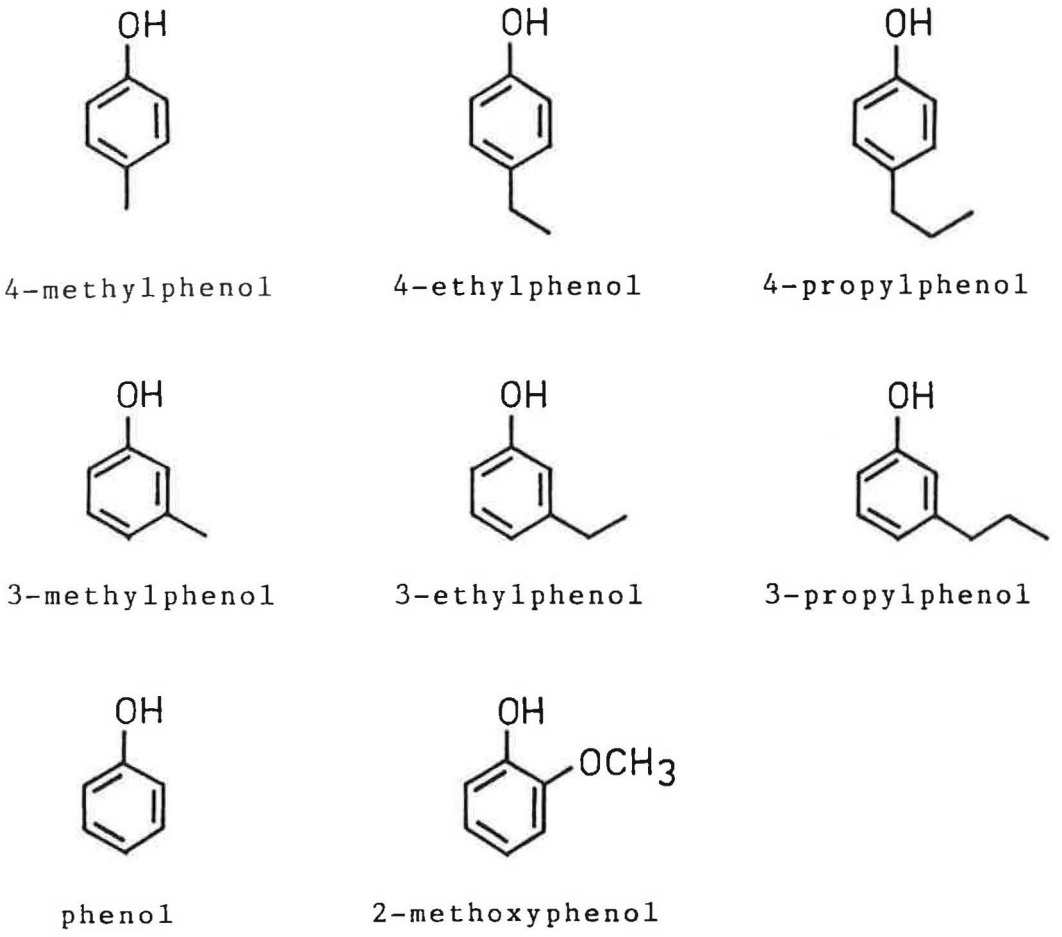


Fig. 3.2.3. Chemical structures of phenols identified in cattle urine

two phenols were well resolved by capillary GC (Bursell *et al.*, 1988).

3.2.4. Composition of the phenolic fractions of different urines.

During the course of this project, the phenolic fractions of urines from various animals were analysed, and the results are summarised in Table 3.2.2.

Up to 8 phenols were detected, including phenol itself and the 3- and 4- isomers of methyl-, ethyl- and propylphenol. In general, 4-methylphenol was the major phenol present, and, whereas for the methylphenols and ethylphenols the 4-isomer predominated over the 3-isomer, in the propylphenols the 3-propylphenol was the major isomer. Trace amounts of 2-methoxyphenol were detected in some samples. Similar results were reported by the ICIPE group (Hassanali *et al.*, 1986), although they did not detect 2-methoxyphenol. The chemical structures of the phenols identified are shown in Fig. 3.2.3.

The one sample of bushbuck urine examined contained 3-methylphenol as the major component. Bushbuck is a favoured host of *G. tachinoides*, and 3-methylphenol has been shown to be an attractant for this species in Burkina Faso (Section 6.2.7.; QR 88/1).

In the samples of attractive ox and buffalo urine analysed initially, the concentration of 4-methylphenol was estimated to be approximately 0.5 mg/ml, and this was taken as a standard for the "natural" concentration in experiments with synthetic phenols.

Samples of urine from Zebu and trypanotolerant N'Dama cattle (four animals each) were analysed to investigate whether there were any signficiant differences in composition that might cause the N'Dama animals to be less attractive to tsetse flies. As shown in Table 3.2.2., there were no major differences in composition or concentration, allowing for the variation between individuals (AR 89).

3.2.3. Composition of acidic fraction

In view of the lack of field activity of the acidic fraction from the urine in the field tests, only two such samples were analysed by GC-MS using a polar capillary column. Components were identified by comparison of their GC retention times and mass spectra with those of synthetic acids. The main components were benzoic acid and phenylacetic acid in approximately 10:1 ratio with a trace of the higher homologue, 3-phenylpropionic acid. Concentration of benzoic acid in the urine was typically 2 mg/ml. The pH of the urine was

TABLE 3.2.2.. Analyses of phenolic fractions of various animal urines

Animal	Origin	Relative concentration[1]								Concn. (µg/ml)	Ref
		2MeO	PhOH	4Me	3Me	4Et	3Et	4Pr	3Pr		
ox	Zimbabwe	0.4	1.4	100	9.9	2.1	1.1	0.8	2.5	500	QR 86/1
ox	Zimbabwe	0.2	0.4	100	1.7	4.1	0.7	0.6	3.7	500	QR 86/1
buffalo	Zimbabwe	tr	1.4	100	1.5	8.8	1.2	0.4	4.3	500	QR 86/1
pig	Zimbabwe	-	3.7	100	2.0	8.0	-	-	-	50	QR 86/4
bushbuck	Côte d'Ivoire	-	2.9	13.4	100	0.7	-	-	-		QR 87/2
camel	Somalia	-	3.7	100	28.0	0.7	2.1	0.08	0.46		QR 88/2
zebu	Gambia	2.2	28.5	100	50.1	22.2	2.0	1.6	6.8	67	AR 90
N'Dama	Gambia	0.5	24.8	100	48.3	22.4	1.3	1.2	5.6	150	AR 90

[1] 2Meo = 2-methoxyphenol; PhOH = phenol; 4Me, 3Me = 4- and 3-methylphenol; 4Et-, 3Et = 4- and 3-ethylphenol; 4Pr, 3Pr = 4- and 3-propylphenol.

typically 8-9, and so these carboxylic acids would be fully ionised and not volatilised.

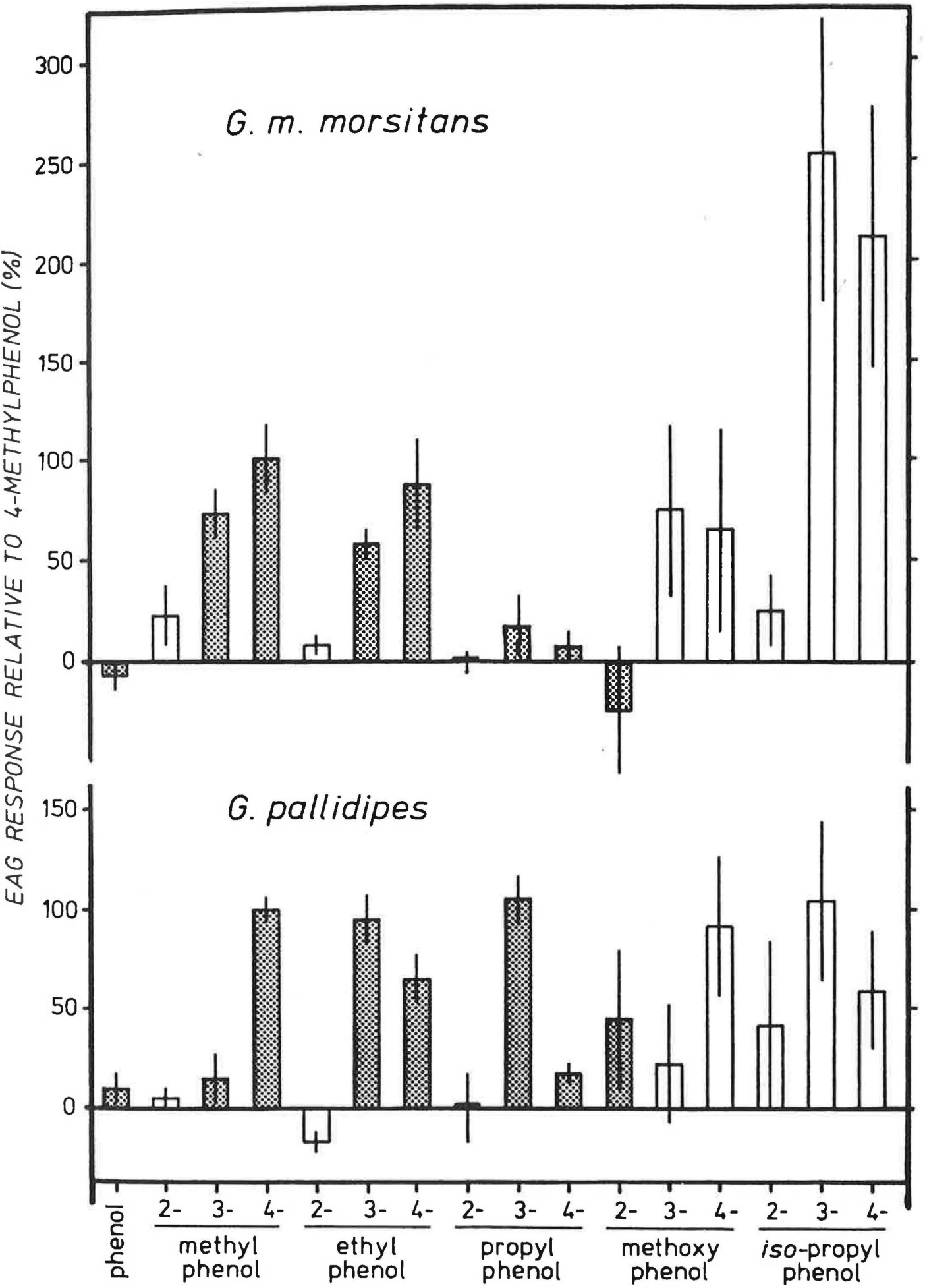
3.2.4. EAG responses of tsetse to synthetic phenols

An EAG dose-response curve for 4-methylphenol against male *G. pallidipes* was recorded (QR 86/2). Based on this, EAG responses of tsetse to phenol and all three positional isomers of the methyl-, ethyl-, propyl- and *iso*-propyl-phenols were recorded. Responses from tsetse species *G. m. morsitans* and *G. pallidipes* are shown in Fig. 3.2.3. (QR 86/4).

Detailed interpretation of these EAG results is probably not justified as they were performed at one dose and it is not known how this relates to quantities experienced in the field. However, for both species, the 4- isomer was the most active of the methylphenols and the 3- isomer of the propylphenols. For the ethylphenols, the 4- isomer was most active against *G. m. morsitans* while the 3- isomer was most active against *G. pallidipes*. In all these alkyl-substituted phenols, the 2- isomers showed only weak activity, and these were never detected in animal urines.

Noteworthy was the high EAG activity of the *iso*-propylphenols, particularly the 3- and 4- isomers. These compounds do not occur naturally, but are readily available commercially.

Fig. 3.2.4. EAG responses of G. m. morsitans and G. pallidipes to synthetic phenols, relative to the response to 4-methylphenol = 100 (10 ng at source; stippled columns for phenols naturally occurring in cattle urine)


G. m. morsitans
G. pallidipes
EAG RESPONSE RELATIVE TO 4-METHYLPHENOL (%)
300
250
200
150
100
50
0
150
100
50
0
phenol
2- 3- 4-
methyl phenol
2- 3- 4-
ethyl phenol
2- 3- 4-
propyl phenol
2- 3- 4-
methoxy phenol
2- 3- 4-
iso-propyl phenol

3.2.5. Field testing of synthetic phenols

After synthesis of 3-propylphenol (Sect. 3.2.6.), a mixture (TF 86/05) of all 8 phenols identified in cattle urine in their naturally occurring ratio was provided for testing in Zimbabwe. A similar mixture (TF 86/06) omitting the 2-methoxyphenol was also provided. The mixtures were dispensed from aqueous solution at the concentration found in the urines analysed (0.5 mg/ml) and at 1/10 and 10x this concentration. They were tested with F3 traps and electrified targets baited with acetone and octenol, and the attractiveness of the synthetic mixtures relative to cattle urine was determined.

The results in Table 3.2.3. show that the mixtures of synthetic phenols were at least as attractive as the natural urines. Addition of natural urine to the synthetic mixture did not significantly increase its attractiveness, indicating that there were probably no other important attractants in the urine.

Catches of *G. pallidipes* in both traps and at targets baited with acetone and octenol were increased by up to three times with the synthetic mixtures. There was little effect on catches of *G. m. morsitans*.

The individual phenols were then tested by adding them to traps already baited with acetone and octenol. Results in Table 3.2.4. show only small effects of the individual phenols on catches of tsetse, the most marked effect being reduction in catch of both species by 2-methoxyphenol.

These results formed the basis for Dr. Vale's work testing combinations of the phenols which showed signs of activity when tested individually (Vale *et al.*, 1988b). A combination of 4-methylphenol and 3-propylphenol is the most attractive blend, increasing catches of *G. pallidipes* in traps by up to six times (Vale *et al.*, 1988b; Table 3.2.6.). Similar results were reported by the ICIPE group (Owaga *et al.*, 1988).

Unfortunately, Dr. Vale reported that the readily available 3-*iso*-propylphenol cannot substitute for 3-propylphenol in synergising the effect of 4-methylphenol, despite its apparently good performance in both EAG and field tests when tested alone.

Table 3.2.3. Catches of tsetse in traps and at electrified targets baited with acetone, octenol and phenol mixtures, Rekomitjie, 1986.

Additional odour[2]		Catch Index[1] GMM	Catch Index[1] GP
1. F3 Traps			
TF 86/05[3]	0.05 mg/ml	1.1	1.5**
	0.5 mg/ml	1.1	3.1***
	5.0 mg/ml	0.9	3.8***
TF 86/06[3]	0.05 mg/ml	1.0	1.5*
	0.5 mg/ml	1.0	2.7***
	5.0 mg/ml	1.1	4.2***
2. F3 Traps			
Ox urine		0.92 ab	2.02 cd
Buffalo urine		0.91 ab	2.23 bc
TF 86/05[3]	0.5 mg/ml	0.96 a	1.72 d
TF 86/05[3]	5.0 mg/ml	0.83 abc	3.07 a
TF 86/05[3]	5.0 + 0.5 mg/ml	0.83 abc	3.05 a
TF 86/05[3]	5.0 mg/ml + ox urine	0.74 c	2.69 abc
TF 86/05[3]	5.0 mg/ml + buffalo urine	0.76 bc	2.72 ab
3. Targets			
TF 86/06[3]	0.05 mg/ml	1.2	1.5*
	0.5 mg/ml	1.0	1.7**
	5.0 mg/ml	1.2	3.2***

[1] *, **, *** denote index differs from unity at 5%, 1% and 0.1% levels; means followed by different letters differ at 5% level of probability.

[2] Traps and targets baited with acetone at 100 mg/hr and octenol at 0.5 mg/hr; phenol solutions dispensed from jam jars 54 mm i.d.

[3] TF 86/05 phenol+3-methyl+4-methyl+3-ethyl+4-ethyl+3-propyl+4-propyl+2-methoxyphenol at 1.4 : 9.9 : 100 : 1.1 : 2.1 : 2.5 : 0.8 : 0.4; TF86/06 same without 2-methoxy.

Table 3.2.4. Catch indices for addition of phenolic compounds to F3 traps baited with acetone and octenol[1]

Phenol[2]	Catch index[3]	
	G. m. morsitans	*G. pallidipes*
phenol	1.15	1.14
2-methyl	1.08	1.14
3-methyl	1.15	1.60 **
4-methyl	1.09	1.14
2-ethyl	0.90	1.05
3-ethyl	1.36	1.42 *
4-ethyl	1.34	1.39 ***
2-propyl	0.73	0.90
3-propyl	1.40	1.82 **
4-propyl	0.75	0.96
2-methoxy	0.39 ***	0.63 **
3-methoxy	1.42 *	1.04
4-methoxy	1.07	1.27*
2-*iso*-propyl	0.60 *	0.74
3-*iso*-propyl	1.21	1.84 ***
4-*iso*-propyl	1.01	1.25

[1] acetone at 100 mg/hr; octenol at 0.5 mg/hr; phenols neat in open bottle diameter 4 cm, release of 4-methylphenol 40-50 mg/day

[2] phenols underlined found in animal urine

[3] *, **, *** indicate means differ from unity at 5%, 1% and 0.1% levels of probability respectively.

3.2.6. Inhibitory effect of 2-methoxyphenol

2-Methoxyphenol was detected in trace amounts in many of the samples of urine analysed, but when added to traps baited with acetone and octenol it significantly reduced catches of both species of tsetse (Table 3.2.2.). This compound was subsequently reexamined to investigate whether it acts as an inhibitor of the action of odour attractants or whether it is actually a repellent, and to determine the magnitude of the effect. It was thus tested with both otherwise unbaited traps

and with traps baited with the current best attractant of acetone, octenol, 4-methylphenol and 3-propylphenol. Acetophenone, another compound found to reduce trap catches (Vale *et al.*, 1980) was included in the test, and the effect of 3-methylindole was also examined (AR 89)

The results in Table 3.2.5. show that 2-methoxyphenol and acetophenone reduced trap catches even in the absence of other odour attractants and thus would seem to be actual repellents. In the presence of the most attractive synthetic lure, both compounds reduced catches to levels at or below those in unbaited traps. Both compounds are readily available and could have value in reducing the challenge of tsetse flies to animals in infested areas.

Table 3.2.5. Effects of 2-methoxyphenol, acetophenone and 3-methylindole on trap catches of tsetse flies, Rekomitjie, October 1989.

Odour[1]	GMM		GP	
	mean[2]	index	mean[2]	index
None	3.59 b	1.00	30.09 b	1.00
AcPh	0.75 d	0.21	3.96 e	0.13
2MeO	1.52 cd	0.42	12.35 d	0.41
Ac + 8:4:1	10.80 a	3.01	99.48 a	3.31
Ac + 8:4:1 + AcPh	3.32 bc	0.93	24.64 bc	0.82
Ac + 8:4:1 + 2MeO	4.48 b	1.25	16.78 cd	0.56
Ac + 8:4:1 + 3-methylindole	12.94 a	3.60	107.38 a	3.57

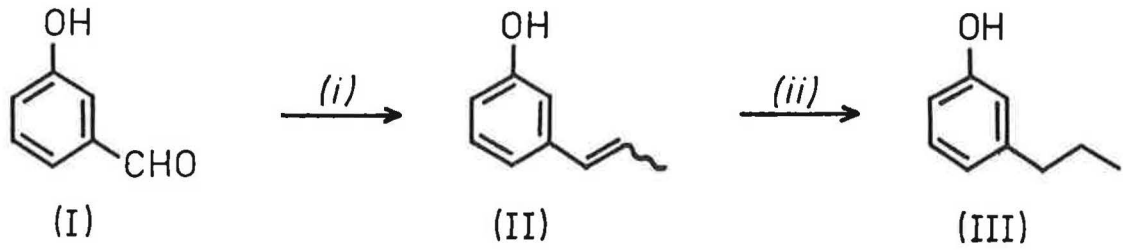
[1] AcPh = acetophenone 9.6 mg/hr; 2MeO = 2-methoxyphenol 6.4 mg/hr; 8:4:1 = 4-methylphenol+octenol+3-propylphenol 1.6 mg/hr; Ac = acetone 100 mg/hr.

[2] detransformed mean catch; means followed by the same letter are not significantly different at the 5% level by DMRT.

3.2.7. Synthesis of 3-propylphenol

Of the eight phenols identified in cattle urine, all are commercially available except for 3-propylphenol.

For initial field testing in Zimbabwe, 3-propylphenol (III) was synthesised at NRI by two routes: the literature route by reductive cleavage of *iso*safrole with sodium and ethanol in xylene (Struntz and Court, 1973), and a route devised at NRI involving Wittig reaction of 3-hydroxy-benzaldehyde (I) with ethyl(triphenyl)phosphonium bromide followed by catalytic hydrogenation of the resultant 3-(1-propenyl)-phenol (II) as shown in Fig. 3.2.4.



(I) (II) (III)

Fig. 3.2.5. Synthesis of 3-propylphenol (i. ethyl(triphenyl)-phosphonium bromide/potassium butoxide/THF; ii. hydrogen/ platinum oxide/ethyl acetate)

3.2.8. Field testing of 3-(1-propenyl)phenol (II)

It was anticipated that commercial production of 3-propylphenol by the latter route might give material contaminated with the precursor 3-(1-propenyl)phenol (II). This closely resembles 3-propylphenol in chemical structure, and it might also be biologically active and interfere with the attractiveness of the 3-propylphenol. However, in trapping tests carried out in Zimbabwe (QR 86/3), it was shown that 10% of 3-(1-propenyl)phenol in 3-propylphenol did not affect the attractiveness of the latter, and mixtures in which the 3-(1-propenyl)phenol replaced 3-propylphenol were almost as attractive as the original.

Table 3.2.6. Catches of tsetse in F3 traps baited with acetone and octenol and phenol mixtures containing 3-(1-propenyl)phenol

Phenol (mg/200 ml water)			GMM		GP	
			detraps	catch	detraps	catch
4Me	3Pr	3-Propenyl	mean[1]	index	mean[1]	index
Experiment 1: (75:25 (*Z*)/(*E*)-3-propenylphenol)						
-	-	-	19.3	1.00	78.5 c	1.00
1000	100	-	23.3	1.21	381.0 a	4.85
1000	100	10	22.0	1.14	356.0 a	4.53
1000	-	100	24.2	1.26	179.4 b	2.29
Experiment 2: (97:3 (*E*)/(*Z*)-3-propenylphenol)						
-	-	-	16.6 b	1.00	75.7 b	1.00
1000	100	-	22.3 ab	1.34	462.6 a	6.11
1000	100	10	22.6 a	1.36	438.1 a	5.79
1000	-	100	23.1 a	1.39	331.9 a	4.39

[1] 8 replicates; catches transformed to log(x+1) for analysis of variance; means followed by the same letter are not significantly different at the 5% level of significance.

3.2.9. Commercial production of 3-propylphenol

Following a tendering exercise run by NRI, Palmer Research was commissioned to produce 1 kg of 3-propylphenol at a price which would include the cost of developing a large-scale method. This material was checked by NRI and shown to be identical with authentic material by GC, GC-MS, IR and TLC. Analysis by GC-MS showed the presence of approximately 1% 3-chlorophenol as the only significant impurity. Palmer has access to large quantities of this 3-chlorophenol which they used as starting material, and it was shown to have no effect on the attractiveness of 3-propylphenol in the field (QR 86/4; 87/1).

Palmer charged £2,700 for this first kilogram. They now manufacture it in 50 kg batches which have been checked at NRI. To date they have supplied 300 kg to Zimbabwe and other African countries, and the current cost is around £900 per kg.

3.2.10. Conclusions and Recommendations

The experimental evidence indicates that the attractiveness of cattle urine to *G. pallidipes* is entirely due to the phenolic fraction which has been shown to contain up to eight phenols. The individual phenols all elicit EAG responses from both *G. pallidipes* and *G. m. morsitans*, but individually they are only weakly attractive in the field. However, a combination of 4-methylphenol and 3-propylphenol only is as attractive as mixtures of all eight phenols, increasing catches of *G. pallidipes* in traps baited with acetone and octenol by up to six times. Catches of *G. m. morsitans* are not significantly increased.

2-Methoxyphenol, found as a very minor component of the phenolic fraction from some urine samples, is a powerful repellent for both *G. pallidipes* and *G. m. morsitans*. It had no such effect on *G. tachinoides* (section 6.2.6.). This compound would be easy to formulate in slow release devices that could be attached to cattle as ear-tags to reduce their attractiveness to savannah species of tsetse.

3-Propylphenol was not previously commercially available, but is now supplied by Palmer Research Laboratories.

A potential impurity in synthetic 3-propylphenol, 3-(1-propenyl)phenol was shown to be almost as effective as 3-propylphenol itself in synergising the attractiveness of 4-methylphenol to *G. pallidipes* in the field. Another structural analogue of 3-propylphenol, 3-*iso*-propylphenol, elicited strong EAG responses from both *G. pallidipes* and *G. m. morsitans*, but was inactive in preliminary field tests. In view of the ready availability of this compound and the demonstrated fact that 3-propylphenol is not unique in its field activity, it is recommended that 3-*iso*-propylphenol be reexamined for behavioural activity in the field.

3.3. IDENTIFICATION OF INDOLE AND 3-METHYLINDOLE IN CATTLE URINE

3.3.1. Detection and identification

During linked GC-EAG analyses of dichloromethane extracts of cattle urine using a polar capillary GC column, two minor components eliciting EAG responses were detected at retention times longer than the phenolic components. After fractionation of the extract, these two components were located in the non-acid fraction, and they were identified as indole and 3-methylindole (skatole) by their mass spectra and GC retention times on both polar and non-polar columns see Fig. 3.4.1.). The two compounds were present in approximately 5:1 ratio and the indole was at 0.5-1% the concentration of 4-methylphenol in the unfractionated extract.

3.3.2. EAG responses of tsetse to indoles

EAG responses of *G. pallidipes* to indole and 3-methylindole (20 ng at source off glass) were 1.9 $\pm$ 0.3 mV and 2.9 $\pm$ 0.7 mV respectively, compared with 6.7 $\pm$ 0.3 mV to 0.5 µl of acetone and 1.2 $\pm$ 0.1 mV to 2 ng at source of octenol. Representative EAG traces are shown in Fig. 3.3.1.

A dose-response curve for 3-methylindole against *G. pallidipes* was recorded and the maximum response of 1.17 $\pm$ 0.2 mV above solvent blank was recorded to 2 ng at source off glass. The mean response of these preparations to 0.5 µl of acetone was 7.71 $\pm$ 1.36 mV.

3.3.3. Field testing of indoles

In initial field tests, polythene vials impregnated with 1 mg of a 5:1 mixture of indole and 3-methylindole were added to Beta traps baited with acetone at 100 mg/hr and octenol at 0.5 mg/hr. There were no significant effects on trap catches of either *G. m. morsitans* or *G. pallidipes*.

Addition of aqueous solutions of the 5:1 mixture of indoles to Beta traps baited with acetone and octenol gave a significant ($P < 0.001$) reduction in catch of *G. pallidipes* females at the highest concentration (Table 3.3.1.).

In 1989, a 4:8:1:4 mixture of 3-methylindole, 4-methylphenol, 3-propylphenol and octenol dispensed from a polythene sachet was evaluated in traps baited with acetone (Table 3.2.5.). Traps baited with this lure caught more tsetse than traps baited with the phenols/octenol mixture only, but the difference was not significant in this case.

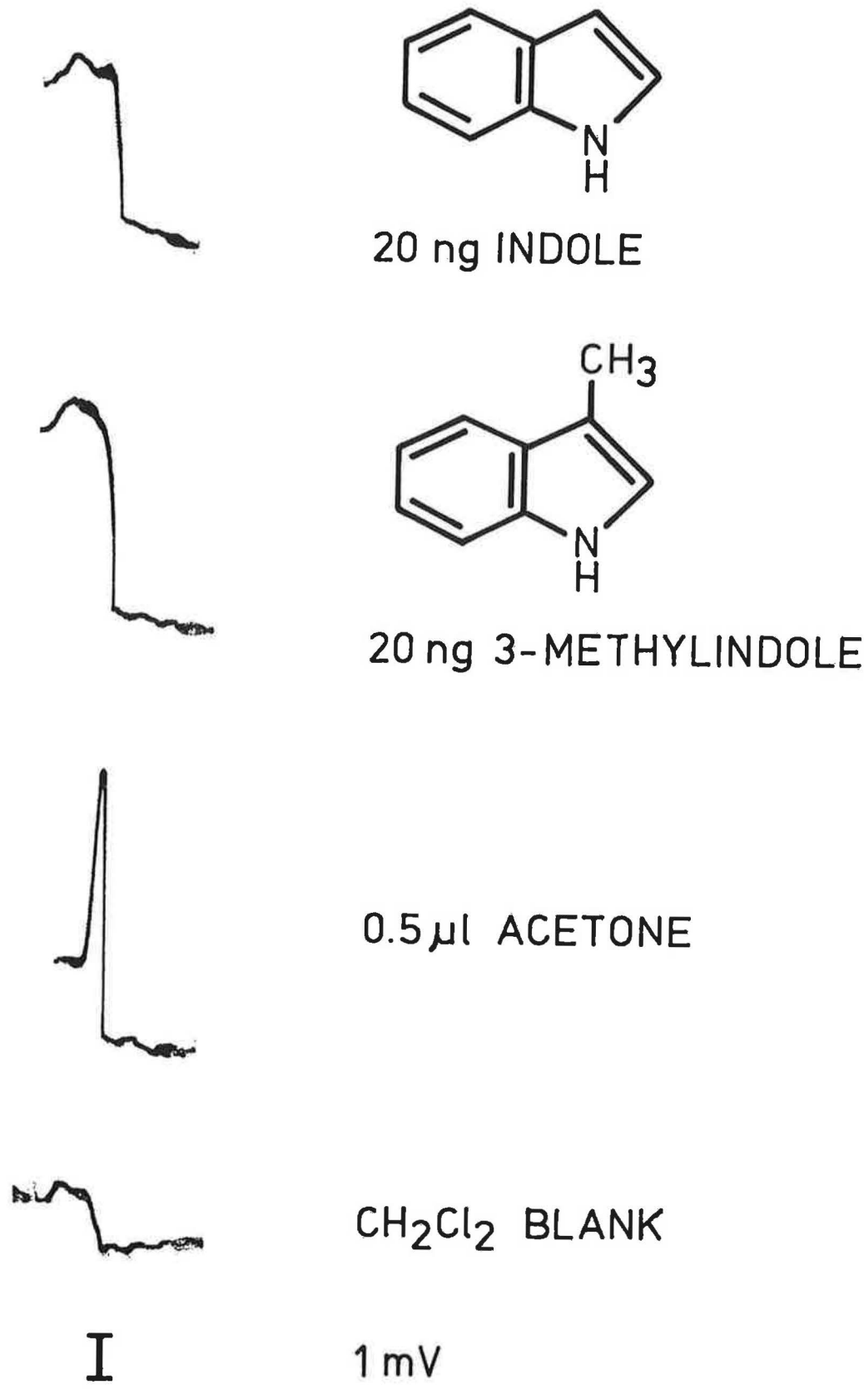


Fig. 3.3.1. EAG responses of G. pallidipes to indole, 3-methylindole and acetone.

Table 3.3.1. Catches of tsetse in Beta traps baited with acetone, octenol and aqueous solutions of indoles.

Additional odour[2]	Detransformed mean catch[1]			
	GMM: male	female	GP: male	female
Nil	0.8	0.4	22.0	22.4
0.5 ppm indoles	1.3	0.7	22.2	23.7
5 ppm indoles	0.7	0.5	19.6	21.7
50 ppm indoles	0.6	0.5	18.4	12.6

[1] catches transformed to log(x+1) for analysis of variance; means for 8 replicates

[2] all traps baited with acetone at 100 mg/hr and octenol at 0.5 mg/hr; 5:1 mixture of indole and 3-methylindole in 200 ml water.

3.3.4. Conclusions and Recommendations

The possible attractiveness of the two indoles to tsetse in the field should be examined further using different release rates and combinations. These compounds are cheap and readily available, and even if they only give a small increase in catch, their inclusion in baits for monitoring and control could be justified. Indole is a component of Swormlure 4, the attractant blend used for New World Screwworm, *Cochliomyia hominovorax* (Mackley and Brown, 1984), and is also an attractant for sorghum shootfly, *Atherigona soccata* (K. Nwanze, ICRISAT, personal communication).

3.4. IDENTIFICATION OF CAROTENOID METABOLITES IN CATTLE URINE

3.4.1. Introduction

In initial analyses of dichloromethane extracts of cattle urine by linked GC-EAG using a polar capillary GC column, the strongest EAG response was elicited by a minor component eluting before phenol which is the first of the phenols to elute. This component was christened Peak 6, as the sixth component of any significance to elute, and was present at levels of 0.5 - 1.0% of the major component in the extract, 4-methylphenol (Fig. 3.2.1.).

3.4.2. Isolation

After chemical fractionation of the crude extract into phenolic, acidic and non-acidic fractions (Section 3.2.2.), the Peak 6 was detected in the non-acidic fraction and was one of the major volatile components (Fig. 3.4.1.). Analysis by GC-MS showed a very characteristic mass spectrum (Fig. 3.4.2.) for Peak 6 with a probable molecular ion at m/z 208 (possibly $C_{13}H_{20}O_2$). The GC-MS analyses also revealed the presence of a second compound, Peak 7, with slightly longer retention time and essentially identical mass spectrum.

GC retention times of Peak 6 and Peak 7 on a polar column were 12.69 and 13.00 respectively, expressed in equivalent chain lengths (ECL) relative to the retention times of straight-chain acetates. Retention times on a non-polar column were 10.26 and 10.46 ECL, suggesting that both compounds were more polar than simple aliphatic acetates, the differences in retention times on the two GC phases being similar to those for unsaturated, aliphatic alcohols.

The non-acidic fraction was purified by liquid chromatography on silica gel, and the Peak 6 and Peak 7 were eluted separately in relatively pure form with 50-60% ether in hexane. This suggested that they were considerably more polar than aliphatic alcohols which would elute with 20% ether in hexane, and gave the first indication that the compounds detected in GC analyses were transformation products of the compounds actually present in the urine.

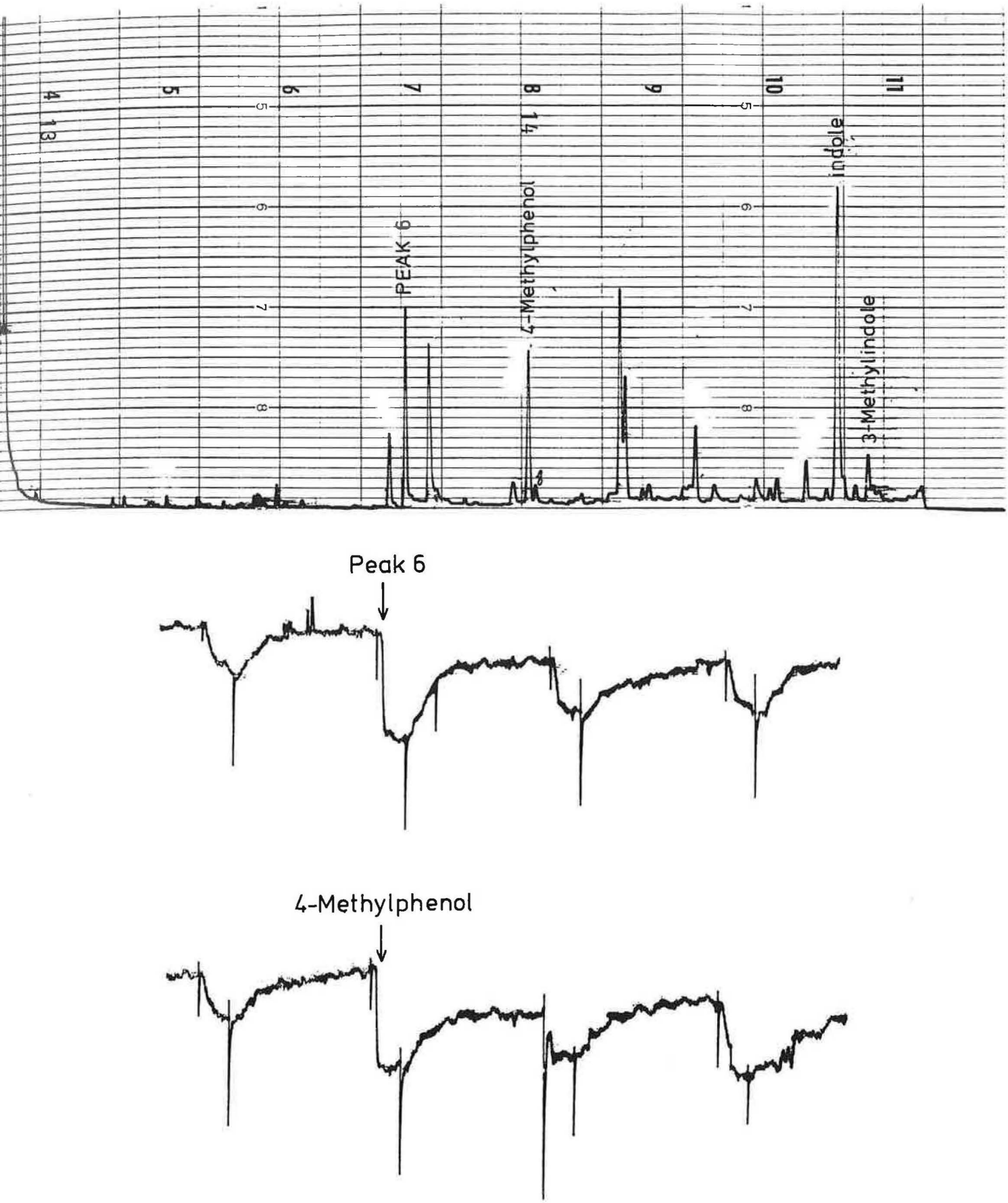
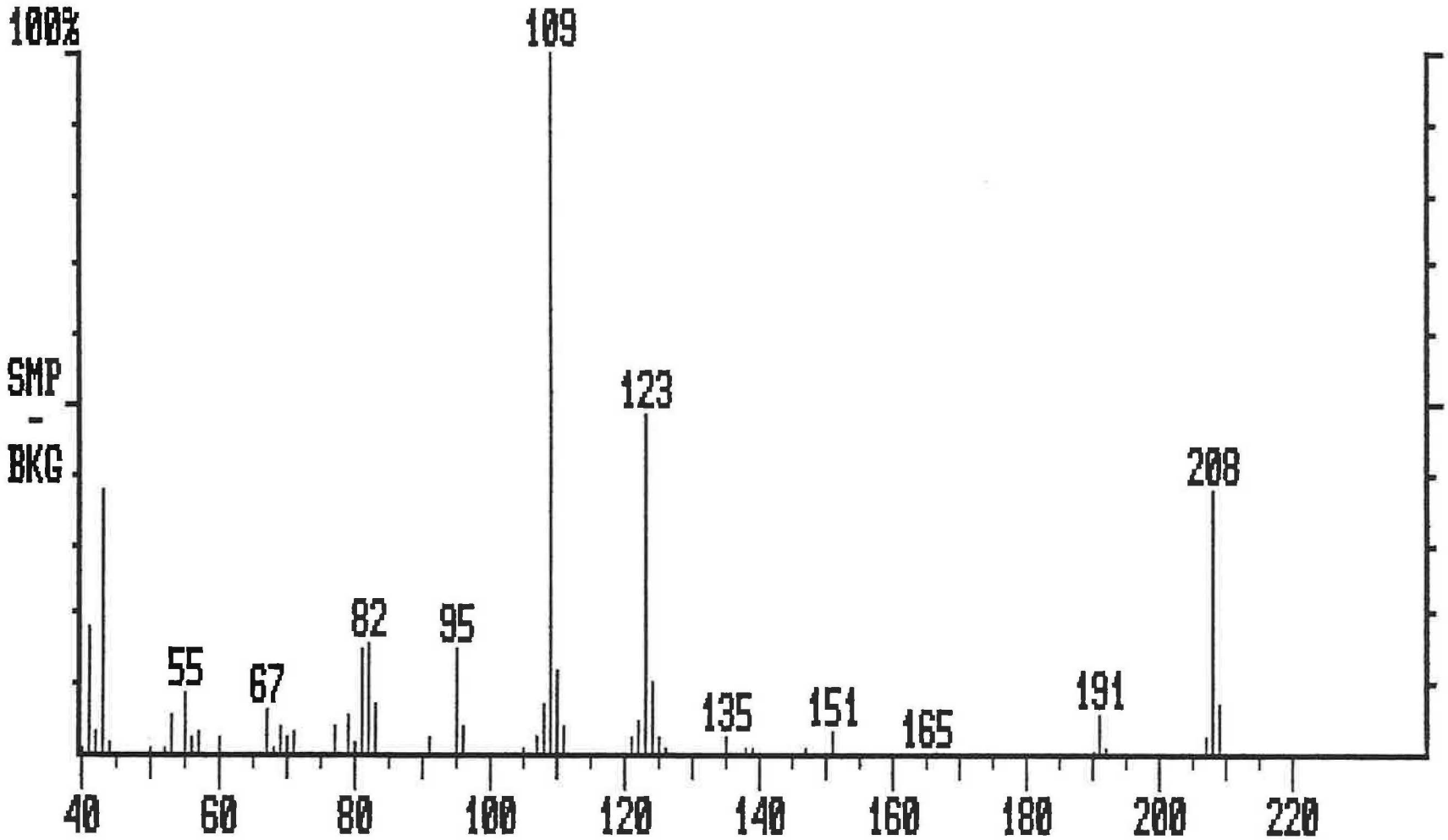


Fig. 3.4.1. Linked GC-EAG analysis of non-acidic fraction of dichloromethane extract of ox urine (polar CP Wax 57CB column)

Fig. 3.4.2. Mass spectrum of Peak 6 from GC-MS analysis



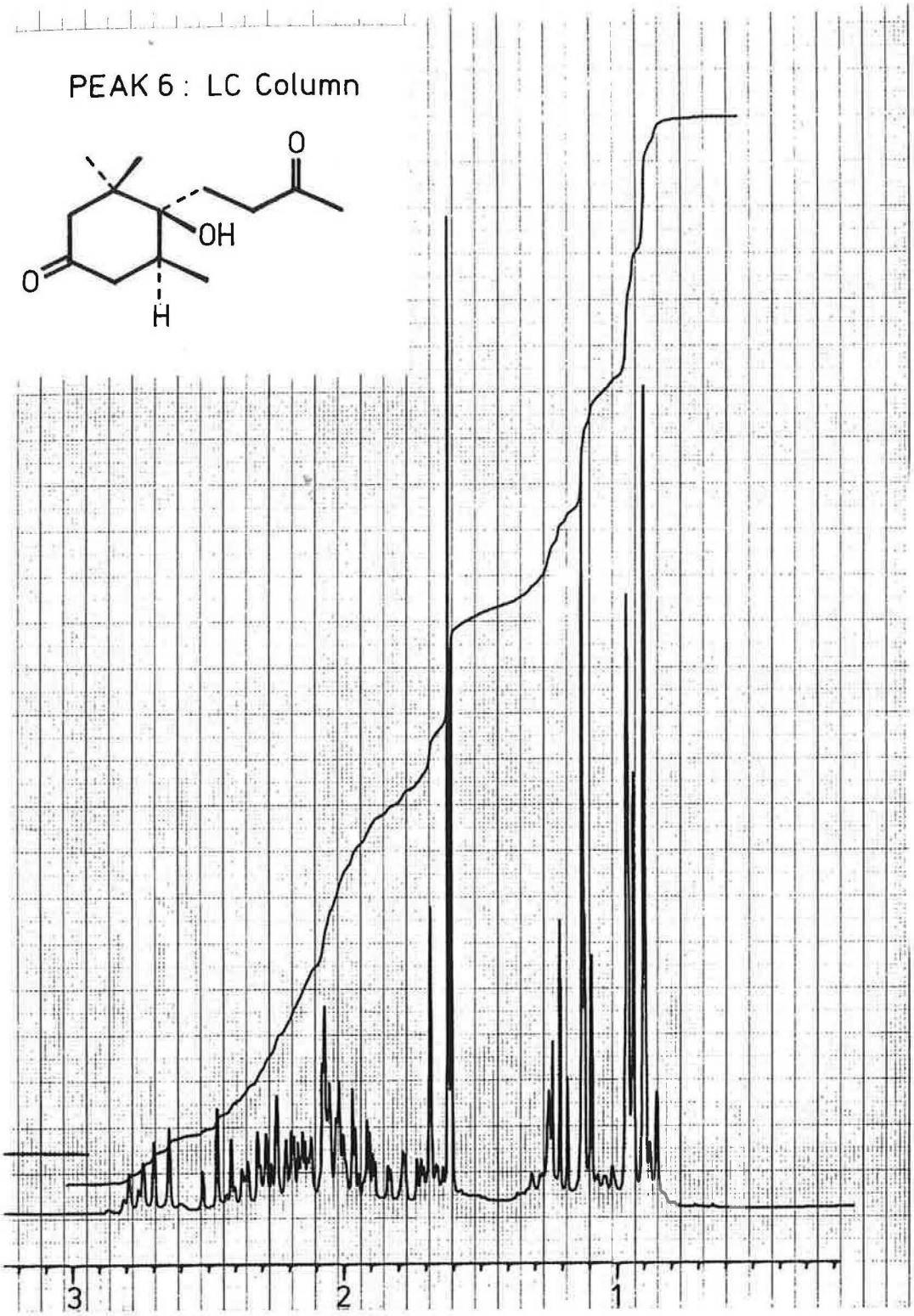
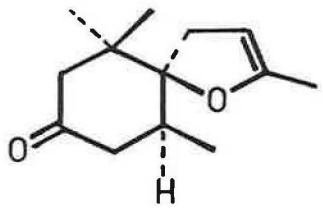


Fig. 3.4.3. 250 MHz ^{1}H NMR spectrum of Peak 6 from LC (actually 6K)

Fig. 3.4.4. 250 MHz ^{1}H NMR spectrum of Peak 6 after distillation



3.4.3. Identification

Approximately 1 mg of Peak 6 purified by LC was obtained, and a ^{1}H NMR spectrum was run (Fig. 3.4.3.). This indicated that impurities were still present, and so the sample was further purified by short-path distillation to give a white, crystalline solid. GC analysis of this was identical with that of the material before distillation, but the NMR spectrum was quite different (Fig. 3.4.4.). In particular, a strong 3H singlet at δ 1.62 in the spectrum of the undistilled material seemed to have shifted downfield to a narrow 3H multiplet at δ 1.77, and a new broad 1Hd singlet had appeared at δ 4.42.

This result confirmed that the compound present in the urine was undergoing a change on heating as in distillation or GC analysis to give the compound actually observed in analysis by GC-EAG and GC-MS. The NMR data suggested that this might involve conversion of a methylketone to a methyl enol ether.

Detailed consideration of the spectral and analytical data and examination of the literature finally led to the finding that the spectral data for Peak 6 was consistent with that reported by Shibagaki *et al.* (1981) for the two isomers of 2,6,10,10-tetramethyl-1-oxaspiro-[4.5]-dec-2-en-8-one (Gough *et al.*, 1987) (Fig. 3.4.5.), carotenoid metabolites isolated from tobacco volatiles by Demole and Berthet (1972).

3.4.4. Synthesis

The two oxaspirone isomers were synthesised at NRI from isophorone by the route of Shibagaki *et al.* (1981) (Fig. 3.4.5.). The *cis* (6) and *trans* (7) isomers were found to be identical with Peaks 6 and 7 by GC retention time, mass spectra, NMR and TLC. The NMR spectra and TLC analyses showed that the two compounds in the urine were identical with hydroxy-ketone precursors to the oxaspirones in the synthesis, *cis* and *trans* 3,3,5-trimethyl-4-hydroxy-4-(3-oxobutyl)-cyclohexanone, and these were christened (6K) and (7K).

The dehydro analogues of (6)/(7) and (6K)/(7K) were also synthesised by a similar route (Fig. 3.4.6.), and christened compounds (T) and (TK) respectively. These compounds were also isolated from tobacco volatiles by Fujimori *et al.* (1981) and Takagi *et al.* (1978). Compounds (6), (7) and (T) are related to theaspirone, an important component of tea odour, but none of these have been reported from animal sources.

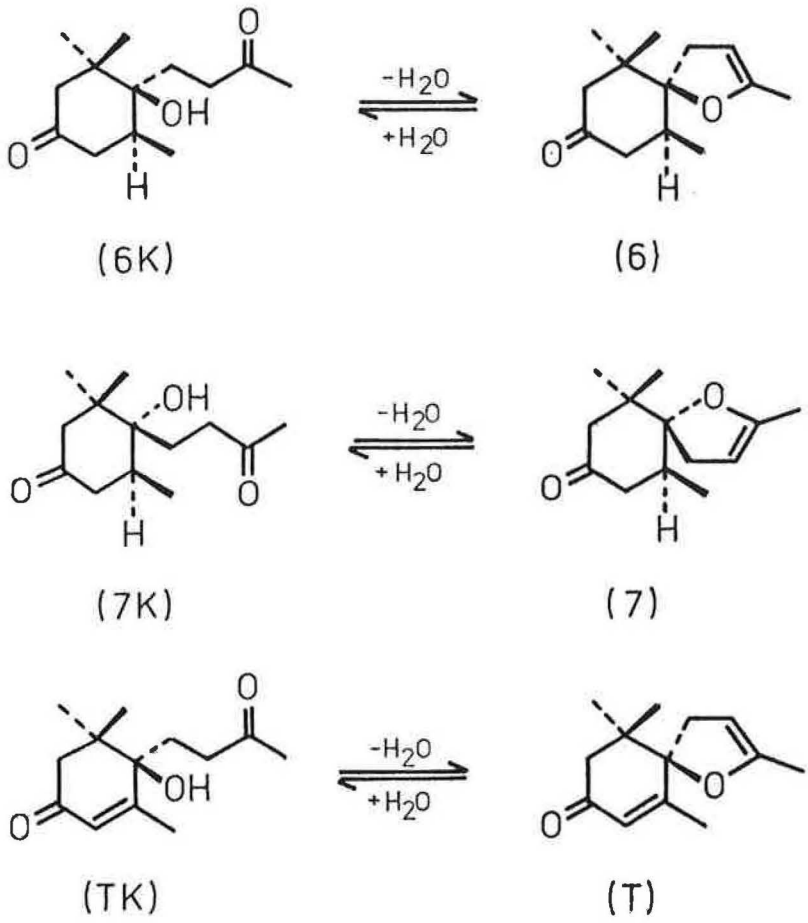


Fig. 3.4.5. Chemical structures of the carotenoid metabolites (6), (7), (6K) and (7K).

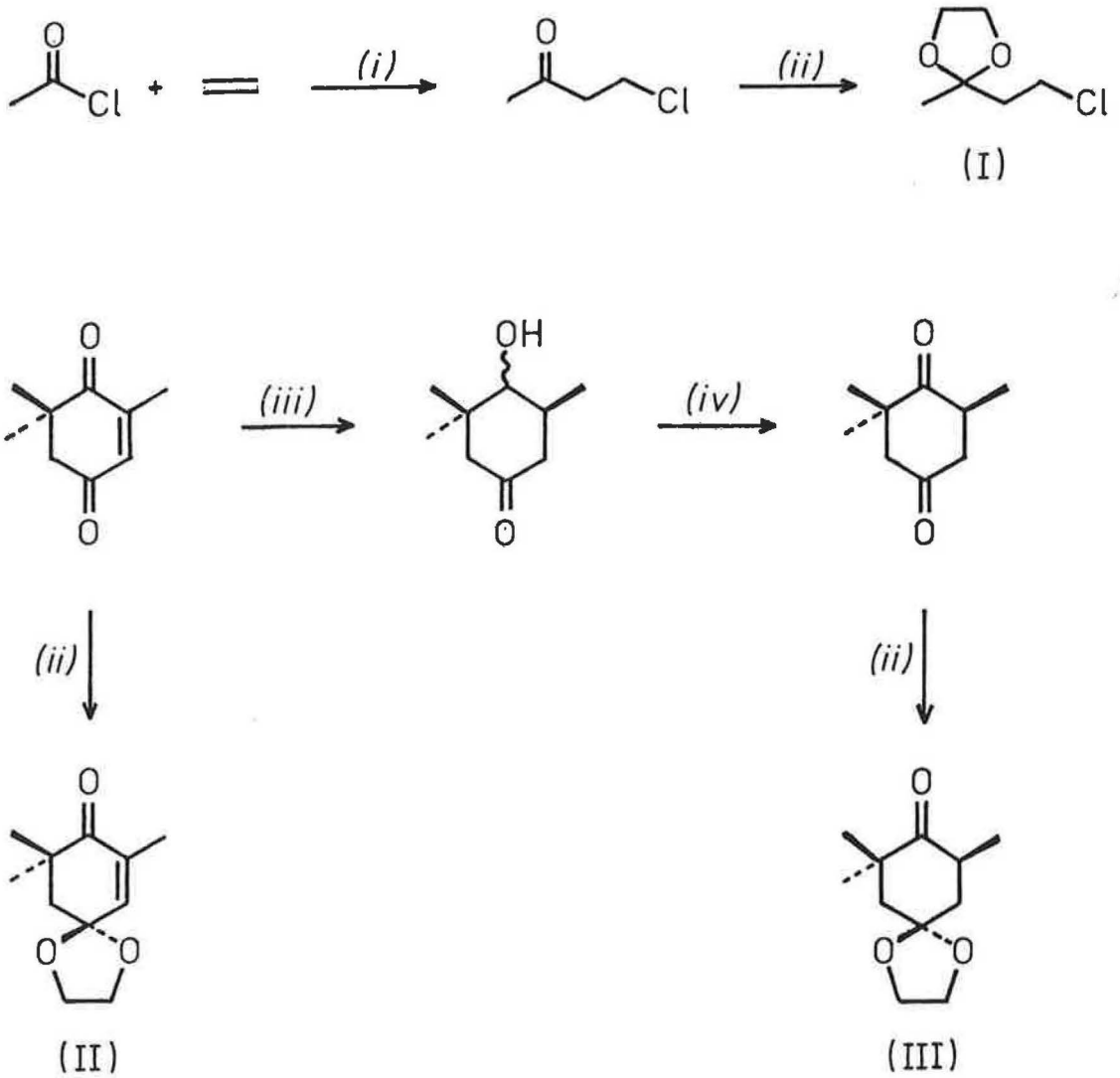


Fig. 3.4.6. Synthesis of precursors (I), (II) and (III). (i. aluminium chloride $CHCl_3$; ii. ethylene glycol/pTSA/benzene; iii. hydrogen/PtO_2/ethyl acetate; iv. pyridinium chlorochromate/dichloromethane)

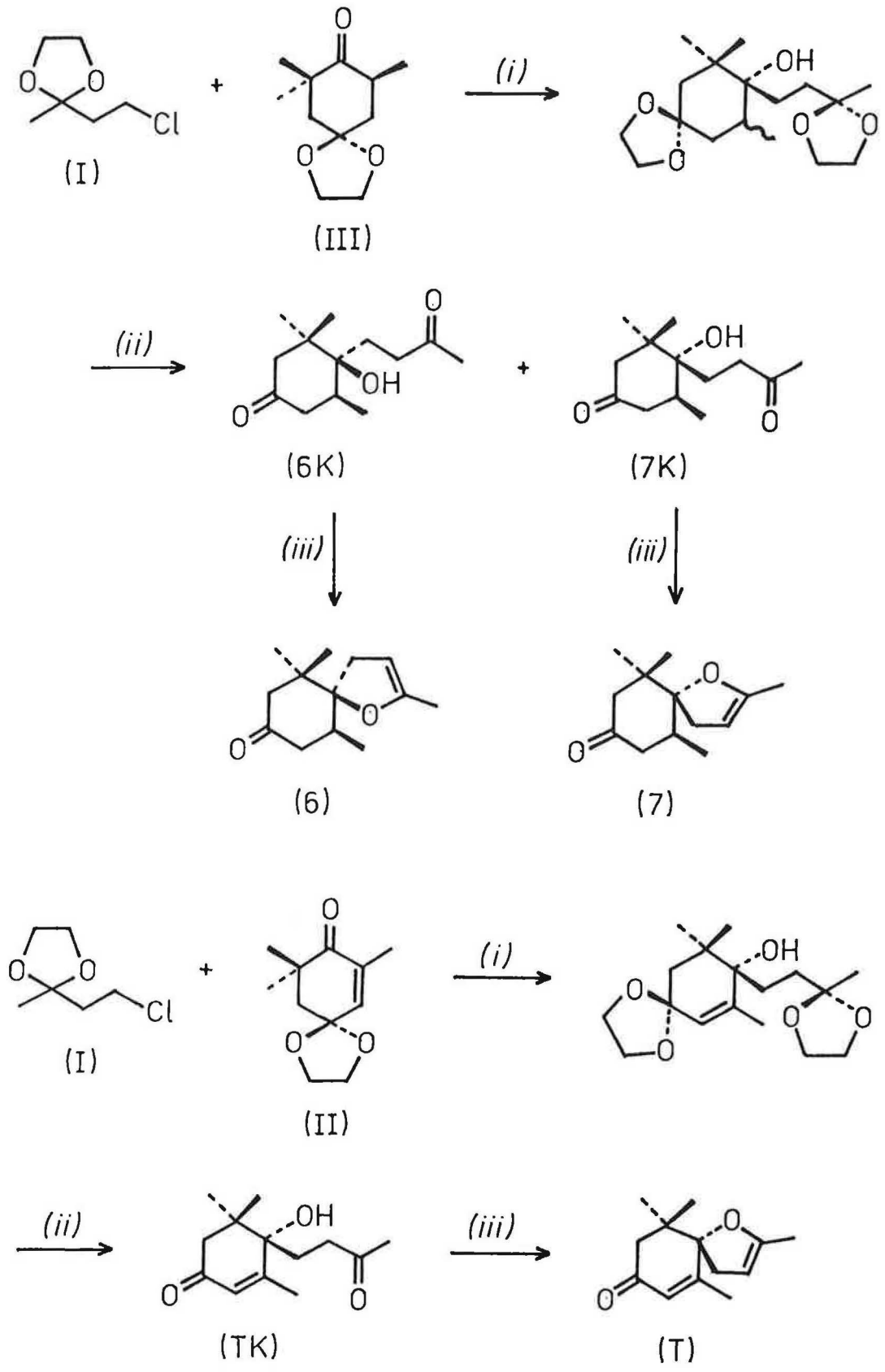


Fig. 3.4.7. Synthesis of (6K), (7K), (6) and (7) and the analogues (TK) and (T). (i. Mg/THF; ii. dil acid/ether; iii. distil)

3.4.5. Biological testing of natural Peak 6 and Peak 7.

In view of the intense EAG activity of Peak 6 observed in linked GC-EAG analyses, small amounts of the natural Peaks 6 and 7 purified by liquid chromatography were supplied for bioassay at TRL and field testing in Zimbabwe. Following the subsequent identification work, these are now known to have been the hydroxyketones (6K) and (7K).

In the field, addition of either compound to Beta traps baited with acetone and octenol significantly reduced catches of *G. pallidipes* (Table 3.4.1.). However, the validity of these results is questionable as, on return of these materials to NRI, the sample of Peak 6 had entirely decomposed although the sample of Peak 7 was intact.

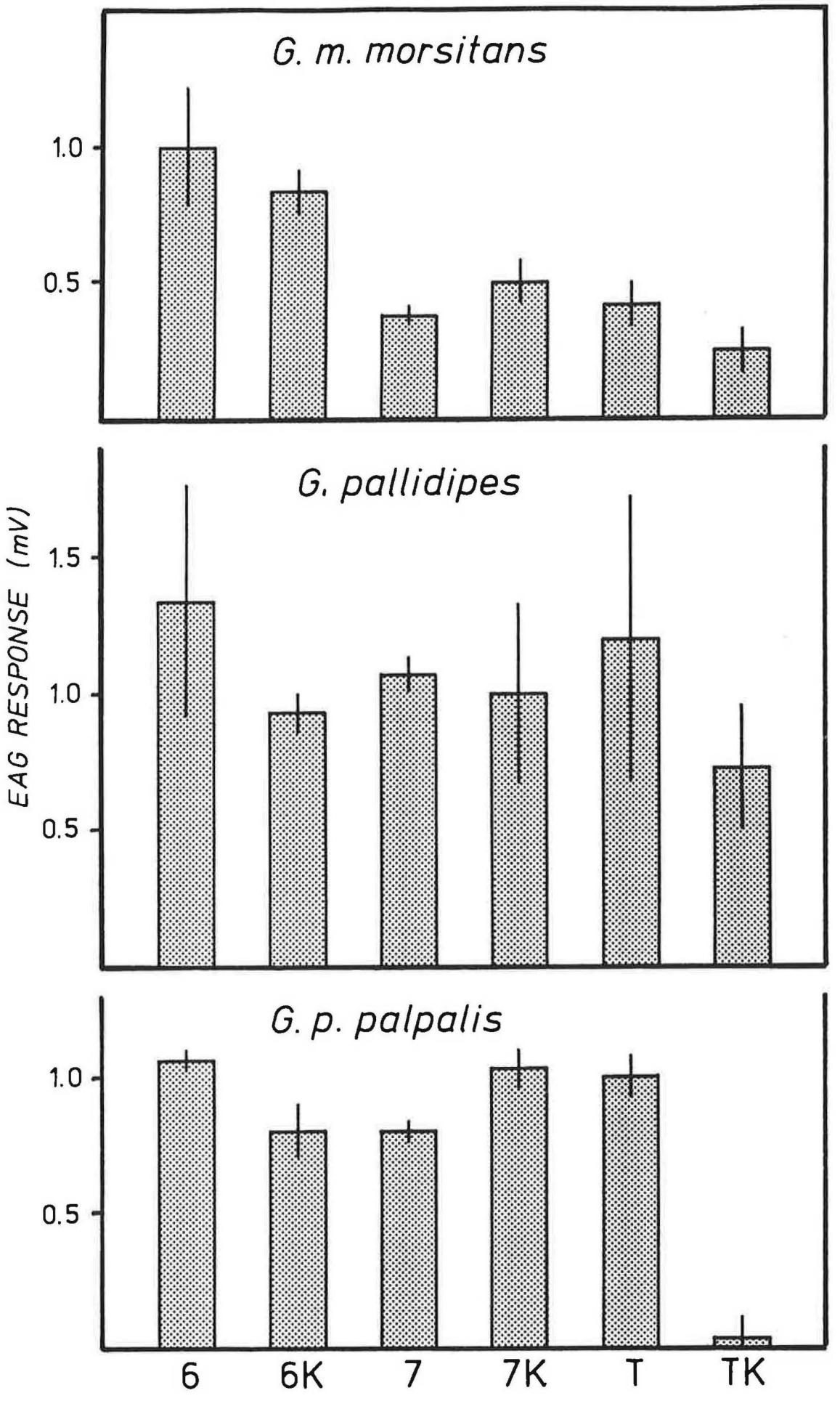
Table 3.4.1. Catches of tsetse in Beta traps baited with acetone, octenol and Peak 6 or Peak 7, Rekomitjie 1986.

	GMM		GP	
Additional odour[1]	mean[2]	index	mean[2]	index
Nil	9.1		108.7a	1.00
Peak 6	9.7		65.0b	0.60
Peak 7	11.4		76.5b	0.70

[1] traps baited with acetone (100 mg/hr) and octenol (0.5 mg/hr); Peaks 6 and 7 0.1 mg on cloth.

[2] detransformed (log (x+1)) means from 12 replicates; means followed by different letters significantly different at 5% level.

In the bioassay, Peak 6 was found to be a strong activator for *G. m. morsitans*, and Peak 7 caused increased upwind orientation.


G. m. morsitans
1.0
0.5
G. pallidipes
EAG RESPONSE (mV)
1.5
1.0
0.5
G. p. palpalis
1.0
0.5
6
6K
7
7K
T
TK

3.4.6. EAG testing of synthetic compounds.

When the synthetic compounds became available, it was possible to test the activity of all the compounds involved: the compounds actually present in the urine, (6K) and (7K); the compounds observed in GC-EAG analyses, (6) and (7); and also the dehydroanalogues (T) and (TK).

Measurement of EAG dose-responses for Peak 6 confirmed the potent EAG activity against *G. pallidipes*, *G. m. morsitans* and *G. palpalis* (QR 86/4). Peak 6 elicited a larger EAG response from *G. pallidipes* than 4-methylphenol at the 10 ng level (QR 86/4).

EAG responses to all six synthetic compounds were compared at the 5 ng level, and all showed pronounced EAG activity against the three tsetse species (QR 86/4) (Fig. 3.4.7.).

Fig. 3.4.7. EAG responses of G. m. morsitans, G. pallidipes and G. p. palpalis to synthetic Peak 6 and analogues (5 ng at source).

3.4.7. Field testing of synthetic compounds

Initial field tests were carried out in 1986 dispensing the synthetic compounds from filter paper in a jam jar at traps baited with acetone and octenol. Cattle urine typically contains less than 0.05 mg/ml of compound (6K), and 1 mg of the synthetic compounds was used, equivalent to at least 200 ml of urine (QR 86/3). The results in Table 3.4.2. show no evidence of any behavioural effect on tsetse under these ocnditions.

Table 3.4.2. Catches of tsetse in traps baited with acetone, octenol and compounds (6), (7), (6K), (7K), (T) and (TK), Rekomitjie, September-October 1986.

Additional odour[1]	Total Catch[2]	
	GMM	GP
Experiment 1		
Nil	63	275
6	63	230
6K	63	270
7	56	284
Experiment 2		
Nil	58	367
7K	70	331
T	73	355
TK	67	306

[1] F3 traps baited with acetone (100 mg/hr) and octenol (0.5 mg/hr); test compound 1 mg on filter paper in jam jar.

[2] latin square run over 4 days.

Polythene vials and rubber septa were evaluated in the laboratory as improved dispensers for these compounds (QR 87/2). As a result of this work, field tests of compounds 6 and 7 were repeated using rubber septa loaded with 5 mg of the test materials and testing them in the presence of synthetic urine phenols as they would be presented naturally (QR 87/2).

The results in Table 3.4.3. also show no evidence for any behavioural effect.

Table 3.4.3. Effects of adding compounds (6) and (7) in the presence of phenols to traps baited with acetone and octenol.

Additional odour[1]	GMM		GP	
	mean[2]	index	mean[2]	index
Nil	14.1	1.00	139b	1.00
phenols	14.4	1.02	441a	3.17
phenols + (6)	11.5	0.82	427a	3.07
phenols + (7)	13.1	0.93	461a	3.32
phenols + (6) + (7)	14.9	1.06	495a	3.56

[1] all traps baited with acetone (100 mg/hr) and octenol (0.5 mg/hr); phenols = 100 mg 4-methylphenol + 10 mg 3-propylphenol in 200 ml water; test compounds 5 mg on rubber septum.

[2] 10 replicates in two latin squares; catches transformed to log(x+1); detransformed means followed by same letter not significantly different at 5% level by DMRT.

3.4.8. Conclusions and Recommendations

Despite the strong EAG responses elicited from tsetse by the synthetic compounds (6), (7), (6K) and (7K), there is no evidence for any behavioural activity in terms of influencing trap catch in the field under the conditions tested.

It should be noted that these field tests were not very extensive involving only single, low doses, and that dispensing systems were not fully optimised. Furthermore, the synthetic materials were racemic whereas the naturally-occurring materials would probably have been homochiral. However, the results agree with the fact that all the attractiveness of urine can be accounted for by the phenolic fraction, with no other major attractive components present.

Thus further work on these compounds would seem not to be justified in relation to the strictly practical aim of finding new tsetse attractants. The compounds would also be expensive

to produce, particularly in homochiral form. Nevertheless, this is the first time these compounds have been identified in urine, and the strong EAG activity towards tsetse is remarkable. It would seem surprising if they do not have some behavioural activity that is not measured by simple trap catch in the field, and there is already evidence for this from the original laboratory bioassays with the natural materials. In this context, it would be informative to determine whether specific receptors for these compounds can be found on the tsetse antennae by single cell recording techniques. In view of the recent findings that 1-octen-3-ol also has behavioural effects on Diptera other than tsetse, it would also be interesting to investigate whether other Diptera respond to these carotenoid metabolites either electrophysiologically or behaviourally.

3.5. IDENTIFICATION OF POTENTIAL TSETSE ATTRACTANTS IN BUSHPIG BEDDING SACKS

3.5.1. Introduction

Bushpig bedding sacks were shown to give small but significant increases in catches of both *G. m. morsitans* and *G. pallidipes* in traps baited with acetone and octenol (Vale *et al.*, 1986b).

3.5.2. Fractionation and analysis of sack extracts

Bushpig bedding sacks from Zimbabwe were soxhelet extracted with dichloromethane, and the extracts fractionated chemically into phenolic, acidic and non-acidic fractions. The phenolic and acidic fractions were examined by GC-MS, and components were identified by comparison of their GC retention times and mass spectra with those of the synthetic compounds (QR 87/1).

3.5.3. Composition of phenolic fraction

Six components were detected in the phenolic fraction, and their relative proportions are shown in Table 3.5.1. Total amounts were small with approximately 0.05 mg 4-methylphenol per sack, and no propylphenols were detected (< 0.5% of 4-methylphenol). Two new phenols were detected and identified as vanillin (4-hydroxy-3-methoxybenzaldehyde) and acetovanillone (4'-hydroxy-3'methoxyacetophenone). Reanalysis of phenolic fractions from urine confirmed that these two compounds were not present.

Table 3.5.1. Composition of phenolic fraction from bushpig bedding sacks

Compound	Relative amount (4-methylphenol = 100)
phenol	21
4-methylphenol	100
3-methylphenol	56
4-ethylphenol	24
vanillin	17
acetovanillone	11

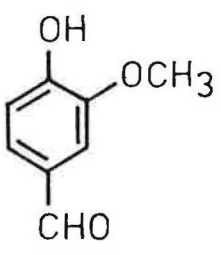
The phenolic fraction was analysed by linked GC-EAG. No significant responses were recorded from *G. pallidipes* to the vanillin or acetovanillone. When the synthetic compounds were tested separately, no significant EAG responses were obtained from *G. pallidipes* to vanillin or acetovanillone at the 10 ng level under conditions where responses to 10 ng 4-methylphenol and 0.5 µl acetone were 1.0 mV and 3.9 mV respectively.

3.5.4. Composition of the acidic fraction

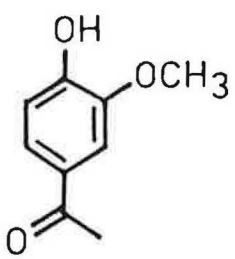
The acidic fraction was similarly analysed by GC-MS, and the compounds detected are listed in Table 3.5.2. In addition to the aliphatic and aromatic carboxylic acids found in the acidic fraction of cattle urine, the acidic compound 1-methylhydantoin was also detected. The synthetic compound elicited no EAG response from *G. pallidipes* when tested as above.

Table 3.5.2. Composition of acidic fraction from bushpig bedding sacks.

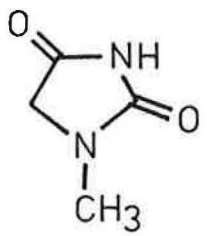
Compound	Relative amount (hexanoic acid = 100)
acetic acid	23
propanoic acid	14
butanoic acid	14
pentanoic acid	26
2-methylbutanoic acid	19
hexanoic acid	100
octanoic acid	54
nonanoic acid	84
benzoic acid	50
phenylacetic acid	12
3-phenylpropanoic acid	12
1-methylhydantoin	43



Vanillin



Acetovanillone



1-Methylhydantoin

Fig. 3.5.1. Chemical structures of compounds identified

3.5.6. Field testing of fractions

Unextracted bedding sack, extracted sack and clean sacking samples impregnated with the crude dichloromethane extract, the phenolic fraction and the acidic extract were returned to Zimbabwe for field testing. When added to F3 traps baited with acetone and octenol, no significant increases in catches of tsetse were observed with any of the samples, even the original sacking as supplied (QR 87/1)

This procedure was repeated (QR 87/3) using four sacks that had been in the bushpig sty in Harare for 10 days and which Dr. Vale had shown to be attractive to tsetse in the field. Aliquots of the crude extract and the fractions equivalent to 1.5 sacks were deposited on pieces of clean sacking and sent to Zimbabwe along with pieces of clean sacking and of the bushpig sacking before and after extraction.

The results in Table 3.5.3. show that none of the samples, not even the original sacks or crude extract, were signficiantly attractive to either species of tsetse in these tests, and no further work was carried out on the sacks during this project.

Table 3.5.3. Catches of tsetse in F3 traps baited with acetone and octenol and bushpig bedding sack and fractions.

	GMM		GP	
Additional odour[1]	mean	index	mean	index
Nil	1.91	1.00	64.9	1.00
clean sack	1.98	1.04	69.6	1.07
unextracted bedding sack	2.39	1.25	76.3	1.18
extracted bedding sack	1.63	0.86	59.3	0.91
crude extract	2.89	1.51	70.6	1.09
non-acidic fraction	3.34	1.75	57.1	0.88
acidic fraction	2.83	1.48	69.8	1.08
phenolic fraction	3.46	1.81	65.5	1.01

[1] all traps baited with acetone (100 mg/hr) and octenol (0.5 mg/hr).

3.5.7. Laboratory bioassay and field testing of vanillin, acetovanillone and 1-methylhydantoin

Vanillin, acetovanillone and 1-methylhydantoin were sent to Prof. Bursell at TRL for bioassay. He reported that, in the presence of carbon dioxide, vanillin caused a significant increase in activation and in upwind orientation over a range of concentrations (3×10^{-4} - 3 µg/litre). Acetovanillone increased upwind orientation but not activation, while 1-methylhydantoin was inactive.

In field tests of the three new compounds found on bushpig bedding sacks, Dr. Holloway found vanillin gave a significant increase in catch of *G. pallidipes* in traps baited with acetone and octenol in Zimbabwe, but this could not be repeated in Zimbabwe or in Somalia by Dr. Torr.

3.5.8. Conclusions and Recommendations

Although bushpig bedding sacks have been reported to increase catches of tsetse in the field and to have an effect additional to that of the urine (Vale *et al.*, 1986b), the effects were only just significant, amounting to at best less than a doubling of catches. In the two operations described above, it proved impossible to take sacks of proven field activity in Zimbabwe, send them to NRI and return them to Zimbabwe and then repeat the field results.

Perhaps not surprisingly, it was also impossible to demonstrate field activity of solvent extracts of these sacks. The "clean" sacks themselves contained large amounts of extractable material, and after exposure to the bushpig they were even dirtier, so that fractionation and analysis of extracts was extremely difficult. In view of these factors, further work on the sacks is not recommended.

In spite of these difficulties, three new compounds probably derived from the bushpig were isolated and identified as vanillin, acetovanillone and 1-methylhydantoin. Vanillin and acetovanillone contain the 2-methoxyphenol moiety and a carbonyl group, both elements found in other compounds with pronounced effects on tsetse behaviour. Although none of the three compounds caused EAG responses from tsetse, there were indications of behavioural activity for the two phenolic compounds in the laboratory bioassay. Field tests gave variable results, and these should be repeated to give a definitive answer.

3.6. WORK ON "OMEGA"

3.6.1. Introduction

In their original work evaluating the importance of carbon dioxide, acetone and 1-octen-3-ol as attractants for tsetse flies produced by oxen, Vale and Hall (1985a) observed that addition of octenol to ox odour that had been passed through a charcoal filter doubled catches of tsetse at 0.05 mg/hr and trebled catches at 0.5 mg/hr. Addition of octenol to synthetic carbon dioxide had little effect on catches of tsetse, and the authors proposed that there was an attractant or synergist for octenol in ox odour which passed through a charcoal filter.

Prof. Bursell subsequently carried out further field work which confirmed that the effect on catches of tsetse of increasing doses of octenol was much greater in the presence of ox odour than in its absence or with carbon dioxide.

Laboratory work at TRL also showed that human or ox breath passed through charcoal and sodalime filters caused an increase in take-off frequency of *G. m. morsitans*. Carbon dioxide caused a similar response, but the filtered breath remained effective at much greater dilutions. Furthermore, when octenol was tested in the presence of carbon dioxide there was no significant effect on the initial direction of flight, but octenol in the presence of filtered breath gave a significant increase in the proportion of flies taking off in an upwind direction.

The compound or compounds responsible for this synergism of the effect of octenol was christened "Omega" by Prof. Bursell. As Omega passed through a charcoal filter, it was likely that it was very volatile and of low molecular weight. This meant that it would be more difficult to trap, store and analyse than less volatile chemicals such as octenol or the phenols. However, if it was of low molecular weight, this limited the number of candidate chemical structures, particularly as it was known to be of animal origin.

Work on identification of Omega during this project thus explored a number of avenues:

(a) candidate chemicals of low molecular weight, likely to be produced by oxen, were supplied for testing in the field as attractants or attractant synergists;

(b) laboratory work was carried out to try to trap Omega and detect it by linked GC-EAG analysis;

(c) field experiments were carried out to confirm the existence of Omega and characterise it further.

3.6.2. Provision of Omega candidates

The following chemicals were supplied for testing in Zimbabwe. Unless otherwise indicated, they were tested by Dr. Vale or Dr. Holloway for the ability to increase catches of tsetse in traps baited with acetone and octenol. None proved to have any effect on tsetse catches under the conditions used.

Candidates of low molecular weight possibly produced by animals or their residues (QR 86/2).

Aldehydes	**Amines**
formaldehyde	ammonia
acetaldehyde	methylamine
propionaldehyde	dimethylamine
butyraldehyde	trimethylamine
acrolein	allylamine
Hydroxycarbonyls	**Alcohols**
glyceraldehyde	methanol
dihydroxyacetone	ethanol
glyoxal	propanol
	butanol
	allyl alcohol

Furfural. During field experiments with ox odour passed through a charcoal filter it was observed that the resulting volatiles had lost all traces of stable smell and had a slightly sweetish odour. This was thought to be reminiscent of the smell of furfural (QR 86/3).

2- and 3-Methylthiophene. 3-Methylthiophene was identified in pig sty volatiles by MAFF scientists at Tolworth. When tested by EAG, these compounds caused significant positive rather than the conventional negative polarisations across antennae of tsetse species *G. pallidipes*, *G. m. morsitans* and *G. palpalis* (QR 86/4).

Dimethylsulphone, Dimethylsulphoxide and Dimethyldisulphide. Dimethylsulphone was detected in dichloromethane extracts of cattle urine at both NRI and ICIPE. It was completely inactive by EAG, but there were reports that ICIPE had shown some field activity against tsetse. These were tested in Zimbabwe by Dr. Hall (QR 86/4).

1,2-Epoxybutane, 1,3-Propane diol, Xylene. These were tested by Dr. Gough in Zimbabwe at low (2 mm opening), medium (5mm) and high (10mm) doses with epsilon traps baited with acetone (500mg/hr) and octenol (0.5mg/hr) (QR 88/3).

3.6.3. Laboratory work on detection of Omega

Prof. Bursell reported that Omega is probably present in human breath, based on bioassay experiments, and this lead was followed up with EAG work at NRI (QR 88/1, 88/2).

A significant EAG response was obtained to human breath blown directly over a *G. m. morsitans* EAG preparation. The response was abolished when the breath was first passed through a large charcoal filter (160 gm; 6-18 mesh). A significant EAG response was also obtained to human breath stored in a 10 litre gas jar and blown out over the EAG preparation with a pulse of nitrogen.

In order to check whether the above responses were due entirely or in part to the carbon dioxide present, EAG responses from *G. m. morsitans* to synthetic carbon dioxide were recorded. These showed a fast negative response, as in a normal EAG, followed by a smaller positive depolarisation. Significant responses could be obtained to 6% and 12% carbon dioxide, but not to 1%. It was thus considered that carbon dioxide contributed little to the EAG response to human breath ($\leq$ 4% carbon dioxide).

Passing the breath through a small filter packed with the polymeric resin Tenax (8 cm x 3 mm i.d.; 100 mg; 50-80 mesh) did not reduce this response, but no response was obtained when the breath was blown through a similar filter packed with activated charcoal (30-72 mesh, 370 mg). Breakthrough tests were carried out by blowing 10 litres of human breath through charcoal filters (3 mm i.d.) of 1, 2, 4, and 8 cm length. When dry air (500 ml/min; 3 sec) was subsequently puffed through these filters over the antennal preparation, a good EAG response was obtained in each case, indicating that breakthrough had occurred even with the longest 8 cm filter. In each case, the EAG response was reduced by first blowing 2.5 litre of dry air through the filter, and was completely abolished by 5 litre of dry air. The EAG activity did not return after sealing and standing the filters for 24 hrs.

EAG responses to human breath were increased by maintaining the antennal preparation in a constant stream of dry air.

Experiments were carried out in which a 100 ml dreschel bottle was filled with a test gas, and the contents blown over an antennal preparation in approx. 35 ml aliquots with laboratory air. The EAG response disappeared after three such puffs, but using human breath in place of laboratory air at least seven such puffs were required before the response disappeared. This disappearance coincided with disappearance of the film of condensation on the walls of the bottle. When the dreschel bottle was filled with human breath blown through anhydrous silica gel (200 gm) dried at 120^{o}C overnight, no EAG response was obtained when the contents were blown over the preparation.

3.6.4. Laboratory experiments to identify "Omega"

Linked GC-EAG analyses were carried out using a fused silica capillary column (10 m x 0.32 mm i.d.) coated with Poraplot Q (Chrompack). The GC-EAG link was modified by taking the column outlet much closer to the antennal preparation and reducing the stimulus flow from 500 ml/min to 200 ml/min to minimise dilution. Air used for the stimulus and for blowing over the antenna between stimuli was dried by passing through freshly dried silica gel. With this equipment, EAG responses from *G. m. morsitans* were obtained to each component of an injected mixture of 400 µg water, 200 µg acetone, 200 µg butanone and 20 ng octenol (splitless injection; oven temperature 70°C for 1 min, then programmed at 10°C/min to 250°C). Significant responses to water down to 40µg injected could be obtained with this modified equipment.

When 100 µl of human breath was injected, only one significant EAG response was obtained at the retention time of water. A peak corresponding to approx. 1 ng at the retention time of acetone was observed, but no EAG response was obtained.

Cattle breath was collected in Tedlar bags at the TRL by the method of Warnes (1989a) and transported directly to NRI by car. In linked GC-EAG analyses using *G. m. morsitans* preparations, only responses at the retention time of water vapour were recorded, even with injection volumes up to 2 ml.

EAG responses of *G. m. morsitans* and *G. pallidipes* to acetone octenol and breath were compared. Relative to the response to octenol the response of *G. pallidipes* to breath was approximately half that of *G. m. morsitans*. In linked GC-EAG runs with water (400 µg), acetone (200 µg) and butanone (200 µg), similar responses were obtained from both species to acetone and butanone, but responses to the water were obtained only from *G. m. morsitans*.

3.6.5. Conclusions from laboratory work

The above results show that water vapour elicits a good EAG response from tsetse. This was the first time that this phenomenon had been observed, and the results suggested that *G. m. morsitans* was more sensitive than *G. pallidipes*. The only significant EAG response obtained to unconcentrated human or ox breath during linked GC-EAG analyses was to water vapour. As water vapour is poorly retained by activated charcoal and would obviously be present in ox odour used in field experiments, it was considered to be a possible candidate for Omega that should be tested in the field.

3.6.6. Field experiments on water vapour

Initial experiments were carried out by Dr. Vale in April 1988. In these, water vapour was evaporated at approx. 500 gm/hr in front of F3 traps baited with acetone (500 mg/hr) and a standard 8:4:1 4-methylphenol + octenol + 3-propylphenol polythene sachet. There was no significant increase in the number of *G. pallidipes* caught. Numbers of *G. m. morsitans* caught were greater with the addition of water vapour, but overall numbers of this species were low and the difference was not statistically significant at the 5% level.

During a field trip to Zimbabwe during September 1988 (QR 88/3), Dr. Gough measured the water output from an underground pit holding 2 oxen (450 kg each) and ventilated at 2000 litre/min, as in previous field experiments on Omega. Humidity was measured at 16-26% in the surrounding bush and 37-41% at the pit outlet. Air in the pits was humidified by 2 corrugated trays (3m x 80cm each). These could be fitted with two cloths (3 m x 1.2 m) hanging above the trays with their ends in the water so they stayed wet. In the open, the two trays alone lost 12 litres per 24 hr, and the trays with cloths lost 25 litres per 24 hr.

Experiments were run in which air from the pit was exhausted 1 m upwind of an epsilon trap baited with acetone and octenol. Two pits were available so that it was possible to interchange treatments on successive days. Relative humidities were measured at the pit outlet as 35% with the trays only and 40% with the trays + cloths.

The results in Table 3.6.1. showed that even with the higher water release, their was no significant effect on catches of either species of tsetse.

Table 3.6.1. The effect of water vapour on catches of tsetse in epsilon traps baited with acetone and octenol (Rekomitjie, September 1988).

Additional odour[1]	GMM mean[2]	GMM index	GP mean[2]	GP index
Experiment 1 (10 replicates)				
Nil	3.7		39.1	
Water (1 litre/hr)	3.6	0.96	52.1	1.34
Experiment 2 (4 replicates)				
Nil	5.7		43.4	
Water (2 litre/hr)	6.8	1.18	45.2	1.04
Experiment 3 (4 replicates)				
Nil	5.0		34.4	
Water (2 litre/hr) + xylene (5 mm hole)	4.2	0.83	29.8	0.87

[1] all traps baited with acetone (500 mg/hr) and octenol (0.5 mg/hr).

[2] detransformed mean; no significant differences between means for each species at 5% level by DMRT.

3.6.7. Field experiments on Omega: measurement of rates of production of phenols and octenol by cattle.

During a field visit to Zimbabwe in May 1987, rates of production of phenols and octenol by cattle were measured as part of an experiment to determine the attractiveness to tsetse of very large doses of natural and synthetic ox odour (QR 87/2).

Cattle were held in the Pilson Pit and volatiles were extracted at up to 120,000 litres/min. Various weights of cattle were used, and experiments were also run with the residues left after removal of the cattle and with an attempted synthetic simulation of the odour from 60 tons of cattle. Volatiles were sampled with Porapak filters in the pit outlets, and the filters were extracted and analysed by quantitative GC on return to NRI.

Results in Table 3.6.2. show that rates of production of the phenols increased with increasing weight of cattle, and that production of the phenols continued from the residues after removal of the cattle.

Two other conclusions from these measurements were as follows. Firstly the synthetic simulation of 60 tons of cattle produced only one third the amounts of the phenols produced naturally. Secondly, octenol was not detected in any of the measurements with the natural odour sources, and this was also found during similar experiments in 1989 (AR 89).

3.6.8. Field experiments on Omega: comparison of attractiveness of natural and synthetic ox odour.

The confirmation that the phenols in ox odour were derived from the ox residues led to an experiment to compare the attractiveness to tsetse of natural ox odour and the best available "semi-synthetic" odour made up of volatiles from cattle residues with other synthetic chemicals known to be produced by the live animals (QR 87/4).

Catches of tsetse with odour from 20 tons of cattle were compared with those with the residues from 20 tons of cattle supplemented by acetone, butanone, octenol and carbon dioxide. Two pits were available for this experiment so that the treatments could be alternated between sites, and catches in nine replicates are shown in Table 3.6.3.

Table 3.6.3. Catches of tsetse and other flies at pits baited with 20 tons of cattle ("Natural") and simulated 20 tons of cattle ("Artificial"), Rekomitjie, October 1987.

	Mean Catch (9 replicates)				
Odour	GMM	GP	biting muscids	non-biting muscids	tabanids
Natural	4.0	628.3	66.9	38.2	0.2
Artificial[1]	2.4	306.2	17.8	6.2	0.1
Nat/Art	1.6	2.1	3.8	6.1	2.0

[1] residues from 20 ton cattle + acetone (200 mg/hr) + butanone (2 mg/hr) + octenol (2 mg/hr) + carbon dioxide 50 litre/min.

Table 3.6.2. Air sampling of phenols and octenol from ox pits in Zimbabwe.

Source	Rate of production (mg/hr)[1]								Ref.
	2MeO	PhOH	4Me	3Me	4Et	3Et	3/4Pr	octenol	
May 1987									QR 87/2
60 tons oxen	12.1	51.6	428	157	31.5	8.7	31.5	-	
10 tons oxen	2.6	7.5	52	37	4.8	1.0	4.6	-	
5 tons oxen	0.8	1.3	7	5.3	0.7	0.2	0.6	-	
60 tons oxen simulation[2]	-	16.8	121	24.8	10.7	-	14.1	14.1	
June 1987									QR 87/2
40 tons oxen		23.5	121	48.9	8.7		12.7	-	
20 tons oxen		13.6	95	36	7.0		6.4	4.0	
10 tons oxen		5.8	22.3	9.1	1.8		1.8	-	
5 tons oxen		2.7	22.2	5.1	0.9		1.0	-	
60 ton oxen residue		30.1	276	117	10.7		13.4	-	
60 ton oxen residue		17.4	128	23.5	8.0		8.0	-	

Table 3.6.2. Air sampling of phenols and octenol from ox pits in Zimbabwe (cont.)

Source	Rate of production (mg/hr)[1]								Ref.
	2MeO	PhOH	4Me	3Me	4Et	3Et	3/4Pr	octenol	
October 1989									AR 89
4 oxen[3] (approx 3 ton)		0.61	1.6	0.3	0.4		0.12	-	
5 sachets[4]			5.6				0.33	0.8	

[1] 2MeO = 2-methoxyphenol; PhOH = phenol; 4Me, 3Me = 4- and 3-methylphenol; 4Et, 3Et = 4- and 3-ethylphenol; 3/4Pr = 3- and 4-propylphenol.

[2] CO_2 7,500 litre/hr, octenol 5 mg/hr, MEK 5 mg/hr, acetone 500 mg/hr, PhOH 5 mg/hr, 4Me 50 mg/hr, 3Me 10 mg/hr, 4Et 5 mg/hr, 3Pr 5 mg/hr.

[3] mean of 5 replicates

[4] polythene sachets, 50 sq. cm., 150µ thick, containing 8:1:4 4Me + 3Pr + Oct

The results showed that the Natural odour was more attractive than the Artificial to both species of tsetse, particularly *G. pallidipes*, suggesting that the Artificial odour was lacking some essential components which are attractants or which synergise the activity of the other attractants. The higher catches of non-tsetse flies with the Natural odour suggested that the dose of carbon dioxide used in the Artificial odour may have been too low. However, the catch of tsetse flies is not very sensitive to dose at these high levels, and it was thought unlikely that this could explain the differences in catches.

3.6.9. Field experiments on Omega: experiment to confirm existence of Omega, 1986

This experiment was designed to confirm the Omega effect, i.e. that ox odour filtered through charcoal increased the attractiveness of acetone and octenol to tsetse, and to determine whether Omega was operative in the absence of carbon dioxide. If the presence of carbon dioxide was required, Omega would not be of great practical use in field operations (QR 86/4).

Three sites were used, all fitted with a standard electrified net and model and baited with high doses of acetone and octenol. At one site, additional odour could be supplied from a pit ventilated with a centrifugal fan built at NRI. Odour from two oxen in the pit could be passed through filters attached to the fan, one containing 1.3 kg of activated charcoal, removing high-boiling components, and another containing 2.5 kg of sodalime which was renewed each day, removing carbon dioxide. Laboratory experiments at NRI had indicated that this quantity of sodalime had the capacity to remove the carbon dioxide produced by two oxen over a two-hour period (QR 86/3), and the carbon dioxide concentration at the pit outlet was measured with a EGM-CO_2 portable meter from Kent Industrial Measurements Ltd.

The effects of different additional odours at the pit site were assessed by taking the geometric mean of the catches at the other two sites as an indication of the general level of catches on that day, and calculating the Catch Ratio of the catch at the pit site relative to that mean. Catch Indices were then calculated as the ratio between the mean Catch Ratio with the additional test odour and the mean Catch Ratio with only acetone and octenol at the pit site, and the results are shown in Table 3.6.4.

Table 3.6.4. Effects of ox odour on attractiveness of acetone and octenol to tsetse, Rekomitjie, September-October 1986.

Additional Odour[1]	No. Reps.	GMM Catch Ratio	GMM Catch Index	GP Catch Ratio	GP Catch Index
Nil	11	1.11	1.00	0.64	1.00
2 oxen	9	2.67	2.41	7.99	12.47
2 oxen + charcoal	7	4.20	3.80	6.35	9.91
2 oxen + charcoal + sodalime	7	2.41	2.18	2.78	4.33
CO_2 (2.5 l/min)	7	2.39	2.16	2.61	4.07

[1] all sites with electrified net and model, acetone (500 mg/hr) and octenol (1 mg/hr); pit ventilated at 2,400 l/min

For *G. pallidipes*, addition of unfiltered odour from two oxen to the acetone and octenol increased catches by 12.5 times. Filtering the ox odour through charcoal reduced this increase to 9.9 times, and further filtering through sodalime reduced this increase to 4.3 times. Synthetic carbon dioxide released in the pit at a rate estimated to be the same as from the oxen increased the attractiveness of acetone and octenol by 4.1. times.

These results were interpreted to indicate that the charcoal filter removed some unidentified attractants from ox odour (12.5x - 9.9x), leaving Omega plus carbon dioxide. Addition of the sodalime filter to this removed the carbon dioxide, the decrease in attractiveness (9.9x - 4.3x) being reasonably consistent with the observed effect of synthetic carbon dioxide (4.1x). Thus Omega was deduced to increase the attractiveness of acetone and octenol to *G. pallidipes* by an exceedingly useful 4.3 times in the absence of carbon dioxide.

Catches of *G. m. morsitans* were, as usual, low and results were more variable. There were no significant differences between the catches with any of the additional natural or synthetic odours.

3.6.10. Field experiments on Omega: experiment to determine the effect of Omega on the best available attractant mixture, 1989

This work was carried out after the discovery of the phenolic attractants for tsetse, and the aim of the experiment was to determine whether Omega could increase the attractiveness of the best available lure consisting of acetone, octenol, 4-methylphenol and 3-propylphenol (AR 89).

Four oxen were used in the same ventilated pit as in 1986, but this was fitted with a chamber in the outlet taking up to 100 kg of sodalime. The pit was ventilated with the centrifugal fan used previously which could be fitted with a filter containing 2 kg of activated charcoal. A more sensitive carbon dioxide monitor, EGA from ADC Ltd., was acquired by NRI, and this was used to ensure that the sodalime filter completely removed the carbon dioxide from the ox odour. It was also used to measure accurately the carbon dioxide production by an ox. Adsorbent filters were used to sample the pit effluent before and after the sodalime and charcoal filters, and these were extracted and analysed for octenol and phenols at NRI.

An electrified screen with flanking nets was positioned at the pit outlet, baited with acetone (500 mg/hr) and five standard polythene sachets containing an 8:4:1 mixture of 4-methyl-phenol, octenol and 3-propylphenol. The air sampling filters showed that this bait released the attractants at 3-4 times the rate of production by the four oxen, so that addition of the natural components produced by the oxen to the synthetic lure would have had little effect on its attractiveness to tsetse.

The different treatments were tested at this one site, and the effects of the treatments were assessed by comparing the catches at this site with those at three other sites. One of these other sites used an electrified net as catching device, the other two used traps, and all three sites were baited with the same synthetic lure of acetone and five sachets used at the treatment site. The catch at the treatment site was expressed as a proportion of the sum of the catch at the treatment site and the detransformed mean (log[x+1]) of the catches at the other three sites. The proportions were transformed to arcsin($\sqrt{x}$) and subjected to analysis of variance.

The results are shown in Table 3.6.5.

Table 3.6.5. Effects of ox odour filtered through sodalime and charcoal on catches of tsetse at an electrified net baited with acetone, octenol and phenols, Rekomitjie, October 1989.

Additional Odour[1]	GMM		GP	
	Ratio[2]	Index[3]	Ratio[2]	Index[3]
4 oxen	3.35 a	1.58	3.94 a	2.76
4 oxen + sodalime	1.59 b	0.75	1.91 b	1.34
4 oxen + sodalime + charcoal	1.53 b	0.72	1.18 c	0.83
CO_2 (10 litre/min)	3.32 a	1.56	3.08 a	2.16
Nil	2.13 ab	1.00	1.43 bc	1.00

[1] all devices baited with acetone (500 mg/hr) and 5 sachets of 8:1:4 4-methylphenol + 3-propylphenol + octenol.

[2] detransformed mean ratio between catch at treatment site and detransformed mean of catches at three control sites; ratios followed by the same letter not significantly different at 5% level.

[3] ratio of catch ratio with treatment to catch ratio with no additional odour at treatment site.

For both species of tsetse, the increase in catch obtained with the unfiltered odour of 4 oxen was the same as the increase with an equivalent amount of synthetic carbon dioxide. This indicated that the oxen were producing no components other than carbon dioxide which could markedly increase the attractiveness of a lure containing acetone, octenol and the phenols. In agreement with this, filtering the ox odour through both sodalime and charcoal reduced catches to the level with no additional odour. However, the catches of *G. pallidipes* with ox odour passed through the sodalime filter only were significantly greater than those with both the sodalime and charcoal filters, suggesting that there may be additional attractants in ox odour which can increase catches of *G. pallidipes* by about 50% and which are trapped on activated charcoal. This effect was not observed for *G. m. morsitans*.

Analyses of the air sampling tubes showed the presence of the urine phenols in the pit effluent. Release was rather lower than had been observed previously (Table 3.6.2.), but in this experiment the pit was cleaned of residues every day. Octenol

was never detected in volatiles from the oxen. When sachets containing synthetic octenol, 4-methylphenol and 3-propylphenol were placed in the pit, the amount of octenol trapped was lower than anticipated, suggesting that this compound was being lost somewhere in the system, and this might possibly explain why small amounts of octenol from the animals were not detected The phenols and octenol were completely removed by the basic sodalime filter. 3-Octanol was released in the pit from a polythene sachet as internal standard. This was not affected by the sodalime filter and not completely removed by the charcoal filter.

During the experiments with the sodalime filter in place, the carbon dioxide concentration in the pit effluent was monitored continuously, and was not allowed to rise above 0.07%, even at the end of an experiment. The carbon dioxide concentration in ambient air is approximately 0.04%.

The concentration of carbon dioxide in the effluent from the pit containing 4 oxen with no sodalime filter was measured at 0.2 - 0.4%. Given the pit ventilation rate of 2000 litre/min, this corresponds to rates of production of 1 - 2 litre/min per animal.

3.6.11. Field experiments on Omega: reexamination of equipment used in 1986 experiments

The availability of an accurate carbon dioxide monitor made it possible to reexamine the sodalime filter that had been used in the 1986 experiments. This was filled with sodalime that had been used in these experiments and stored in a sealed tin since then. It was shown that this filter did not remove carbon dioxide effectively from the airstream, and thus it was probable that, at least towards the end of each day during 1986, there was significant breakthrough of carbon dioxide. Although the capacity of the sodalime had been calculated and measured to have been sufficient, the flow rate may have been too rapid to allow efficient contact and absorption.

3.6.12. Conclusions and Recommendations

The field experiment carried out in 1989 indicated that ox odour does not contain any components which can greatly increase the attractiveness to tsetse of the current best lure of acetone, octenol and the phenols.

The measurements made in 1989 indicated that in the 1986 experiments there was probably appreciable breakthrough of carbon dioxide through the sodalime filter used, at least towards the end of each day's experiment. In these 1986 experiments, addition of unfiltered ox odour to electrified nets baited with acetone and octenol increased catches of

G. pallidipes by 12.5 times. Addition of the charcoal filter would have removed at least some of the phenols in the ox odour, and the increase in catch was reduced to 9.9 times. Further addition of the sodalime filter would have probably removed all the phenols and some of the carbon dioxide, reducing the increase in catch to 4.3 times. This was similar to the increase of 4.1 times observed with addition of synthetic carbon dioxide to the acetone and octenol, not unreasonably since catches are known not to be very sensitive to the actual level of carbon dioxide, certainly within the limits of accuracy of this experiment.

Effects observed previously may have been due to breakthrough of carbon dioxide and possibly also some of the phenols in natural ox odour.

It is recommended that the 1989 experiments should be repeated to obtain a larger, more reliable data set, and to confirm that ox odour really does not contain unidentified components that can greatly increase - by four times - the catch of tsetse by the current best lure.

The 1986 experiments used the "incomplete" lure of acetone and octenol only. For completeness, any further experiments should include additional treatments to check whether unidentified components of ox odour can greatly increase the catch of tsetse by this "incomplete" lure.

These further experiments would also firm up the evidence that ox odour does contain some components other than the phenols and octenol which can give small, but useful, increases in catch of *G. pallidipes* and which are trapped on charcoal. These components may have been identified already - e.g. indole, 3-methylindole, Peak 6, Peak 7 - or further analytical work may be necessary to detect and identify them.

3.7. IDENTIFICATION OF POTENTIAL TSETSE ATTRACTANTS IN EXTRACTS OF CHARCOAL USED TO TRAP OX VOLATILES

3.7.1. Introduction

Activated charcoal used to trap ox volatiles during field experiments on Omega during 1986 was extracted with dichloromethane, and the resultant extract was tested to check whether any significant tsetse attractants had been trapped and recovered.

As shown in Table 3.7.1., the extract almost doubled catches of *G. pallidipes* in F3 traps baited with acetone and octenol.

Table 3.7.1. Effect of adding charcoal extract to F3 traps baited with acetone and octenol, Zimbabwe, May 1987.

	GMM		GP	
Bait[1]	mean[2]	index	mean[2]	index
acetone + octenol	11.3	1.00	153.8	1.00
acetone + octenol + extract	9.2	0.81	295.0	1.92 ***

[1] acetone at 100 mg/hr and octenol at 0.5 mg/hr; extract equivalent to 0.2 ox hr on cloth.

[2] 10 replicates; mean is detransformed mean; *** indicates index significantly different from unity at 0.1% level.

3.7.2. Analysis

In the laboratory, the crude charcoal extract was highly active when tested by EAG against *G. pallidipes*. Analysis of the extract by GC-MS using a polar GC column showed two main components, labelled (B) and (C). Compound (C) was easily identified as tributyl phosphate, and compound (B) had a mass spectrum similar to those of methyl ethers of di-*tert*-butylhydroquinones. However, in linked GC-EAG analyses with a polar GC column, neither of these components, or any other, showed EAG activity.

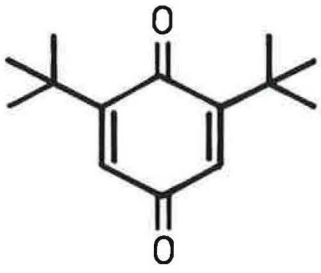
The extract was fractionated by liquid chromatography (LC), and fractions were tested for EAG activity. Most activity was

located in two adjacent fractions, and analysis of these by GC-MS with a polar column showed the presence of compound (B).

Analysis of the same fractions with a non-polar GC column showed two main peaks - compound (B) and a new compound (A) with slightly shorter retention time, present at approximately 14 mg/kg of charcoal. The ratio of (A):(B) was 100:43.

3.7.3. Identification of EAG-active component

This new compound (A) was identified as 2,6-di-*tert*-butyl-1,4-benzoquinone by comparison of its GC retention time and mass spectrum with those of the synthetic compound. The synthetic compound elicited a strong EAG response from *G. pallidipes* when presented alone or by linked GC-EAG (Fig. 3.7.1.)



2,6-DI-*tert*-BUTYL-1,4-BENZOQUINONE

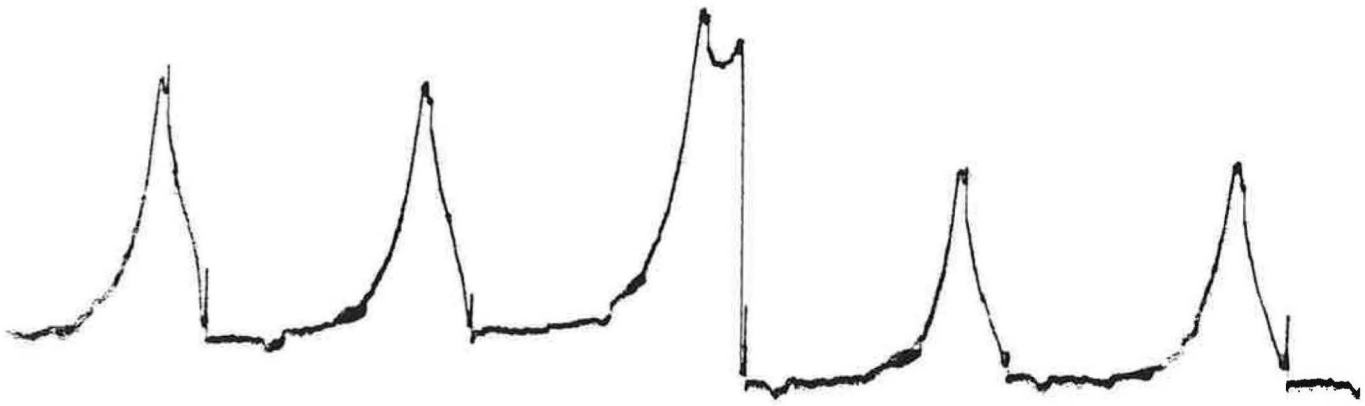


Fig. 3.7.1. EAG response of G. pallidipes to 2,6-di-tert-butyl-1,4-benzoquinone (A) from charcoal in linked GC-EAG run.

Although compound (B) had shown no EAG activity, some further attempts were made to identify it. The remainder of the charcoal was extracted and the extract fractionated more carefully by LC to give fractions containing only (B). Infrared and NMR spectra were recorded and these showed fairly conclusively that the compound was another, symmetrical di-*tert*-butyl-benzoquinone, i.e. probably 2,5-di-*tert*-butyl-1,4-benzoquinone, or the 3,6-di-*tert*-butyl- or 4,5-di-*tert*-butyl-1,2-benzoquinones (QR 87/3).

3.7.4. Field testing

Aliquots from the LC fractions equivalent to 200 gm of charcoal were field tested in Zimbabwe by Dr. Holloway along with the crude extract, but none showed any attractiveness to tsetse.

Tributyl phosphate (C) was tested in Zimbabwe at three release rates with F3 traps baited with acetone at 100 mg/hr and octenol at 0.5 mg/hr, but there was no significant effect on catches of either species of tsetse (QR 87/2).

The EAG-active compound, 2,6-di-*tert*-butyl-1,4-benzoquinone (A), would have been present at approximately 150 µg in the original tests with the crude charcoal extract (Table 3.7.1.). The synthetic compound was tested in Zimbabwe as for (C) above, using 1 mg dispensed from cloth. Also tested were the analogues 3,5-di-*tert*-butyl-1,2-benzoquinone and 2,5-di-*tert*-butyl-1,4-hydroquinone, but none showed any effect on catches of tsetse. These compounds were also tested alone at the same level with biconical traps in Côte d'Ivoire, but catches of *G. tachinoides* were unaffected (QR 87/2).

3.7.5. Conclusions and Recommendations

It is unlikely that any of these three compounds, (A), (B) and (C), is derived from the oxen. They are known as antioxidants or their degradation products, and it is possible they came from plastic tubing used as ducting for the ox odour. The EAG activity of 2,6-di-*tert*-butyl-1,4-benzoquinone (A) is remarkable, and, in spite of the unsuccessful field tests with the synthetic material and the difficulty of repeating the results originally obtained with the crude charcoal extract, it is recommended that this compound be re-examined in the field for any behavioural effect on tsetse.

3.8. INVESTIGATION OF BEHAVIOUR-MODIFYING CHEMICALS FOR TSETSE IN OX SEBUM

3.8.1. Introduction

Laboratory bioassay work carried out by Warnes (1989b) at the TRL showed that solvent extracts of sebum from cattle hair increased flight activity of both *G. m. morsitans* and *G. pallidipes* around targets as though the flies were searching for feeding sites. In other experiments the sebum was found to increase the frequency of probing of both species.

3.8.2. Fractionation of sebum extract

For field testing, approximately 2 kg of cattle hair was collected by Dr. Warnes and washed with dichloromethane at NRI. The extract was concentrated to 1000 ml, and half of this was chemically fractionated into phenolic, acidic and non-acidic fractions (QR 87/4).

The phenolic fraction was analysed by capillary GC for known phenols, and the relative compositions are shown in Table 3.8.1. The phenolic fraction was extremely dilute, with the 4-methylphenol at only 1 mg/litre (cf. 500 mg/litre in urine), and it was not possible to determine the relative amounts of 3- and 4-propylphenol (QR 87/4).

Table 3.8.1. Composition of the phenolic fraction from sebum washings.

Phenol	Relative concentration (4-methyl = 100)
phenol	5.0
3-methylphenol	6.4
4-methylphenol	100
3-ethylphenol	3.5
4-ethylphenol	4.4
3/4-propylphenol	2.3

3.8.3. Field testing of sebum extract and fractions.

The extract and fractions were field tested by Dr. Warnes at Rekomitjie in September 1988 and April 1989 (Warnes, 1990). Solutions were sprayed onto cloth targets and used with a variety of electrified devices and traps, all baited with acetone at 500 mg/hr and a standard sachet containing a 8:1:4 mixture of 4-methylphenol, 3-propylphenol and octenol. Results were variable, but catches of both *G. m. morsitans* and *G. pallidipes* at electrified targets were generally higher in the presence of sebum, often significantly so. Although there was sometimes an increase in the proportion of *G. pallidipes* landing, the predominant effect of sebum was to increase the total number caught, i.e. to attract more flies to the region of the target.

Testing of the fractionated sebum extract sprayed onto targets gave increases in catch of both species with the phenolic and non-acidic fractions, but the acidic fraction had no effect.

The phenolic fraction was so dilute in known phenols that it would not be expected to increase the attractiveness of the phenol sachet used in all these tests. Spraying the target with an equivalent amount of a mixture of synthetic phenols in the proportions shown in Table 3.8.1. did not give significant increases in catches, confirming that the phenolic fraction of sebum might contain additional, unidentified attractants.

Positioning a cloth screen sprayed with sebum extract next to a F3 trap gave increased catches in the trap, supporting the conclusion that the sebum acts to attract flies from a distance.

3.8.4. Analysis of phenolic fraction.

The non-acid fraction contained large amounts of waxy material and was not investigated further during this project.

GC-EAG analyses of a sample of the phenolic fraction used in the field tests with a polar or non-polar capillary GC column gave three responses. The first two responses occurred at the retention times of 1-octen-3-ol and 4-methylphenol respectively, and these assignments were confirmed by GC-MS analysis using a polar column.

The third response occurred at retention times of 18.36 ECL and 16.2 ECL (relative to retention times of straight-chain acetates) on the polar and non-polar columns respectively, and amounts of components eluting at these retention times were extremely small (QR 87/4).

3.8.5. Further analyses.

In order to obtain more material, a further 1 kg of ox hair was washed with dichloromethane, and the washings concentrated and chemically fractionated as above. The phenolic fraction was chromatographed on Florisil eluted with a gradient of ether in pentane. Ninety fractions were collected and 5 µl aliquots of alternate fractions bioassayed by EAG with a male *G. m. morsitans* preparation.

Fractions around 55 were the most active and shown to contain the phenols by GC-MS analysis. Significant responses were also found around fractions 40 and 70. Fraction 40 was found to contain 1-octen-3-ol by GC-MS.

Aliquots from fractions 35-75 were combined and analysed by GC-MS and GC-EAG. The major peak at ECL 19.00 on a polar GC column was identified as (*E*)-3,7,11,15-tetramethyl-2-hexadecen-1-ol (Fig. 3.8.1.), trivial name *trans* phytol, by its GC retention time and mass spectrum. This must presumably have been present as a result of incomplete separation of the phenolic and non-acidic fractions due to emulsification during the separatory process.

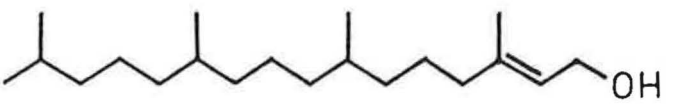


Fig. 3.8.1. <u>trans</u> Phytol

A commercial sample of phytol (*trans* : *cis* 60:40) was inactive by EAG against *G. m. morsitans* at the 10 ng level off glass.

Linked GC-EAG analysis of the fractions 35-75 on a polar column gave six responses. Four of these were shown to be due to 1-octen-3-ol, 4-methylphenol, 3- and 4-ethylphenol and 3- and 4-propylphenol. The other two responses were due to components eluting just after 1-octen-3-ol and at 18.36 ECL, the latter presumably corresponding to the unknown component detected previously. Amounts of these two components were too small for further identification (QR 87/4).

3.8.6. Discussion and Recommendations

The only new compound identified from sebum has been phytol, but no EAG response was observed to this in linked GC-EAG analyses, and no response was obtained to the synthetic material. Two unidentified components eliciting EAG responses from *G. m. morsitans* were detected in linked GC-EAG analyses of the phenolic fraction, but the amounts were extremely small and further work was not attempted in the absence of conclusive evidence for their importance in the field.

It is recommended that chemical support for this work be continued by providing sebum extracts and fractions in order to establish more definitively the effects of these on tsetse behaviour in the field.

3.9. CONCLUSIONS AND RECOMMENDATIONS

3.9.1. Overview

Table 3.9.1. summarises the 21 new compounds investigated during this Project with an indication of the biological activity observed as measured by EAG response, laboratory bioassay and field testing and described in the relevant sections above. The Table also shows whether further work on the compound is recommended in this Report.

3.9.2. Urine phenols (Section 3.2.)

The 3- and 4-*iso*propyl phenols elicited strong EAG responses from *G. pallidipes* and *G. m. morsitans*. They are both readily available commercially, and, in fact, commercial 3-*iso*propyl phenol was found to be a 60:40 mixture of the 3- and 4-isomers. It is recommended that they be retested for field activity.

3.9.3. Urine indoles (Section 3.3.)

Indole and 3-methylindole caused strong EAG responses from tsetse and showed activity in the laboratory bioassay. There was some indication of attractiveness in the field and it is recommended that they be retested with both traps and targets at various release rates.

3.9.4. Peak 6 (Section 3.4.)

The carotenoid metabolites (6), (7), (6K) and (7K) elicited strong EAG responses from tsetse and were active in the laboratory bioassay, but they showed no activity in the field. Although it is unlikely that these compounds would ever be cost-effective as attractants because of their chemical complexity, it would be interesting to carry out further behavioural work on these unusual compounds in the laboratory and/or field. If possible, homochiral compounds should be synthesised and used in this work.

3.9.5. Compounds from bushpig bedding sacks (Section 3.5.)

Further work on the bushpig bedding sacks is not recommended because of the difficulty of analysing these, the variability of the results and the fact that the field effect is at best small.

Vanillin and acetovanillone showed no EAG activity, but behavioural activity in the laboratory bioassay was reported. Field tests gave variable results, but there was some

Table 3.9.1. Summary of compounds investigated.

	Isolated from	Biological activity			more work?	Section
		EAG	Lab	Field		
1. Phenol	urine	+		0	N	3.2.
2. 3-Methylphenol	urine	+		+	N	3.2.
3. 4-Methylphenol	urine	+	+	++	N	3.2.
4. 3-Ethylphenol	urine	+		+	N	3.2.
5. 4-Ethylphenol	urine	+		+	N	3.2.
6. 3-Propylphenol	urine	+	+	++	N	3.2.
7. 2-Methoxyphenol	urine	+	+	--	N	3.2.5.
8. 3-*iso*-propylphenol	synthetic	++		0	Y	3.2.
9. 4-*iso*-propylphenol	synthetic	++		0	Y	3.2.
10. 3-(1-propenyl)phenol	synthetic			+	N	3.2.8.
11. Acetophenone	synthetic	+		--	N	3.2.5.
12. Indole	urine	++		+?	Y	3.3.
13. 3-Methylindole	urine	++		+?	Y	3.3.
14. Peak 6	urine	++	+	0	(Y)	3.4.
15. Peak 7	urine	++	+	0	(Y)	3.4.
16. Vanillin	pig sack	0	+	0	Y	3.5.3
17. Acetovanillone	pig sack	0	+	0	Y	3.5.3.
18. 1-Methylhydantoin	pig sack	0	0	0	N	3.5.4.
19. Water	breath	+	0	0	N	
20. 2,6-di-*tert*-butyl-1,4-benzoquinone	charcoal	+		0	Y	3.7.1.
21. (*E*)-Phytol	sebum	0		0	N	3.8.5.

indication of attractiveness, and this should be checked definitively. Both compounds are readily available, and unlike the other phenols, have a pleasant smell to the human nose.

3.9.6. "Omega" (Section 3.6.)

The latest field experiments indicated that ox odour does not contain any unidentified components which can markedly (x 4) increase the attractiveness of the known attractants. This important result should be checked definitively by carrying out more replicates, now the technology has been developed. This work would also confirm whether there are really other components in ox odour which can give a modest (50%) increase in catch and which are adsorbed by a charcoal filter.

3.9.7. Compounds from charcoal filters (Section 3.7.)

The extract of the charcoal used to filter ox odour showed significant attractiveness to tsetse in the field, and 2,6-di-*tert*-butyl-1,4-benzoquinone was the only compound in the extract which elicited an EAG response from *G. pallidipes*. In field tests this compound was unattractive. Although it is unlikely to have been produced naturally, it is readily available and should be retested.

3.9.8. Behaviour modifying chemicals from ox sebum (Section 3.8.)

trans Phytol has been identified in sebum extracts but this is inactive by EAG and in the field. Traces of EAG-active compounds have been detected in extracts, but separation and analysis of these materials is difficult. Chemical support for this work should be continued in order to define better the behavioural effects of ox sebum on tsetse in the field.

3.9.9. The significance of electroantennography (EAG)

Of the 21 compounds listed in Table 3.10.1., 16 elicited a significant EAG response from tsetse. Of these, nine showed some behavioural effect in the laboratory bioassay, but only four - 4-methylphenol, 3-propylphenol, 2-methoxyphenol and acetophenone - showed marked behavioural activity in field tests, with possible weaker effects for five others - 3-methylphenol, 3-ethylphenol, 4-ethylphenol, indole and 3-methylindole.

These discrepancies may well be due to the fact that the laboratory and/or field tests do not measure the specific behaviour influenced by the stimulus, or that the tests are not performed under appropriate conditions - e.g. 4-methylphenol and 3-propylphenol are only weakly attractive

to *G. pallidipes* when presented individually but are highly attractive when presented together.

It is also likely that EAG responses, which are the summed change in resting potential when receptors on the antennae are stimulated, can be relatively non-specific with a wide range of compounds causing EAG responses when the antenna is exposed to a sufficiently high dose in tsetse. This is why EAG is of limited value in random screening for behaviourally-active compounds for tsetse. On the other hand, the summation effect of EAG over all receptors on the antenna is essential for detecting unknown stimulants in mixtures by linked GC-EAG , and this latter technique becomes a powerful means of detecting the active components when used to analyse mixtures which have been demonstrated to be behaviourally-active (Cork *et al.* 1990).

However, once a stimulatory compound has been identified, if it could be shown that the compound stimulates a specific type of receptor this would make it more likely that the compound produces some behavioural effect.

Recording from single antennal receptor cells has been used extensively with Lepidoptera and Coleoptera (Wadhams *et al.*, 1990) as well as other insects such as mosquitoes (Davis, 1988). Recording from single receptors on tsetse antennae has been carried out in Prof. Boeck's laboratory at the University of Regensburg, Germany, (Boeck, personal communication) and it is recommended that this technique be investigated as a means of providing further information on the specificity of receptors for compounds found to elicit EAG responses in linked GC-EAG analyses of behaviourally-active mixtures.

4. DEVELOPMENT OF DISPENSING SYSTEMS FOR USE WITH TSETSE ATTRACTANTS IN THE FIELD

4.1. DISPENSERS FOR PHENOLS AND OCTENOL

4.1.1. Introduction

At the time of the start of this project, the lure used for tsetse with traps and targets consisted of acetone and octenol. Acetone was released from an open bottle, but sealed, slow release dispensers were developed by NRI for the octenol. One consisted of a bottle sealed with a rubber septum so that the octenol diffused out through the septum, and the other was a sealed polyethylene vial filled with a solution of octenol in paraffin oil (Vale and Hall, 1985a).

During identification of the urine phenols as tsetse attractants, field testing utilised release of the neat materials from open bottles or release from aqueous solution. As the phenols are hygroscopic, it was impossible to determine release rates from the neat liquids reliably by simple weighing. Also, of course, it was impossible to determine release rates from aqueous solution by weighing because of concommitant evaporation of the water. Thus techniques were developed at NRI for trapping and quantitative analysis of phenols and other tsetse attractants given off from any dispensing system under standard conditions. This made it possible to correlate release rates of attractants from these early dispensing systems with their attractiveness to tsetse in the field, and hence to design more practical, long-lived dispensers which gave the optimum release rates.

Work was also carried out to develop more convenient, sealed dispensing systems to replace the open bottle system for the acetone component of the tsetse lure.

4.1.2. Release of neat liquids from open containers

Release rates were measured by placing the dispenser in a silanised glass container, and drawing air into the container through an activated charcoal filter, over the dispenser and out through a filter containing the polymeric adsorbent Porapak Q. The whole apparatus was maintained in a room thermostatted at 27^{o}C. Samples could also be maintained in a wind tunnel in the same room with windspeed 8 kph. Volatiles trapped on the Porapak were recovered by eluting with dichloromethane, and the resulting solutions analysed quantitatively by gas chromatography against an internal standard.

Release rates of a variety of phenols were measured from open bottles with different apertures, corresponding to those used in field experiments. Representative examples are shown in Table 4.1.1. (QR 86/2, 86/4, 87/3).

Table 4.1.1. Release rates of phenols from different diameter open bottle dispensers at 27°C, measured by entrainment.

Phenol	dia.	Release Rate (mg/24 hr)[1]				
		2 mm "low"	8 mm "medium"	20 mm "high"	35 mm "very high"	50 mm
4-methyl				5.8	(42.0)[2]	(50.2)[2]
3-ethyl		0.35	0.59	2.4	(0.36)[2]	(0.40)[2]
4-ethyl					(0.51)[2]	(0.78)[2]
3-propyl		0.15	0.29	0.91	(0.36)[2]	(0.40)[2]
2-methoxy				7.7		
[Relative areas		1	16	100	306	529]

[1] values are means of at least four measurements; "low" etc. are release rate designations used in field work (Vale *et al.*, 1988b)

[2] release from a mixture containing phenol + 3-methyl + 4-methyl + 3-ethyl + 4-ethyl + 3-propyl + 4-propylphenol at 1.4 : 9.9 : 100 : 2.1 : 1.1 : 0.8 : 2.5

In the alkylphenols, release rate decreased with increasing molecular weight and boiling point as expected. 2-Methoxyphenol has a similar boiling point (205°C) to that of 4-methylphenol (202°C), but release is significantly faster, probably because of internal hydrogen bonding in the molecule. Release rate was qualitatively but not apparently quantitatively related to area of the dispenser opening.

4.1.3. Release of phenols from aqueous solution (QR 87/3)

The more detailed fieldwork with the phenols utilised aqueous solutions of the synthetic compounds, simulating the natural urine (Vale *et al.*, 1988b). These experiments showed that mixtures of 4-methylphenol and 3-propylphenol were at least as

attractive as the natural urine or aqueous solutions of all eight phenols found in urine. Release rates were measured for these two phenols from 10:1 mixtures in aqueous solution, and these were correlated with attractiveness to tsetse in the field.

Table 4.1.2. Release rates of 4-methylphenol and 3-propylphenol from a 10:1 mixture in aqueous solution at 27°C and corresponding increases in catch of tsetse.

Concentration	Release (mg/24hr)[1]		Ratio	Catch Index[2]	
	4-methyl	3-propyl	4Me/3Pr	GMM	GP
0.5 mg/ml	3.9	0.54	7.2	1.34*	4.33***
5.0 mg/ml	33.9	4.36	7.8	1.29	5.96***

[1] measured by entrainment; means of four 3-hr exposure periods; 200 ml solution in jars 54 mm i.d. x 120 mm high.

[2] indices for addition of solutions to F3 traps baited with acetone at 500 mg/hr and octenol at 0.5 mg/hr; *, *** indicate indices differ from unity at 5% and 0.1% levels.

Results in Table 4.1.2. show that a release rate for 4-methylphenol of at least 4 mg/day under laboratory conditions gave a four-fold increase in trap catch of *G. pallidipes*. Higher release rates gave higher catches of *G. pallidipes*, but there was evidence from these and other experiments that high release rates of the phenols decreased catches of *G. m. morsitans*.

4.1.4. Release of phenols from polyethylene vials

Although effective at increasing catches of tsetse, the aqueous solutions of phenols did not constitute a convenient, easy-to-handle, long-lived dispensing system. Experience with insect pheromones had shown that polymeric matrices such as polyethylene or rubber impregnated with these compounds can provide long-lived, slow-release dispensing systems, and these were investigated for use with the phenolic tsetse attractants.

Polyethylene vials impregnated with 4-methylphenol gave low release rates, and the effects of adding plasticisers were examined (QR 86/2).

Table 4.1.3. Release rates of phenols from polythene vials with diluent/plasticiser at 27°C.

Phenol	plasticiser[2]	Release rate (mg/24hr)[1]	
		DOP	Cereclor
3-methyl		0.082	0.242[3]
3-ethyl		0.070	0.197
3-propyl		0.070	0.188

[1] measured by daily weight loss over 70 days.

[2] 100 mg of phenol in 0.5 ml plasticiser; DOP = dioctylphthalate.

[3] this value was checked by entrainment giving 0.308 ± 0.071 mg/24hr.

Results in Table 4.1.3. show that the release rate could be increased by use of the chlorinated hydrocarbon plasticiser, Cereclor S45, but that the release rate was still much less than that required (cf. Table 4.1.2.). Release was linear over the 70-day period of measurement.

The polythene vial dispensers were tested in the field at Rekomitjie, and results are shown in Table 4.1.4. (QR 86/3). Although catches in traps baited with acetone and octenol were doubled by addition of the polythene vials, these increases were less than those achieved with higher release rates (cf Table 4.1.2.).

Table 4.1.4. Catches of tsetse in F3 traps baited with acetone and octenol and polythene vials containing phenols

Polythene vial contents[2]	Total tsetse catch[1]	
	Detrans mean	Catch Index
Nil	129.1	1.00
500 mg 4-methylphenol + 2.5% 3-propylphenol	283.1	2.19
500 mg phenol mix[3]	269.4	2.09
100 mg phenol mix[3] + 100 mg Cereclor	262.2	2.03

[1] four replicates in Latin square; catches transformed to log(x+1).

[2] polyethylene vials 35mm x 8mm x 1.5mm thick.

[3] phenol + 4-methyl + 3-methyl + 4-ethyl + 3-ethyl + 4-propyl + 3-propyl 1.5 : 100 : 9.9 : 2.1 : 1.1 : 0.8 : 2.5.

4.1.5. Release of phenols from polyethylene tubing

In order to increase the release rates from the sealed, polythene vial type of dispensers, the surface area for release was increased by using a length of polythene tubing.

Release rates for mixtures of 4-methylphenol, 3-propylphenol and octenol were measured from 25 cm lengths of tubing 1mm i.d. x 2 mm o.d. and 3 mm i.d x 5 mm o.d. (QR 86/4) and from different lengths of tubing 7 mm i.d. x 10 mm o.d. (QR 87/1). These measurements showed that a 12.5 cm length of polythene tubing 7 mm i.d. x 10 mm o.d. containing 4 gm of 4:1:2 or 4:1:4 mixtures of 4-methylphenol + 3-propylphenol + octenol gave the required initial release rates of approx. 20 mg/day, 1-2 mg/day and 5-10 mg/day respectively under laboratory conditions (Table 4.1.5.), and these dispensers performed well in the field (Table 4.1.6.) (QR 87/2).

Table 4.1.5. Release rates from polyethylene tubes[1] at 27°C by entrainment.

Day	Release rate (mg/day) 4-methyl	3-propyl	octenol	Total
4:1:2 4-methylphenol + 3-propylphenol + octenol				
14	20.6	2.5	5.4	28.5
19	20.7	2.1	4.6	27.4
29	9.1	1.1	1.8	12.0
58	4.5	0.3	1.4	6.2
4:1:4 4-methylphenol + 3-propylphenol + octenol				
22	17.0	0.9	7.3	25.2
23	15.0	1.0	7.9	23.9

[1] tubing 12.5 cm x 7 mm i.d. x 10 mm o.d.

Table 4.1.6. Catches of tsetse with addition of polythene tube dispensers for phenols and octenol to F3 traps baited with acetone (Rekomitjie, May 1987).

Additional odour[2]	GMM mean[2]	GMM Index	GP mean[1]	GP Index
Nil	14.0 b	1.00	154 c	1.00
4:1:4 mix in tube[2]	15.8 b	1.13	665 a	4.32
4:1:2 mix in tube[2]	19.2 a	1.37	691 a	4.49
200 ml aqueous soln. 0.5 mg/ml 10:1 4-methyl + 3-propyl, + octenol 0.5 mg/hr	13.6 b	0.97	441 b	2.87

[1] 8 replicates; detransformed mean catch/day; means followed by same letter significantly different at 5% level.

[2] all traps with acetone (100 mg/hr); 4-methylphenol+3-propylphenol+octenol in tubing 12.5 cm x 7 mm i.d. x 10 mm o.d.

However, plotting weight loss of these polythene tube dispensers over extended periods confirmed the decline in release rate shown in Table 4.1.5. above (e.g. Fig. 4.1.1.).

The decline in release rate was associated with hardening of the polythene tubing. Neither of these effects was prevented by adding the plasticiser Cereclor to the contents (QR 87/2). Furthermore, release rate determinations on tubes loaded with the individual components showed that the same drop in release rate occurred with the phenols or with octenol (QR 87/3).

4.1.7. Release of phenols from polyethylene "drip-bags"

Attention was turned to other types of sealed dispensing system for the phenols and octenol. Small polyethylene "drip bags" (10 cm x 6 cm; 1 mm thick) were tested, partially filled with 20 ml of a 4:1:4 or 8:1:4 mixture of 4-methylphenol, 3-propylphenol and octenol (QR 87/3). In the laboratory windtunnel at 27°C, release rates reached a maximum after approx 5 days and showed only a slight decline over the 130 days of measurement (Fig. 4.1.2.). However, total release rates for the two mixtures were only 4.6 mg/day and 5.0 mg/day respectively, as measured by weight loss. Relative release rates of the individual components were measured by entrainment (Table 4.1.7.).

Table 4.1.7. Release of phenols and octenol from small polythene "drip bag" dispensers measured by entrainment at 27°C.

Mixture	Release rate (mg/day)				Ratio
4Me : 3Pr : Oct	4Me	3Pr	Oct	Total	4Me : 3Pr : Oct
4 : 1 : 4	3.26	0.38	2.14	5.78	8.6 : 1 : 5.6
8 : 1 : 4	4.78	0.31	1.21	6.30	15.4 : 1 : 3.9

Fig. 4.1.1. Release of a 4 : 0.8 : 2 mixture of 4-methylphenol + 3-propylphenol + octenol from polythene tube (12.5cm x 7mm i.d. x 10mm o.d.)

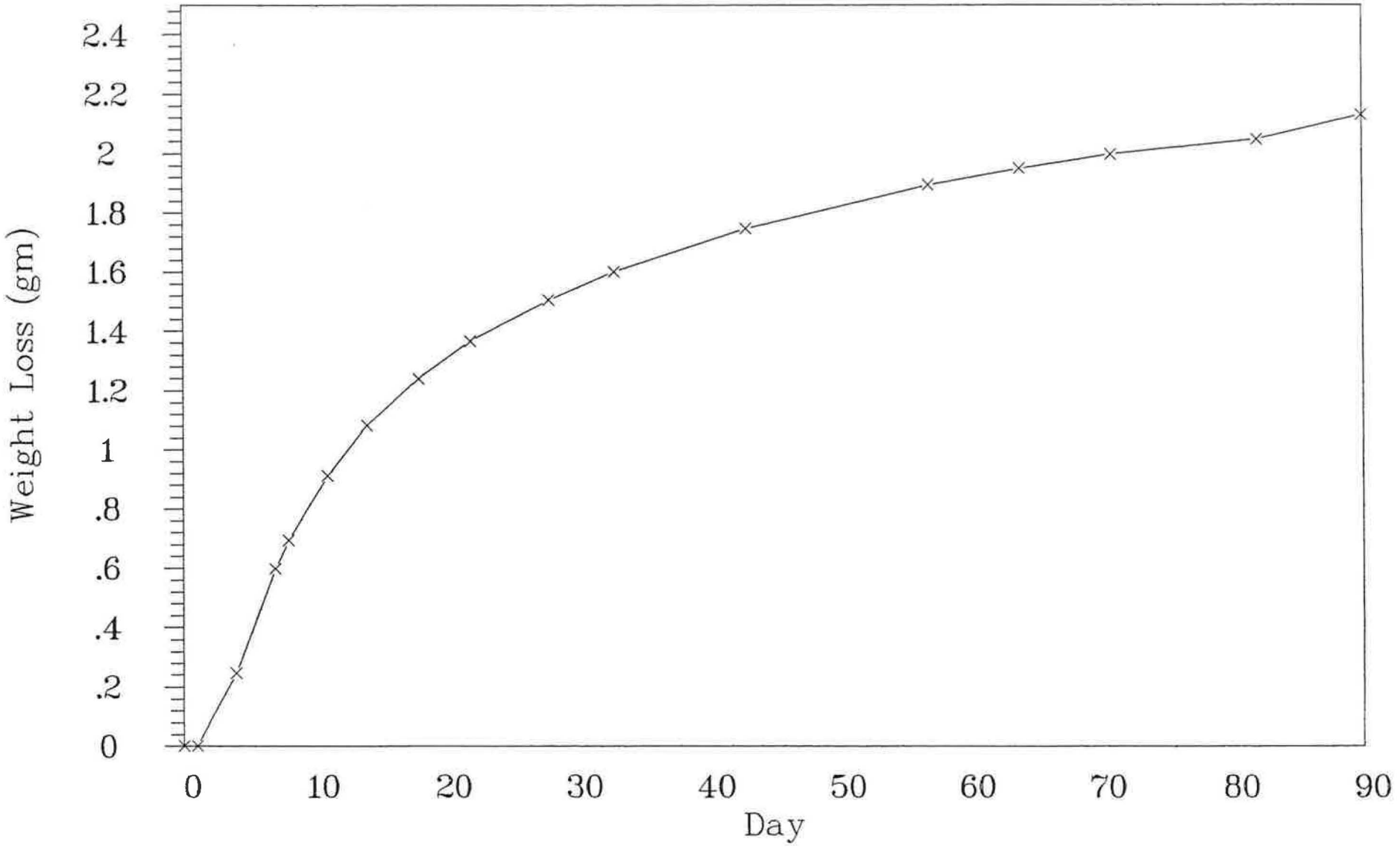
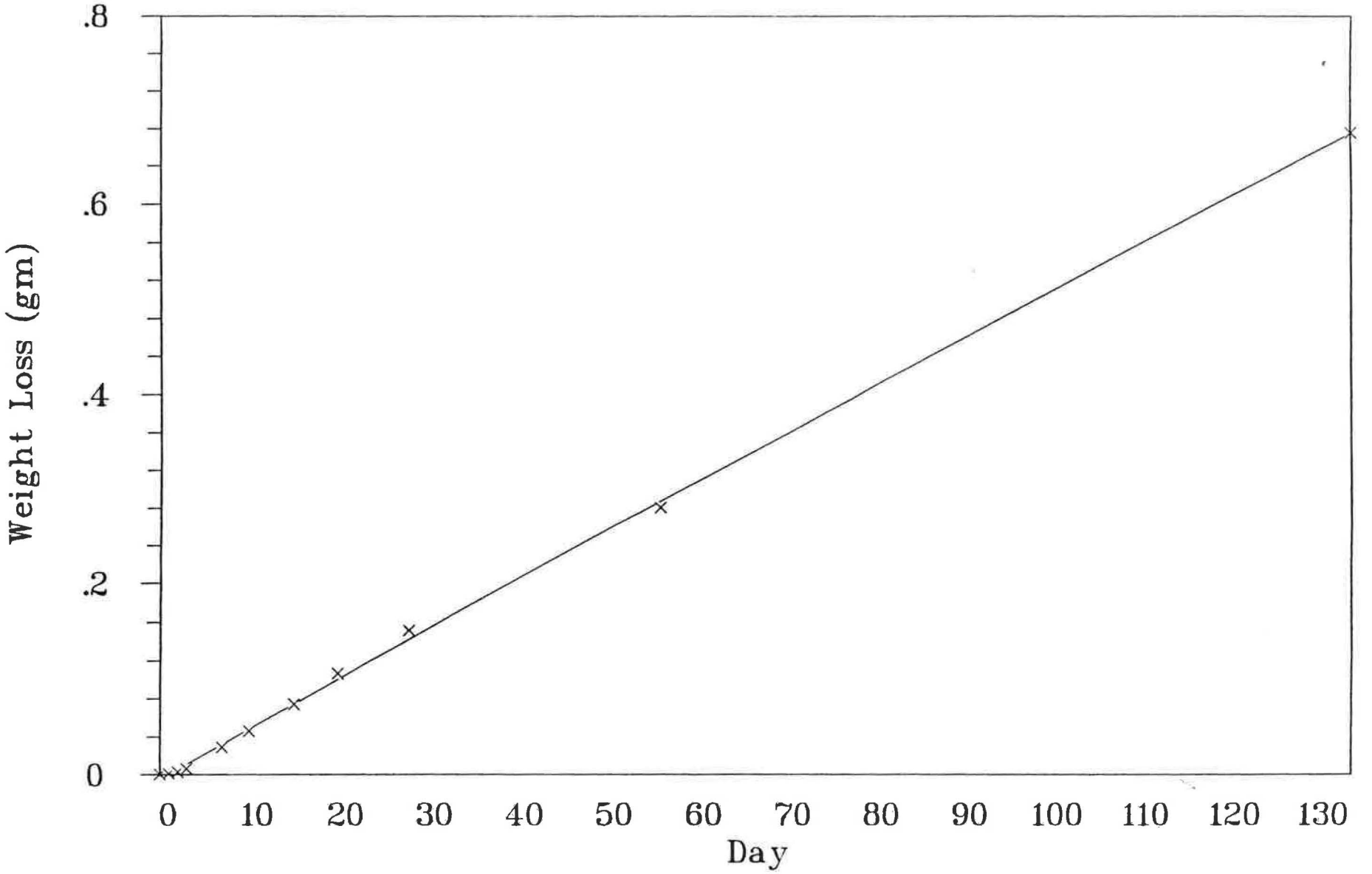


Fig. 4.1.2. Release of a 8 : 1 : 4 mixture of 4–methylphenol + 3–propylphenol + octenol from small polythene drip bags



4.1.8. Release of phenols and octenol as individual components from polyethylene sachets

In order to increase the release rate, devices with thinner walls were constructed from polythene layflat tubing. This could be made into sachets with a heat sealer.

Sachets were made up from layflat tubing 150 μ thick, with total surface area 50 sq. cm. (4.3 cm x 5.8 cm). These were filled with 4 ml of 4-methylphenol, 3-propylphenol or octenol, and realease rates measured by weight loss in the windtunnel at 27°C (QR 87/3). Release was completely linear over the experimental period of 80 days, and rates for the three compounds were 15.4 mg/day, 9.9 mg/day and 26.7 mg/day respectively.

4.1.9. Release of mixtures of phenols and octenol from polyethylene sachets

Release rates of various mixtures of 4-methylphenol, 3-propylphenol and octenol from the sachets were measured under laboratory conditions by both weight loss and entrainment. Weight loss measurements showed release rates declined only slightly over the 218 days of measurement (e.g. Fig. 4.1.3.), at which point approximately 65% of the initial contents had been released. Release rates of each component, as measured by entrainment, are shown in Table 4.1.8.

As expected, release of the 3-propylphenol was slower than that of the other components, and so the proportion of this in the material released increased with ageing of the sachet. Interestingly, the octenol was released at a similar, if anything slower, rate to that for 4-methylphenol from the mixture. This is markedly different from the relative release rates for the individual components (section 4.1.8.) where octenol was released from the sachet much faster than the 4-methylphenol.

In parallel with these laboratory experiments, trapping experiments were carried out in Zimbabwe to determine which three-component mixture was the most effective and the effect of ageing on the attractiveness. Sachets containing 4-methyl-phenol, 3-propylphenol and octenol were added to F3 traps baited with acetone. Sachets were aged in Zimbabwe at higher temperatures than that used in the laboratory experiments, and those aged for 77-85 days had lost over 70% of their contents.

Catch indices are shown in Table 4.1.9. All the blends tested gave highly significant ($P<0.001$) increases in catch of *G. pallidipes*. The 32:1:32 blend with the lowest proportion of 3-propylphenol gave the lowest catches throughout, but there were no obvious, consistent differences between the other blends or effects of ageing. Catches of *G. m. morsitans* were often increased, but it was not possible to show statistical

Fig. 4.1.3. Release of a 8 : 1 : 4 mixture of 4–methylphenol + 3–propylphenol + octenol from polythene sachet (50cm2; 150um thick)

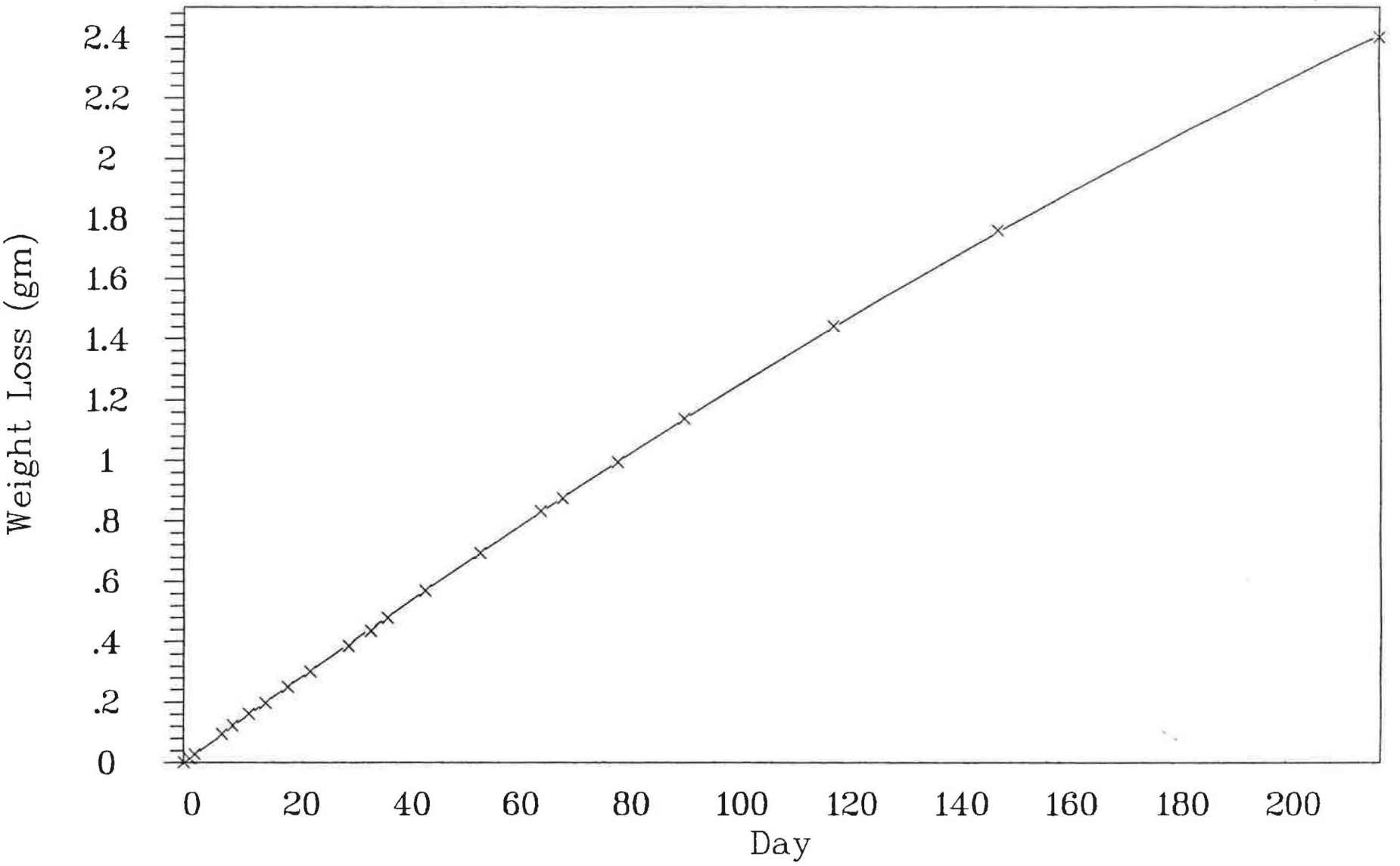


Table 4.1.8. Release rates of phenols and octenol from polythene sachets at 27°C.

	Release rate (mg/day)							
	Ratio 4-methylphenol (4Me) : 3-propylphenol (3Pr) : octenol (Oct)							
	32:1:32	32:1:16	16:1:16	16:1:8	8:1:8	8:1:4	4:1:4	4:1:2
DAY 7-8[1]								
4Me	6.3	9.1	6.7	9.0	6.6	9.1	6.8	8.6
3Pr	0.10	0.11	0.23	0.23	0.38	0.52	0.80	1.00
Oct	5.7	2.9	5.6	3.0	5.4	3.0	5.4	2.6
Total	12.1	12.1	12.5	12.2	12.4	12.6	13.0	12.2
Ratio	63:1:57	83:1:26	29:1:24	39:1:13	17:1:14	18:1:6	9:1:7	9:1:3
DAY 36-38[1]								
4Me	6.4	9.1	6.0	7.6	6.2	8.0	6.2	7.7
3Pr	0.10	0.13	0.20	0.21	0.40	0.50	0.78	0.95
Oct	5.6	3.0	5.3	2.6	5.3	2.5	4.9	2.6
Total	12.1	12.2	11.5	10.3	11.9	11.0	12.0	11.2
Ratio	65:1:58	70:1:23	30:1:26	36:1:12	15:1:13	16:1:5	8:1:6	8:1:6
DAY 85[1]								
4Me	6.2	7.3	5.4	7.0	5.6	7.5	5.7	7.2
3Pr	0.12	0.12	0.22	0.22	0.42	0.53	0.82	1.07
Oct	4.5	2.6	4.8	2.5	4.5	2.6	4.5	2.5
Total	10.8	10.1	10.5	9.8	10.6	10.6	11.1	10.8
Ratio	51:1:37	59:1:21	25:1:22	32:1:11	13:1:11	14:1:5	7:1:6	7:1:2

Table 4.1.8. Release rates of phenols and octenol from polythene sachets at 27°C (cont.)

	Release rate (mg/day)							
	Ratio 4-methylphenol (4Me) : 3-propylphenol (3Pr) : octenol (Oct)							
	32:1:32	32:1:16	16:1:16	16:1:8	8:1:8	8:1:4	4:1:4	4:1:2
DAY 159[1]								
4Me	5.0	6.8	5.2	6.3	4.7	5.9	5.0	6.2
3Pr	0.11	0.11	0.20	0.20	0.34	0.42	0.92	1.09
Oct	4.0	2.4	4.4	2.6	3.8	2.1	4.2	2.5
Total	9.1	9.3	9.8	9.1	8.8	8.5	10.1	9.8
Ratio	45:1:36	62:1:21	22:1:26	31:1:13	14:1:11	14:1:5	5:1:5	6:1:2
DAY 218[1]								
4Me	5.0	6.4	5.3	6.0	5.3	6.5	4.4	5.5
3Pr	0.16	0.23	0.26	0.26	0.48	0.57	1.05	1.20
Oct	4.2	2.8	4.0	2.6	4.4	2.5	3.6	2.2
Total	9.4	9.4	9.6	8.9	10.2	9.6	9.1	8.9
Ratio	31:1:26	28:1:12	20:1:15	23:1:10	11:1:9	11:1:4	4:1:3	4.6:1:1.8
Day 1-80 weight loss	14.0	12.9	14.3	12.1	15.0	12.6	13.9	12.9

[1] measured by entrainment

Table 4.1.9. Catch indices for sachet dispensers aged under field conditions added to F3 trap baited with acetone, Rekomitjie April 1987.

	Catch Index[1]							
	Ratio 4-methylphenol (4Me) : 3-propylphenol (3Pr) : octenol (Oct)							
	32:1:32	32:1:16	16:1:16	16:1:8	8:1:8	8:1:4	4:1:4	4:1:2
DAY 0-8								
GMM	0.93	0.93	1.13	1.18	1.99	1.27	1.56	1.37
GP	2.39	2.81	3.13	3.14	2.77	2.99	3.82	3.56
Total	2.34c	2.75bc	3.06abc	3.07abc	2.73bc	2.94abc	3.74a	3.48ab
Ratio[2]	0.67	0.79	0.88	0.88	0.78	0.84	1.07	1.00
DAY 41-48								
GMM	1.69	2.42	2.49	2.01	1.96	2.50	1.83	1.94
GP	2.55	2.61	3.03	2.31	2.26	2.54	3.10	3.40
Total	2.48a	2.56a	2.95a	2.26a	2.20a	2.49a	3.01a	3.29a
Ratio[2]	0.75	0.78	0.90	0.69	0.67	0.76	0.91	1.00
DAY 77-85								
GMM	0.98	1.21	0.95	1.16	0.88	0.98	1.39	0.83
GP	2.59	3.23	3.55	3.35	3.53	3.59	3.20	3.37
Total	2.51b	3.13a	3.42a	3.24a	3.40a	3.46a	3.11a	3.24a
Ratio[2]	0.77	0.96	1.05	0.99	1.05	1.07	0.96	1.00

[1] catch index relative to trap baited with acetone (500 mg/hr) only; indices followed by same letter in same row not significantly different at 5% level.
[2] ratio of catch index to index for 4:1:2 mixture.

significance because of the low numbers. No decreases in catch of this species were observed, an important point as decreases have been observed with high doses of the phenols.

4.1.10. Release of compounds from polythene sachets in the field.

The effect of temperature on the release rate of compounds from the polythene sachets was not investigated under controlled, laboratory conditions. Field release rates of some compounds were measured during experiments carried out in 1989 (AR 89), and results are shown in Table 4.1.10.

Table 4.1.10. Release rates from polythene sachets at Rekomitjie, October 1989.

Time period	Temp. range	Mean release rate (mg/hr)[1]			
		8:1:4	2MeO	AcPh	3Oct
Day 0730-1900 hr	27-35°C	1.6	6.4	10.5	4.2
Night 1900-0730hr	21-27°C	0.4	1.6	2.7	0.9

[1] sachets 50 sq. cm., 150µ thick; 8:1:4 = 4-methylphenol + 3-propylphenol + octenol; 2MeO = 2-methoxyphenol; AcPh = acetophenone; 3Oct = 3-octanol.

4.1.11. Effects of thickness and surface area on release from polyethylene sachets

Release rates from the polythene sachets are dependent upon the surface area of the sachet and the thickness and density of the polythene. Thus, release from the "standard" sachet prepared from Zimbabwean layflat tubing 150 µ thick with 50 sq. cm surface area was doubled by doubling the surface area (QR 87/3).

A doubling of release rate was obtained using layflat tubing of UK origin: this had a thickness of 100-120 µ and presumably was a different density of polythene giving more rapid release for an equivalent thickness (QR 87/4). Relative release rates of the three components were not affected by these changes (Table 4.1.10).

Table 4.1.11. Release rates from polythene sachets of different sizes and materials at 27°C, measured by entrainment.

Origin	Area (sq.cm)	Thick (μ)	Release rate (mg/day)[1] 4Me	3Pr	Oct	Total	Ratio 4Me:3Pr:Oct
Zimbabwe	50	150	8.00	0.47	2.39	10.86	17 : 1 : 5
Zimbabwe	100	150	16.19	0.95	5.50	22.64	17 : 1 : 6
UK	50	110	16.00	1.05	5.20	22.25	15 : 1 : 5

[1] all sachets contained 4 ml of a 8:1:4 mixture of 4-methylphenol (4Me), 3-propylphenol (3Pr) and octenol (Oct).

In field tests, the faster release of the UK sachets gave higher catches of tsetse as shown in Table 4.1.11. Total release rates were measured by weight loss of the sachets shielded in blue bags, as on targets, in Harare. Mean release rates were 43.0 mg/day for the Zimbabwe sachets and 80.2 mg/day for the UK sachets (QR 87/4).

Table 4.1.12. Catches of tsetse in F3 traps baited with acetone and phenols and octenol in different polythene sachets

Additional odour[1]	GMM mean[2]	GMM Index	GP mean[2]	GP Index
Nil	0.60	1.00	15.38	1.00
Zimbabwe sachet (150μ)	0.99	1.64	41.45	2.55***
UK sachet (110μ)	1.47	2.44*	53.52	3.48***

[1] all traps baited with acetone (500 mg/hr); sachets 50 sq. cm containing 4-methylphenol + 3-propylphenol + octenol 8:1:4

[2] 12 replicates; detransformed mean; *, *** indicate indices differ from 1.00 at 5% and 0.1% levels

4.1.12. Conclusions and Recommendations

Sealed polythene devices provide convenient dispensers for octenol and the phenolic tsetse attractants.

Polythene tubes are initially highly effective, but the release rate declines on ageing. However, these tubes have been adopted as standard in Burkina Faso (Filledier and Mérot, 1989b).

Sachets made from polythene layflat tubing are cheap and easy to make. They provide linear release of the contents, the release rate being dependent upon the surface area of the sachet and the thickness and density of the polythene. Sachets constructed of 150 µ polythene of 50 sq. cm. surface area and containing 4 ml of a 8:1:4 mixture of 4-methylphenol, 3-propylphenol and octenol have been adopted as a standard dispenser in the Region. These increase catches of *G. pallidipes* in F3 traps baited with acetone by 3-4 times and last for at least 3 months under field conditions in Zimbabwe.

4.2. SEALED DISPENSERS FOR ACETONE AND BUTANONE (MEK)

4.2.1. Introduction

The bait used for traps and targets in Zimbabwe includes an acetone dispenser consisting of a glass bottle with a 5 mm hole drilled in the lid to give a release rate of 100 mg/hr under field conditions. Although cheap and readily available, these dispensers are very prone to breakage and spillage. There is thus a need for dispensers which are less susceptible to breakage and spillage and which can be made up centrally and easily transported to trap and target sites.

Various sealed dispensing systems were examined during the course of this project. These were made from commercially-available bottles or tubing in polythene or polypropylene. Acetone does not diffuse well through polythene, presumably because of the high polarity of acetone. Butanone (MEK) was shown to be an effective replacement for acetone as a tsetse attractant at lower release rates of 7 - 70 mg/hr (Vale and Hall, 1985a). Moreover, this compound diffuses more readily through polythene, probably because it is less polar, and its release rate from several of the dispensers was measured.

4.2.2. Measurement of release rates in the laboratory

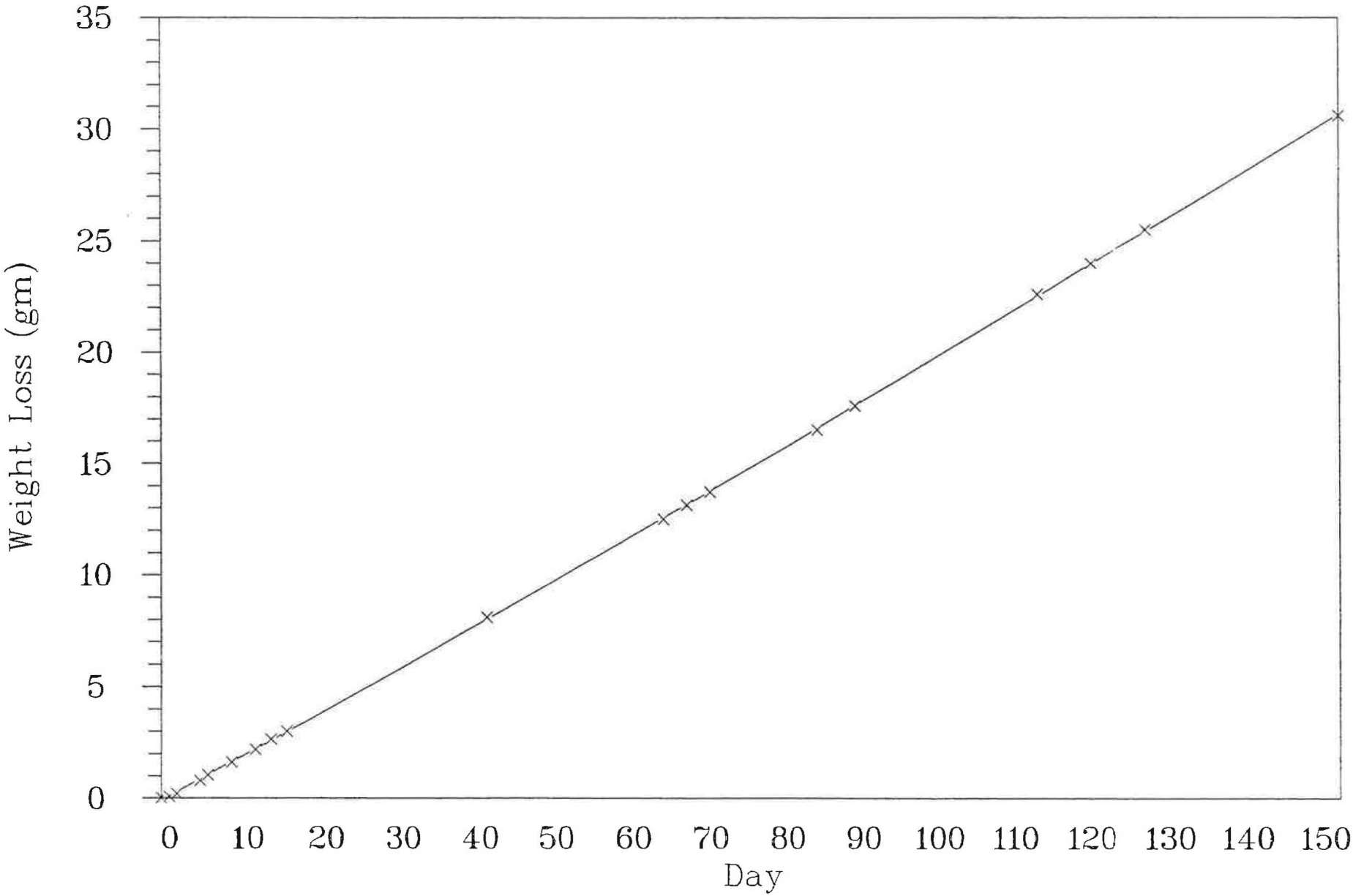
Release rates of acetone and/or butanone from a variety of dispensers were measured by weight loss in the laboratory windtunnel at 27^{o}C and 8 kph windspeed. Various dispensers were tested during the Project, and the results are summarised in Table 4.2.1.

The thin, layflat polythene sachet released acetone at only 13 mg/hr. Although this could be increased by increasing the surface area of the dispenser, it was found that such sachets filled with acetone burst under field conditions. The more robust polythene bottle released acetone at only 3 mg/hr, while many of the other dispensers tested released butanone at rates greater than 7 mg/hr (168 mg/day) in a linear manner (e.g. Fig. 4.2.1. for TT Containers 500 ml).

Table 4.2.1. Release rates of acetone and butanone (MEK) from sealed dispensers at 27°C.

Dispenser	Material	Dimensions (mm)	Contents	Release rate (mg/day)	Days measured	Ref.
TT Containers	polythene	100 x 55 (250 ml)	100 ml MEK	230	13	QR 86/4
TT Containers	polythene	100 x 55 (250 ml)	100 ml MEK + 10% Cereclor	158	153	QR 86/4
TT Containers	polythene	130 x 70 (500 ml)	250 ml MEK	199	153	QR 87/1
Scientific Supplies	polythene	100 x 50 (200 ml)	100 ml MEK	90	13	QR 86/4
Scientific Supplies	polythene	100 x 50 (200 ml)	100 ml acetone	75	13	QR 86/4
Betix drip bag	polythene	180 x 100	200 ml MEK	444	90	QR 87/1
Layflat tubing	polythene	11.6 x 4.3 x 150μ	25 ml acetone	310	70	QR 87/3
Steripak	polypropylene	500 ml	250 ml MEK	291	91	QR 87/4
Steripak	polypropylene	1000 ml	500 ml MEK	506	91	QR 87/4

Fig. 4.2.1. Release of butanone from polythene bottle (TT Containers; 500ml)



4.3.1. Field testing of butanone dispensers

Several of these dispensers were field tested in Zimbabwe by adding them to Epsilon traps baited with octenol (QR 88/3).

Table 4.2..2. Catches of tsetse in Epsilon traps baited with octenol and different dispensers for acetone or butanone (MEK), Rekomitjie, September 1988.

Dispenser	Chemical	Release[1] (mg/hr)	GMM		GP	
			mean[2]	index	mean[2]	index
Experiment 1						
standard	acetone	500	5.3	1.00	85.6	1.00
Bettix drip	MEK	25	4.8	0.89	84.9	0.99
Steripak 500	MEK	27	4.9	0.93	73.7	0.86
TT 500	MEK	27	4.8	0.91	72.2	0.84
Experiment 2						
standard	acetone	500	3.8	1.00	62.7	1.00
Steripak 1000 (thin wall)	MEK	52	3.8	0.99	61.5	0.98
Steripak 1000 (thick wall)	MEK	44	3.6	0.96	58.0	0.92
TT 250	MEK	17	3.8	1.00	46.4	0.74

[1] calculated from daily release rates measured in field, max 37°C, min 22°C; all traps baited with octenol at 0.5 mg/hr.

[2] 8 replicates each experiment; detransformed mean catch/day

As shown in Table 4.2.2., nearly all the dispensers tested performed as well as the standard bottle acetone dispenser releasing at 500 mg/hr, higher than that used in field operations. Only the small, 250 ml TT Containers bottle gave catches of *G. pallidipes* significantly lower ($P<0.05$) than that with the acetone standard.

4.2.4. Conclusions and Recommendations

Although the Bettix drip packs cost over £1.00 each, even when ordered in bulk, the TT Containers 500 ml polythene bottles cost £0.30 with screwtop and the Steripak 500 ml polypropylene bottles cost £0.50 with lid. The latter two devices can thus be recommended as reasonably cheap, re-usable dispensers for butanone which are convenient to transport and handle and which should last for many months in the field.

4.3. CONCLUSIONS AND RECOMMENDATIONS

Satisfactory sealed dispensers have been developed for both the phenol/octenol components and the acetone/butanone components of baits for use with traps and targets in the field. These are highly effective, last for at least three months in the field and are practical to manufacture and use.

One aspect of both these dispensers that has not been investigated in detail is the effect of temperature on release rates. The available comparisons of laboratory and field release rates confirm that release rates increase with increasing temperature, and the results indicate that this increase is rather marked (e.g. Section 4.1.10; 4.2.3.). It is recommended that laboratory studies of release rates from the dispensers at different temperatures be carried out so that field behaviour of the dispensers, in particular the longevity, can be predicted more accurately.

5. COLLABORATION WITH THE TSETSE RESEARCH LABORATORY

5.1. GENERAL

The post-doctoral fellow working on this Project, Dr. Andrew Gough, was employed through the TRL and Bristol University. Close collaboration was maintained with the TRL throughout the Project by telephone and visits between NRI and TRL.

We are extremely grateful to Dr. Tony Jordan, Director of TRL, for his meticulous handling of the administration of this part of the Project and for his advice, support and enthusiasm in conducting the scientific work.

5.2. PROVISION OF TSETSE FLIES

All tsetse flies used in this Project - *G. m. morsitans*, *G. pallidipes* and *G. palpalis* - were supplied from colonies at the TRL, maintained with funding from ODA.

5.3. COLLABORATION WITH Prof. BURSELL

As part of the tripartite arrangement between Zimbabwe, NRI and TRL, close collaboration was maintained with Prof. Einar Bursell for laboratory bioassay of the behavioural activity of new compounds supplied by NRI.

This covered bioassay phenols from urine (Section 3.2.), the carotenoid metabolites Peak 6 and Peak 7 from urine (Section 3.4.), compounds from pig sacks (Section 3.5.) and extensive investigations of "Omega" (Section 3.6.).

Following the retirement of Prof. Bursell in June 1989, this work was carried on by Dr. Chris Green at TRL.

5.4. COLLABORATION WITH Dr. WARNES

Assistance was given to Dr. Martin Warnes in his work on behaviour modifying chemicals for tsetse in ox sebum (Section 3.8.). This involved preparation of large quantities of sebum extract for laboratory and field work and preliminary fractionation of these extracts.

5.5. MAINTENANCE OF A SOURCE OF OX ODOUR

During the second two years of this Project, funding was provided to TRL for the setting up and maintenance of two calves in a calf box for exclusive use of tsetse workers at TRL and NRI.

This proved invaluable in securing a supply of ox odour for bioassay work by Prof. Bursell and Dr. Warnes and EAG work at NRI, particularly that on "Omega" (Section 3.6.).

6. LIAISON WITH TSETSE CONTROL OPERATIONS IN WEST AFRICA

6.1. INTRODUCTION

As a result of the successful work on tsetse attractants in Zimbabwe, there has been great interest in developing attractants for other tsetse species throughout Africa, and particularly in West Africa. Before this project started, contact had already been made with tsetse workers in West Africa, and the WHO funded laboratory work at NRI on *G. palpalis* during September 1984 - January 1986. The WHO also funded a visit by Dr. Vale and Dr. Hall to the Congo in November 1984.

Throughout this project, close liaison was maintained with the following tsetse workers in West Africa funded by European organisations.

(a) Dr. Philippe Mérot and Dr. Heinz Politzar,
Centre des Récherches sur les Trypanosomoses Animales (CRTA), Bobo Dioulasso, Burkina Faso.
(funded jointly by IEMVT and GTZ).

(b) Dr. Werner Küpper,
Lutte contre les Trypanosomiase et les Tsetse, BP 45, Korhogo, Côte d'Ivoire.
(funded by GTZ)

(c) Dr. Claude Laveissière,
Organisation Commune de Coordination de Lutte contre les Grandes Endemies (OCCGE), Institut Pierre Richet, BP 1500, Bouake, Côte d'Ivoire.
(funded by ORSTOM)

Lures were also sent to Dr. Thomas Jaennson at the University of Uppsala, Sweden, for testing in Guinea-Bissau.

6.2. CRTA, BOBO DIOULASSO, BURKINA FASO

6.2.1. Introduction

In previous work carried out by the CRTA, insecticide impregnated traps and screens placed along 650 km of riverine areas at one device every 100 m reduced populations of *G. tachinoides* and *G. palpalis gambiensis* by 94% and 88% respectively. Subsequent widespread releases of sterile male flies led to eradication of these species over an area of 3,500 sq. km. Traps and screens were used to provide a barrier to

reinvasion (Cuisance *et al.*, 1984; Politzar and Cuisance, 1984; Cuisance *et al.*, 1990).

These unbaited traps and screens were ineffective against the savannah species *G. morsitans submorsitans.* However, it was shown that baiting these devices with acetone and octenol increased catches of this species by up to 6.7 times, and odour-baited traps and targets were used to provide a barrier to reinvasion at the acceptable density of 4 per sq. km. (Politzar and Mérot, 1984).

Although traps and targets were being used against the three species of tsetse in Burkina Faso, it was recognised that their cost-effectiveness might be improved by the incorporation of attractive odours. Materials and information were provided from NRI and Zimbabwe on an informal basis, and in 1985 the EC DG XII funded a project based at the CRTA to determine whether tsetse flies of the *palpalis* group respond to odours, and, if so, to attempt to identify the attractive components.

Dr. Jean Baptiste-Galey was recruited to assist with this work, and he was briefed at NRI and in Zimbabwe prior to taking up his position at the CRTA.

6.2.2. Visit by Dr. Hall, 1986.

The EC DG XII funded a visit by Dr. Hall to the CRTA in June 1986. The main objective of this was to ensure that techniques and methods used for research on tsetse attractants were similar to those used in Zimbabwe so that results from the two countries could be compared (QR 86/2; Hall, 1986).

A work plan was agreed and this formed the basis for future research as follows:

(a) demonstration that host odours other than carbon dioxide are attractive to *G. tachinoides*;

(b) testing of animal urines for attractiveness before and after chemical fractionation;

(c) testing of known components of host odour and urines for attractiveness;

(d) testing of suitable dispensing systems.

6.2.3. Attractiveness of host odours.

Using the techniques developed in Zimbabwe and at NRI, it was shown that *G. tachinoides* is attracted to electrified nets by the odours of cattle, pigs and men exhausted from an underground pit. There was a reasonable dose-response relationship between

the numbers of flies caught and the number of animals used, with four cows increasing the catch by 79%. The attractiveness of the odour was greater than that of an equivalent quantity of synthetic carbon dioxide, and passing the odour through a charcoal filter reduced its attractiveness to a level similar to that of the carbon dioxide. These results indicated that cattle odour contained attractants for *G. tachinoides* other than carbon dioxide, and that these were trapped on a charcoal filter. Similar trends were observed with *G. m. submorsitans*, but the numbers of flies caught were much smaller and the differences in catch not significant (Mérot *et al.*, 1986).

6.2.4. Attractiveness of carbon dioxide

In the above experiments, carbon dioxide at 3 litre/min increased catches of *G. tachinoides* at electrified nets by only 1.16 times. In biconical traps there was a positive dose-response relationship between catch of *G. tachinoides* and carbon dioxide release rate, with catches being increased by 3.2 times at 20 litres/min (Galey *et al.*, 1986).

6.2.5. Attractiveness of urines

Details of the methods for fractionation and testing of urines were supplied. When used to bait biconical traps, urine from Baoulé cattle was found to be more attractive than urine from Zebu cattle, and pig urine was unattractive: catch indices were 3.2, 2.4 and 0.9 respectively (QR 86/3; Filledier *et al.*, 1988).

After fractionation of the urine, the phenolic fraction was the only fraction with significant activity, but in these latter tests the urine gave only small increases in catch up to 1.32 times (Filledier and Mérot, 1989a). This small increase was attributed to ageing of the urine as the same samples were used throughout (Filledier and Mérot, 1989b). With *G. pallidipes* in East Africa, urine was reported to become more attractive with ageing (Owaga, 1985; Vale *et al.*, 1986).

6.2.6. Attractiveness of phenols.

Individual phenols and mixtures of the phenols in the relative proportions found in cattle urine were provided to the CRTA.

Catches of *G. tachinoides* in biconical traps were signficantly increased by a mixture of all the phenols found in cattle urine (TF 86/05), the same mixture without 2-methoxyphenol (TF 86/06) and a mixture of 4-methylphenol with 2.5% 3-propylphenol (TF 86/68). Catches of *G. m. submorsitans* were increased by all except TF 86/05 which contained 2-methoxyphenol (Table 6.2.1.).

Table 6.2.1. Catches of tsetse in biconical traps baited with phenolic mixtures.

Bait[2]	*G. tachinoides*		*G. m. submorsitans*	
	total[1]	index	total[1]	index
TF 86/05[3]	1831	1.60**	263	0.88
TF 86/06[4]	1895	1.66**	411	1.37
TF 86/68[5]	1816	1.59**	379	1.27
Nil	1143	1.00	299	1.00

[1] totals for 16 replicates in 4 latin squares; ** indicates indices differ from unity at 1% level.

[2] all phenol mixtures dispensed from 1 mg/ml in water.

[3] phenol + 3-methyl + 4-methyl + 3-ethyl + 4-ethyl + 3-propyl + 4-propyl + 2-methoxy 1.4:9.9:100:1.1:2.1:2.5:0.9:0.4.

[4] as TF 86/05 without 2-methoxy

[5] 4-methylphenol + 2.5% 3-propylphenol

Some individual phenols in polythene vial dispensers were provided and tested with biconical traps. Results in Table 6.2.2. show no activity for 3-ethyl- or 3-propylphenol. 2-Methoxyphenol had no effect on catches of *G. tachinoides*, but significantly reduced catches of *G. m. submorsitans* as for the Zimbabwe species *G. m. morsitans* and *G. pallidipes* (QR 86/4).

Mérot continued evaluation of the phenols individually and in mixtures, and reported that 3-methylphenol was the most important (QR 88/1; Filledier and Mérot, in press).

Table 6.2.2. Catches of tsetse in biconical traps baited with phenolic compounds in polythene vial dispensers

Bait[1]	*G. tachinoides*		*G. m. submorsitans*	
	total[2]	index	total[2]	index
3-ethylphenol	1246	1.25	117	0.85
3-propylphenol	1087	1.09	130	0.95
2-methoxyphenol	1089	1.09	73	0.53
Nil	1000	1.00	137	1.00

[1] 100 mg + 0.5 ml Cereclor in vial 35 x 8 x 1.5 mm

[2] 12 replicates in 3 latin squares.

6.2.7. Effect of phenolic attractants on cattle odour

Passing ox odour through a charcoal filter had been shown to reduce its attractiveness to *G. tachinoides* and *G. m. submorsitans*. Following the recommendations made during Dr. Hall's visit, the effects of adding known attractants for the Zimbabwe species of tsetse to the cattle odour were investigated (Mérot *et al.* 1988).

As shown in Table 6.2.3., addition of the mixture of phenols excluding 2-methoxyphenol found in cattle urine (TF 86/06) to ox odour passed through a charcoal filter restored catches of both *G. tachinoides* and *G. m. submorsitans* to the levels caught with untreated ox odour. A mixture of synthetic carbon dioxide and the phenol mixture TF 86/06 was similarly almost as attractive as the untreated ox odour. These results suggest that the phenols constitute the main, if not only attractive components trapped on the charcoal (QR 86/4).

Table 6.2.3. Catches of tsetse at electrified nets baited with natural and synthetic odours

Bait	*G. tachinoides*		*G. m. submorsitans*	
	total[1]	index	total[1]	index
3 oxen	3007	1.64**	179	1.61
3 oxen + charcoal filter + TF 86/06[2]	2603	1.42**	202	1.82
CO_2 (1.5 l/min) + TF86/06[2]	2771	1.51**	164	1.48
Nil	1839	1.00	111	1.00

[1] total catch over 12 replicates in 3 latin squares;
** indicates indices differ from unity at 1% level

[2] composition as in Table 4.2.1.; 1 mg/ml in water.

6.2.8. Attractiveness of acetone and octenol

Following on from the experiments in Table 4.2.3., the effects of adding acetone and octenol to the ox odour at rates higher than would be produced naturally were examined. The results in Table 6.2.4. show that octenol further increases the attractiveness of ox odour passed through charcoal and "reconstituted" by addition of the urine phenols. Acetone at these levels did not increase the attractiveness and, if anything, even seemed to reduce the effect of the octenol (QR 86/4, 87/1).

Table 6.2.4. Catches of tsetse at electrified nets baited with ox odour supplemented with phenols, acetone and octenol.

Bait[1]	*G. tachinoides*		*G. m. submorsitans*	
	total[2]	index	total[2]	index
Experiment 1				
3 oxen	1306	1.36**	181	1.79
3 oxen + charcoal filter + TF86/06 + acetone + octenol	2092	2.19**	199	1.97
CO_2 + TF86/06 + acetone + octenol	1901	1.99**	186	1.84
Nil	957	1.00	101	1.00
Experiment 2				
3 oxen	839	1.90	138	1.45
3 oxen + charcoal filter + TF 86/06 + octenol	1239	2.81	202	2.13
CO_2 + TF86/06 + octenol	1155	2.62	190	2.00
Nil	441	1.00	95	1.00
Experiment 3				
3 oxen	1171	1.78	115	1.47
3 oxen + charcoal filter + TF86/06 + acetone	1006	1.53	93	1.19
CO_2 + TF86/06 + acetone	945	1.43	122	1.56
Nil	659	1.00	78	1.00

[1] TF 86/06 as in Table 4.2.1., 1 mg/ml in water; octenol 0.5 mg/hr; acetone 500 mg/hr.

[2] each experiment 8 replicates in 2 latin squares.

With biconical traps, catches of *G. tachinoides* were increased by the phenol mixture, and further increased by the addition of octenol to this, although octenol by itself was unattractive. Addition of acetone to octenol or to the phenols + octenol caused a marked reduction in catches. Numbers of *G. m. submorsitans* caught were very small (Table 6.2.4.; QR 87/1).

Table 6.2.5. Catches of tsetse in biconical traps baited with phenols, acetone and octenol.

Bait[1]	*G. tachinoides*	
	total[2]	index
TF 86/06	899	1.79
octenol	516	1.03
octenol + acetone	152	0.30
TF 86/06 + octenol	1016	2.02
TF 86/06 + octenol + acetone	514	1.02

[1] TF 86/06 as in Table 4.2.1. at 1 mg/ml in water; octenol at 0.5 mg/hr; acetone at 500 mg/hr

[2] 12 replicates in 2 latin squares.

Mérot reported that 3-methylphenol was the most attractive phenol for *G. tachinoides*, and that this phenol by itself potentiated the attractiveness of octenol (QR 88/1).

6.2.9. Dispensers for attractants.

Polythene vials were tested as dispensers, as in Table 6.2.2.

Polythene tubing was sent to the CRTA for manufacture and testing of dispensers for the phenols and octenol. Following discovery that a 3:1 blend of 3-methylphenol and octenol was the most attractive for *G. tachinoides*, this mixture was tested in the polythene tubing with biconical traps. Two traps were used, one baited and one unbaited. The positions were alternated every seven days and trap catches compared over 16 weeks to investigate the effect of ageing of the dispenser. Marked flies

were also released at intervals and the numbers caught in the baited and unbaited traps compared.

Table 6.2.6. Comparison of catches of G. tachinoides in biconical traps baited with a polythene tube dispenser containing 3:1 3-methylphenol and octenol and in unbaited traps.

	Wild flies			Marked flies		
Weeks	Baited	unbaited	ratio	Baited	unbaited	ratio
0-2	1575	621	2.54	41	8	5.12
3-4	2075	821	2.53	81	31	2.61
5-6	1940	906	2.14	109	67	1.63
7-8	1509	605	2.49	90	44	2.05
9-10	1858	710	2.62	102	62	1.65
11-12	891	590	1.51	81	39	2.08
13-14	614	381	1.61	67	39	1.72
15-16	610	465	1.31	15	18	0.83

The tube dispensers remained attractive for approximately 10 weeks. After this the attractiveness declined, presumably due to the fall in release rate observed in laboratory experiments (Section 4.1.5.). These tubes were proposed as a cost-effective dispenser for the attractants as the traps and screens are reimpregnated with insecticide every 10 weeks (Filledier and Mérot, 1989b).

6.3. GTZ, KORHOGO, COTE D'IVOIRE

6.3.1. Introduction

GTZ carried out a successful control programme against *G. palpalis* and *G. tachinoides* along the Bandama River in northern Côte d'Ivoire. This used impregnated biconical traps at 600 m intervals along the river bank at the edge of the gallery forest, and fly populations were reduced by over 95% (Küpper *et al.*, 1984).

G. longipalpis and *G. medicorum* are also important species in Côte d'Ivoire, and investigations were carried out to determine whether odours could be used to increase the effectiveness of traps for any of these species.

6.3.2. Briefing of Jochan Spath

Jochan Späth, who was assigned to assist Dr. Kupper was briefed at NRI before taking up his post (QR 87/1) and after the 24th Tsetse and Trypanosomiasis Seminar at Bristol in September 1987.

Throughout the project, he was supplied with chemicals and polythene vials, tubes and sachets for dispensers.

6.3.3. Visit by Dr. Gough

Dr. Gough visited Korhogo in June/July 1987 with travel and subsistence provided by GTZ.

The main objective of this visit was to demonstrate the techniques used for testing natural and synthetic odours used in Zimbabwe, and to ensure experimental designs and methods of data analysis used were comparable with those used in Zimbabwe.

The methods developed at NRI for extraction and chemical fractionation of animal urines were demonstrated.

A method for quantitative analysis of phenols was developed utilising the gas chromatographs with electron capture detectors available at Korhogo. This method involved conversion of the phenols to their chloroacetate esters prior to analysis.

6.3.4. Results

The results of the subsequent work carried out were described by Jochan Späth at the 20th ISCTRC meeting held in Mombassa, Kenya, 10-14 April 1989, and will form the basis of his Ph.D. thesis. Dr. Chris Green of the TRL carried out field work at Abokouamikro, near Yamoussoukro, during a consultancy visit in July/August 1988. Materials for his work on odours were supplied by NRI, and his results were incorporated in this summary.

Cattle urine increased catches of *G. longipalpis, G. tachinoides* and *G. medicorum* in biconical traps, and the phenolic fraction accounted for most of this attractiveness.

Of the natural urines tested, bushbuck urine was most attractive to *G. longipalpis*. This was shown at NRI to contain 3-methyl-phenol as the major component (Table 3.2.2.).

As recommended by NRI, the attractiveness of the blend of eight synthetic phenols found in cattle urine was compared with those of the corresponding mixtures with each phenol left out in turn. These experiments showed that omission of 3-methylphenol or 4-methylphenol caused the greatest loss in attractiveness.

Tests using the individual phenols as bait confirmed that 3-methylphenol and 4-methylphenol were the most attractive for all the above three species.

The effects were tested of adding acetone dispensed from a bottle at 500 mg/hr and/or octenol at 0.5 mg/hr to the urine phenols. The results suggested that acetone and to a lesser extent octenol increased catches of *G. longipalpis*; octenol increased catches of *G. tachinoides*; and acetone decreased catches of *G. medicorum* while octenol had little effect.

Results are summarised in Table 6.3.1. (Späth and Küpper, 1989).

6.3.5. Conclusions

As a result of this work, sachet dispensers were recommended containing 3-methylphenol only for *G. medicorum* and a 2:1:1 mixture of octenol, 3-methylphenol and 4-methylphenol for *G. tachinoides* and *G. longipalpis*.

Table 6.3.1. Catch indices for tsetse in Côte d'Ivoire

Bait	Catch Index[1]		
	longipalpis	*tachinoides*	*medicorum*
cattle urine	1.3	1.7*	-
phenolic fraction	1.3	1.5	-
phenolic fraction x 10	1.5	1.9**	2.9*
bushbuck urine	2.5**		
domestic pig urine	1.9*		
warthog urine	1.5		
hartebeest urine	1.4		
cattle urine	1.1		
8-phenol mix	1.6*		
" - 4-propyl	1.4		
" - 3-propyl	1.3		
" - 4-ethyl	1.3		
" - 3-ethyl	1.4		
" - 4-methyl	0.8		
" - 3-methyl	0.6*		
" - 2-methoxy	1.4		
8-phenol mix	1.6	1.1	2.9
" + acetone	3.4**	1.3	0.6
" + octenol	2.1	1.5**	2.3
" + acetone + octenol	2.4*	1.5**	0.5

[1] *, ** denote treatment mean differs from control mean at 5% and 1% levels respectively.

6.4. INSTITUT PIERRE RICHET, BOUAKE, COTE D'IVOIRE

6.4.1. Attractants for G. palpalis

All the urine phenols, 8- and 7-component mixtures, indole, 3-methylindole, 2-phenylehtanol, dimethylsulphone, benzyl alcohol, benzoic acid, phenylacetic acid, 3-phenylpropionic acid and octenol were sent to Dr. Claude Laveissiere in Côte d'Ivoire. These were tested with biconical traps but showed no attraction for *G. palpalis* flies.

Using materials supplied by NRI, Cheke and Garms (1988) in Liberia reported catches of *G. p. palpalis* were doubled by acetone and octenol, but a mixture of all eight urine phenols had no effect.

6.5. UPPSALA, SWEDEN / GUINEA-BISSAU

6.5.1. Introduction

At the request of Dr. Thomas Jaenson of the Department of Zoology, Uppsala University, Sweden, 200 standard polythene sachet dispensers (120 µ thick) containing 4 ml of the 8:1:4 mixture of 4-methylphenol, 3-propylphenol and octenol were supplied by NRI for testing in Guinea-Bissau (QR 88/1).

6.5.2. Results

The sachets were tested with biconical traps alone, in combination with acetone at 500 mg/hr and in combination with acetone and N'Dama cow urine (0-3 days old) dispensed from a glass bottle with 17 mm opening.

Catches of *G. longipalpis* males and females were doubled by the sachets combined with acetone, and further addition of urine had no effect. Catches with acetone, urine or a combination of acetone and urine were not significantly different from those in unbaited traps (Jaenson *et al.*, 1991).

6.5.3. Conclusions

These results are consistent with those reported above from Côte d'Ivoire by Späth and Küpper (1989) who showed that acetone increased catches of *G. longipalpis* by a mixture of all the urine phenols. In Côte d'Ivoire, addition of octenol decreased catches, but in Guinea-Bissau the phenols and acetone were not tested in the absence of octenol. The lack of attractiveness of

the urine in Guinea-Bissau may have been due to the low release rate expected from the dispenser.

6.6. CONCLUSIONS AND RECOMMENDATIONS

As a result of liaison between this Project and tsetse workers in West Africa, significant progress has been in research on attractants for West African species of tsetse.

Although there is no evidence for olfactory attraction of *G. palpalis*, catches of *G. tachinoides* in traps or at electric nets can be at least doubled by addition of 3-methylphenol and octenol, and these compounds along with carbon dioxide probably account for most of the attractiveness of host odours to this species. Polythene tubes have been recommended as practical dispensers for 3-methylphenol and octenol in the field in Burkina Faso.

A combination of 3-methylphenol, 4-methylphenol and octenol has been recommended as an attractant for *G. longipalpis*, and 3-methylphenol has been reported to increase catches of *G. medicorum*.

Although the increases in catches of these species of tsetse flies caused by olfactory attractants are not as spectacular as those with savannah species, they are useful and the chemicals involved are cheap and readily available. The results so far have been somewhat variable, at least in part because populations of these species are generally low and because there does seem to be very marked variability in catches at different sites and times. Nevertheless, it is recommended that collaboration with these organisations in West Africa is continued with exchange of results and materials as previously.

7. FIELD AND LIAISON VISITS BY PROJECT PERSONNEL

7.1. VISITS FUNDED BY THE PROJECT

7.1.1. Field and liaison visits

Twelve field and/or liaison visits were funded by the Project (Table 7.1.).

These included eight field/liaison visits by Dr. Hall and/or Dr. Gough to Africa, results of which are inlcuded in the technical sections of this report.

They also included three liaison visits by Dr. Vale to the UK for discussions at NRI, TRL, Imperial College, Oxford University, Salford University and Wellcome (QR 86/4, 87/3, 88/4).

7.1.2. Liaison visit to other European laboratories

During the final year of the Project, the Project funded Dr. Hall and Dr. Alan Cork to accompany Dr. Vale on visits to four European laboratories to discuss possible collaboration in work on tsetse attractants (AR 89). The laboratories were:

Dr. W. Takken, Wageningen Agricultural University, The Netherlands;

Dr. C.J. Persoons, TNO Division of Technology for Society, Delft, The Netherlands;

Dr. C.J. den Otter, State University of Groningen, The Netherlands;

Prof. J. Boeckh, Regensburg University, Germany.

At Wageningen, discussions were held with Dr. Takken and Mr. Luc Willemse about the latter's work programme in Zimbabwe.

As a result of the visits, it was concluded that Dr. Persoons and Dr. den Otter did not have any particular facilities that could be used to introduce new elements into or to augment the work on tsetse attractants at NRI.

It was recommended that collaboration with Prof. Boeckh should be considered to explore the potential of recording from single receptor cells on tsetse antennae. Prof. Boeck had already made a start on this work, and his knowledge, equipment and enthusiasm were impressive.

Table 7.1. Overseas visits funded by the project

Dates	Person[1]	Place	Purpose	Ref.
17/09/86 - 03/10/86	DRH	Zimbabwe	liaison, field	QR 86/3
17/09/86 - 16/10/86	AJEG	Zimbabwe	field	QR 86/3
07/12/86 - 17/12/86	GAV	UK	liaison	QR 86/4
27/03/87 - 04/04/87	AJEG	Lome, Togo	ISCTRC conference	QR 87/1
09/05/87 - 23/05/87	AJEG	Zimbabwe	field	QR 87/2
10/09/87 - 24/09/87	GAV	UK	liaison	QR 87/3
06/03/88 - 11/03/88	DRH, AJEG	Kenya	KETRI conference, field	QR 88/1
03/12/88 - 16/12/88	GAV	UK	liaison	QR 88/4
05/10/89 - 19/10/89	DRH	Zimbabwe	liaison, field	AR 89
10/12/89 - 14/12/89	GAV, DRH, AC	Netherlands, Germany	liaison	AR 89

[1] DRH = D.R. Hall; AJEG = A.J.E. Gough; GAV = G.A. Vale; AC = A. Cork

In the first instance, known tsetse attractants and repellents would be tested to determine which act on specific receptors. Secondly, compounds previously found to produce and EAG response from tsetse could be tested to check whether they act on specific receptors and are thus likely to have some, as-yet undiscovered, behavioural effect. Thirdly, if cells were found that did not respond to any previously identified compounds but responded to natural attractants such as cattle odour or urine volatiles, these receptors could be investigated as leads to new behaviourally-active compounds.

7.2. VISITS FUNDED BY OTHER SOURCES

7.2.1. Visit by Dr. Hall to CRTA, Burkina Faso, 1986.

Dr. Hall visited the CRTA at Bobo Dioulasso, Burkina Faso in 1986, funded by IEMVT with a grant from the EC DGXII. The aim of this visit was to ensure that studies on tsetse attractants at CRTA were carried out in a way that made possible valid comparisons of the results with those obtained in the Region (Section 6.2.).

7.2.2. Visit by Dr. Gough to Côte d'Ivoire

Dr. Gough visited the GTZ project in Côte d'Ivoire as a consultant paid for by GTZ in 1987. The objective of this visit was similar to that above (Section 6.3.).

8. DISSEMINATION OF RESULTS

8.1. REFEREED PAPERS AND REVIEWS

Six refereed papers describing work from the Project were published by Dr. Gough and/or NRI staff, as follows.

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

Cork, A., Beevor, P.S., Gough, A.J.E. and Hall, D.R. (1990). Gas chromatography linked to electroantennography: a versatile technique for identifying insect semiochemicals. in "Chromatography and Isolation of Insect Hormones and Pheromones". Eds. A.R. McCaffery and I.D. Wilson. Plenum Press, New York. pp. 271-279.

Hall, D.R. (1990a). Use of odor attractants for monitoring and control of tsetse flies. in "Behavior-Modifying Chemicals for Insect Management", ed. R. Ridgeway. Marcel Dekker, New York. pp. 517-530.

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Jaenson, T.G.T., Barreto dos Santos, R.C. and Hall, D.R. (1991). Attraction of *Glossina longipalpis* (Diptera: Glossinidae) in Guinea-Bissau to odour-baited biconical traps. *Journal of Medical Entomology*, **28**, 284-286.

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

8.2. CONFERENCES AND MEETINGS

Seven presentations were given by Project staff at Conferences or meetings, as follows.

19th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC). Lome, Togo, 27 March - 4 April 1987 (Gough *et al.*, 1987).

24th Trypanosomiasis Seminar. British Society for Parasitology, University of Bristol, 17-18 September 1987. (Hall).

Practical Applications of Insect Pheromones and other Attractants. American Entomological Society, Boston, USA, December 1987. (Hall, 1990a).

Regional Development and Implementation of Tsetse Control Strategies for Eastern Africa with Emphasis on Targets and Traps. Kenya Trypanosomiasis Research Institute (KETRI), Muguga, Nairobi, Kenya, 7-8 March 1988 (Hall).

Chemical Signals in Vertebrates V. Oxford University, 8-10 August 1988. (Hall, 1990b).

Chromatography and Isolation of Insect Hormones and Pheromones. Royal Entomological Society and Chromatography Society, University of Reading, March 1989. (Hall; Cork *et al.*, 1990).

Tsetse Workshop. NRI, December 1989. (Hall).

8.3. ACKNOWLEDGEMENTS

As a further measure of the impact of this Project, assistance by Project staff was acknowledged in 10 other papers: Bursell (1987); Cheke and Garms (1988); Filledier and Mérot (1989a); Filledier and Mérot (1989b); Galey *et al.* (1986); Mérot *et al.* (1986); Mérot *et al.* (1988); Späth and Küpper (1989); Torr *et al.* (1989); Warnes (1990).

9. BUDGET SUMMARY

A Contract was originally agreed between the RTTCP and NRI to cover the three years April 1986 - March 1989. This provided funding for a post-doctoral fellow and for field and liaison visits by project personnel between the UK and Africa, and also the setting up and maintenance of a calf box at TRL. NRI staff were funded by ODA R&D, and TRL staff involved were funded by ODA R&D and ODA TC. Costings given in the original contract were updated in Annual Work Programmes and Cost Estimates agreed by the RTTCP.

The Project was extended for a fourth year, April 1989 - March 1990 and provided additional funding for staff at NRI and TRL.

Actual and allocated expenditures are summarised in Table 9.1. Over the four years April 1986 - March 1990, the funds provided by the RTTCP amounted to £120,135.

Table 9.1. Budget summary 1986–1990 (UK £).

	01/04/86-31/03/87	01/04/87-31/01/88	01/02/88-31/03/89	01/04/89 31/03/90	TOTAL
POST-DOCTORAL FELLOW					
Recruitment	596				596
Remuneration	13,706	11,797	21,471	1,456	48,430
UK travel, etc.	114	654	438		1,206
Overseas trips (field, conference)					
travel		2,317	2,000	4,317	4,317
subsistence, etc.	560	1,039	1,061		2,661
NRI bench fee			3,500		3,500
TRL STAFF TIME				5,233	5,233
NRI STAFF TIME				10,593	10,593
TRL CALF BOX					
capital			3,275		3,275
maintenance			2,815	2,800	5,615
UK SCIENTIST-IN-CHARGE LIAISON/FIELD					
travel	966			1,723[1]	2,689
subsistence	800			1,090[1]	1,890

Table 9.1. Budget summary 1986-1990 (UK £) (continued)

	01/04/86-31/03/87	01/04/87-31/01/88	01/02/88-31/03/89	01/04/89 31/03/90	TOTAL
ZIMBABWE SCIENTIST-IN-CHARGE LIAISON					
travel	896	515	500		1,911
subsistence	2,411	1,769	1,808		5,988
ADMINISTRATION					
TRL	1,204	1,089	4,474	712	7,479
Bristol University	1,293	1,171	4,511	765	7,740
NRI	874	2,753	1,076	2,309	7,012
TOTAL EXPENDITURE	**£23,421**	**£23,103**	**£46,931**	**£26,680**	**£120,135**
TOTAL ALLOCATION	**£32,300**	**£33,920**	**£51,854**	**£50,077**	**£168,151**

[1] also includes liaison trip by Drs. Hall and Cork to the Netherlands and Germany.

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11. ABBREVIATIONS

AR	Annual Report
CRTA	Centre de Recherches sur les Trypanosomoses Animales, Bobo Dioulasso, Burkina Faso.
DMRT	Duncan's Multiple Range Test
DVS	Department of Veterinary Services
EAG	electroantennography
EC	European Community
GC	gas chromatography
GMM	*Glossina morsitans morsitans*
GP	*Glossina pallidipes*
LC	Liquid chromatography
NMR	Nuclear magnetic resonance
MS	mass spectrometry
NRI	Natural Resources Institute (formerly Overseas Development Natural Resources Institute, ODNRI, and its component organisations including the Tropical Development and Research Institute, TDRI)
OCCGE	Organisation Commune de Coordination de Lutte contre les Grandes Endemies (OCCGE), Institut Pierre Richet, BP 1500, Bouake, Côte d'Ivoire.
Octenol	1-octen-3-ol
ODA	Overseas Development Administration
QR	Quarterly Report
R&D	Research and Development
RTTCP	Regional Tsetse and Trypanosomiasis Project for Malawi, Mozambique, Zambia and Zimbabwe
SAT	Sequential aerosol technique
TC	Technical Cooperation
TLC	Thin layer chromatography
TRL	Tsetse Research Laboratory