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Improved attractants for enhancing tsetse fly suppression

Final report of a co-ordinated research project
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FOREWORD

The Insect Pest Control sub-programme of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has worked for many years on developing the sterile insect technique (SIT) for tsetse fly control. The work included:

(a) research and methods development, which comprised in-house R&D at the Entomology Unit of the FAO/IAEA Agriculture and Biotechnology Laboratory, as well as research collaboration with other specialists through six previous and ongoing Coordinated Research Projects (CRPs) that focussed on general development of tsetse SIT, field application, using radiation and isotopes to develop diets for mass-rearing haematophagous insects for SIT release and to study disease transmission by these vectors, automation for tsetse mass-rearing for use in sterile insect technique programmes and genetic applications to improve the SIT for tsetse control/eradication including population genetics;

(b) the developments of standards and guidelines for various aspects of area wide integrated pest management (AW-IPM) against the tsetse and trypanosomosis (T&T) problem and the close interaction with other relevant partners working on T&T; and

(c) support to feasibility assessments and, where feasible, the application of the SIT component as part of an AW-IPM campaign against T&T in affected Member States.

As the efficiency of the SIT increases with reducing densities of the target insect pest populations, these are usually suppressed by other means prior to the "mopping-up" SIT phase. For several tsetse fly species the bait technology, consisting of visually attractant devices that can be further enhanced by odour blends, can be a useful and efficient tool in support of tsetse suppression, but also for tsetse monitoring or the maintenance of (temporary) artificial barrier systems to prevent a reinfestation of tsetse from adjacent infested areas into the intervention zone. For some of the economically important tsetse fly species no, or insufficiently efficient, attractants were available for using the bait technology.

This TECDOC reports on the results of a CRP established by FAO and IAEA in 1996 and completed in 2002 on the systematic screening and laboratory and field testing of known and new candidate attractant compounds and odour blends for use in the suppression or monitoring of a tsetse population and in barrier systems.

The IAEA wishes to thank I. Ujváry of the Chemical Research Centre, Hungarian Academy of Sciences for his assistance in compiling this TECDOC. The IAEA officer responsible for this publication was U. Feldmann of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

EDITORIAL NOTE

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SUMMARY

At the initiation of this co-ordinated research project (CRP), the available visually attractant devices and odours for entomological monitoring and for suppression of tsetse fly populations were not equally effective against all economically important tsetse fly species. For species like *G. austeni*, *G. brevipalpis*, *G. swynnertoni* and some species of the PALPALIS-group of tsetse flies no sufficiently effective combinations of visual or odour attractants were available for efficient suppression and standardized monitoring as part of an operational integrated intervention campaign against the tsetse and trypanosomosis (T&T) problem.

The Co-ordinated Research Project on Improved Attractants for Enhancing the Efficiency of Tsetse Fly Suppression Operations and Barrier Systems used in Tsetse Control/Eradication Campaigns involved (a) the identification, synthesis and provision of candidate kairomones, their analogues and of dispensers; (b) laboratory screening of synthesised candidate kairomones through electrophysiological studies and wind tunnel experiments; (c) field tests of candidate kairomones alone or as part of odour blends, in combination with available and or new trap designs; and (d) analysis of hydrocarbons that influence tsetse sexual behaviour. The CRP accomplished several main objectives, namely:

- The screening of new structurally related compounds, including specific stereoisomers, of known tsetse attractants resulted in the identification of several new candidate odour attractants with promising potential.
- An efficient two-step synthetic method was developed for the pilot plant scale production of 3-*n*-propyphenol, synergistic tsetse kairomone component.
- Electrophysiological experiments complemented with wind tunnel studies provided an efficient basis for the laboratory screening of candidate attractants prior to the initiation of laborious field tests.
- New traps were identified and modifications of existing traps were tested for some species that previously were difficult to monitor.
- The combination of some plant volatiles with new and standard host odours constitute attractant odour blends for several tsetse species for which previously no odour attractants were known.
- The novel hydrocarbons identified and characterized from several tsetse species specifically influence (stimulate or inhibit) the sexual behaviour of tsetse males and offer useful tools in rearing / control operations.

An incorporation of specific operational research with identified host and habitat odour blends for *Glossina fuscipes fuscipes*, *G. palpalis gambiensis* and *G. swynnertoni* should be considered as part of ongoing tsetse intervention projects that are supported by IAEA-TC and other partners. Thus the findings under this CRP can be refined for large scale field application and eventually benefit integrated tsetse intervention campaigns through availability of improved standard tools for fly population monitoring, for tsetse suppression operations and for the establishment of (temporary) barrier systems.

1. INTRODUCTION

1.1. Significance of tsetse and trypanosomosis in sub-Saharan Africa

Tsetse flies infest 36 African countries and a total land area of at least 8.7 million km² in Africa. Throughout this area the disease transmitted by the tsetse fly, trypanosomosis, has a devastating effect on livestock and man. Tsetse flies are largely responsible for an uneven distribution of cattle in Africa, leading to overgrazing and severe environmental degradation in some areas and preventing the introduction of productive farming and livestock systems in other areas. Tsetse and trypanosomosis is a problem that is closely linked with rural poverty, which is why the tsetse fly is frequently referred to as the "poverty insect". Direct losses in meat production and milk yield and the costs of programmes to control trypanosomosis are estimated to be between US $0.6 and 1.2 billion each year [1]. If the trypanosomosis problem would be removed from Africa it is estimated that benefits to overall agricultural production would gradually rise to US $4.5 billion per year [2]. According to the World Health Organization, over 55 million people living in rural areas of sub-Saharan Africa are at risk of contracting sleeping sickness (human trypanosomosis). Some 30,000 new cases are reported annually but this does not reflect the real epidemiological situation because of poor surveillance. The estimated number of infected persons ranges between 300,000 and 500,000 [3].

1.2. Traditional tsetse control methods

Different methods are available for tsetse and trypanosomosis control or eradication[1] that have all their specific advantages and limitations. Intervention measures that were successful in the past such as bush clearing or elimination of wild animals on which tsetse depend for food have been abandoned for environmental reasons. The indiscriminate use of persistent insecticides for aerial spraying is also restricted. The currently available and environmentally acceptable methods for interventions are:

- Parasite control through
  - — the use of trypanocidal drugs; and
  - — (to a limited extent) the promotion of trypanotolerant livestock.
- Vector intervention using
  - — traps and insecticide treated devices (there are considerations to treat the devices with entomopathogenic fungi or insect growth regulators, including juvenile hormone analogues), in some cases baited with odour attractants;
  - — special formulations of insecticides on livestock;
  - — the sequential aerosol technique (SAT = repeated aerial spraying of ultra low volume formulations of non-persistent insecticides); and
  - — the sterile insect technique (SIT).

Although the strategies for using these options may vary considerably, only a combination of several of the above methods, preferably as part of an area wide integrated pest management

[1] Although the term "eradication" is often understood as the extinction of a species from the earth, in this document it stands for localized complete removal of a population of a pest species, i.e. creation of a sustainable pest-free zone that — except for quarantine measures — does not require further pest control. The pest species concerned may still exist at other places (in the laboratory colonies or distant habitats).

(AW-IPM) approach, can effectively and sustainably alleviate the tsetse and trypanosomosis problem and, if possible, create a tsetse fly free zone and foster the establishment of viable agricultural systems.

1.3. The role of SIT in area wide tsetse control

The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has played a major role in promoting AW-IPM and SIT. The success stories of SIT against key insect pests of agricultural and livestock systems are well documented [4, 5, 6, 7, 8, 9]. Pilot efforts to apply the SIT against vectors of major diseases like tsetse flies were successfully implemented in Burkina Faso and Nigeria but were not sustainable as the planning of operations was not part of a larger scale sub-regional intervention and agricultural / livestock development strategy and the selection of the areas was not based on the area wide principle. Between 1994 and 1996 tsetse were eradicated from Unguja Island of Zanzibar [10]. Particularly the Zanzibar experience, which integrated the SIT with entomological and veterinary monitoring as well as conventional tsetse suppression using insecticides on cattle and cloth targets, highlighted the unavailability of adequately attractive baits for efficient suppression and entomological monitoring of some tsetse species, such as *G. austeni*.

1.4. Control technologies relying on tsetse behaviour

The past 30–40 years of research on the biology and ecology of tsetse have started to yield some successes in the fight against human and animal trypanosomosis in Africa [11]. In particular, the improved bait technology has become vital in monitoring and control operations against several *Glossina* species in many tsetse-infested areas [12]. Nevertheless, the available bait technology is not similarly efficient for all tsetse fly species: whereas the placement of four odour-baited traps or targets per km^2 may be sufficient to reduce *Glossina pallidipes* and *G. morsitans morsitans* populations in Zimbabwe within only 3–4 months to 3–5% of their original apparent population density [13], some 40–70 targets per km^2 had to be placed for 1.5 years in the Jozani forest of Zanzibar to reduce the *G. austeni* target population to 18% of its original population density [10]. This suggests that for species like *Glossina austeni* and in dense habitats the available bait technology may reach less than 1% of the efficiency recorded, for example, for *G. pallidipes* in the open savannah. Nevertheless, the two extremes highlight that the bait technology, consisting of visually attractant devices that can be further enhanced by odour blends can be a useful and efficient tools in support of tsetse suppression, tsetse monitoring or barrier systems. A further advantage of the use of baits and targets is that their impact on non-target species or the environment appears to be minimal, localised and temporary [14]. If an efficient bait technology may not be available for a target tsetse fly species, this species can be suppressed with other means, for example SAT or use of pour-on formulations of insecticides on livestock, but the entomological monitoring operations and, possibly, barrier systems would still require or substantially benefit from improved visual or odour attractants.

Therefore, with the assistance of collaborators and consultants, the Joint FAO/IAEA Division and the IAEA's Research Contracts Administration Section implemented between 1995 and 2002 a Co-ordinated Research Project (CRP) on "*Improved Attractants for Enhancing the Efficiency of Tsetse Fly Suppression Operations and Barrier Systems used in Tsetse Control/Eradication Campaigns*".

The following tables lists the species that were studied in under this CRP:

Table 1.1. List of *Glossina* species studied in the laboratory (electrophysiological studies or wind tunnel experiments)

A. Electrophysiological studies		
Sub-region	Group	Species
East Africa	*morsitans*	*G. pallidipes* Austen 1903
	fusca	*G. brevipalpis* Newstead 1910
West Africa	*palpalis*	*G. palpalis gambiensis* Vanderplank 1949

B. Wind tunnel experiments		
Sub-region	Group	Species
East Africa	*morsitans*	*G. pallidipes* Austen 1903
West Africa	*palpalis*	*G. palpalis gambiensis* Vanderplank 1949

Table 1.2. List of *Glossina* species studied in the field

Country	Group	Species
East Africa		
Kenya	*morsitans*	*G. austeni* Newstead 1912
		G. pallidipes Austen 1903
	fusca	*G. brevipalpis* Newstead 1910
Tanzania	*morsitans*	*G. morsitans centralis* Machado 1970
		G. pallidipes Austen 1903
		G. swynnertoni Austen 1923
	fusca	*G. brevipalpis* Newstead 1910
Uganda	*palpalis*	*G. fuscipes fuscipes* Newstead 1910
West Africa		
Burkina Faso	*palpalis*	*G. palpalis gambiensis* Vanderplank 1949
		G. tachinoides Westwood 1850
Mali	*morsitans*	*G. morsitans submorsitans* Newstead 1910
	palpalis	*G. palpalis gambiensis* Vanderplank 1949
		G. tachinoides Westwood 1850

1.5. Objective

The specific research objectives under this CRP were to identify, test and refine, particularly for "difficult" tsetse species, for which no or inefficient attractants are available:

- traps or targets that may be used for population monitoring, pre-release fly population reduction and in (temporary) barrier systems for preventing re-infestation of tsetse into tsetse-free areas;

- appropriate technology for tsetse population monitoring using attractants from previous work or new candidate chemicals for use in integrated area wide SIT schemes;

- locally available inexpensive sources of chemical and visual attractants and alternative, less expensive methods for synthesizing conventional attractants; and

- other attractant or odour-based mechanisms that may be used for, or are relevant to, tsetse SIT operations. This includes the assessment of known or suspected sex pheromone components of conspecific tsetse of different populations in order to better understand these pheromone systems and also to ensure that proper biological interaction between different populations is likely to occur when SIT is contemplated or used, i.e. lab colony versus wild flies.

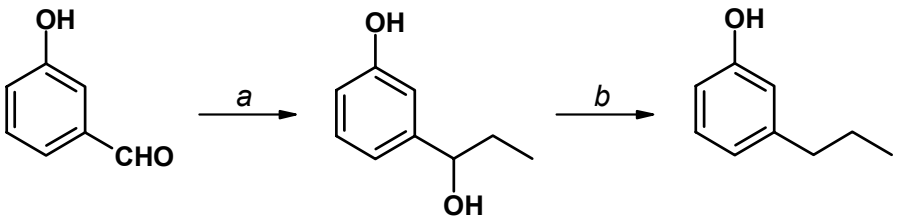
2. DEVELOPMENT AND PROVISION OF KAIROMONES, THEIR ANALOGUES AND DISPENSERS

*2.1. Large scale synthesis of 3-*n*-propylphenol*

Among various phenols, identified as attractive principles of buffalo and cattle urine, 3-*n*-propylphenol (henceforth propylphenol) is a synergistic minor component [15, 16, 17, 18]. This compound is used in artificial host odours, such as the 1:4:8 combination of propylphenol, racemic 1-octen-3-ol (henceforth octenol) and *p*-cresol (4-methylphenol). Since propylphenol is not readily available commercially in bulk quantities at a reasonable price the CRP participants decided to explore the development of an inexpensive method for the production of this kairomone on a large scale. Previous syntheses of propylphenol used propiophenone, safrole, 3-benzyloxybenzaldehyde or 3-bromoanisole starting materials [15, 16, 19, 20, 21, 22]. These compounds, however, are not readily accessible or obtained by reactions unsuitable for large-scale synthesis of the target phenol at an acceptable cost. A more straightforward method, starting from 3-hydroxy-benzaldehyde and using *Grignard*-reaction for the construction of the *n*-propyl side chain, was reported earlier but no experimental details were given [23, 24].

Therefore, a two-step synthesis of propylphenol was developed that is suitable for the large-scale preparation of this important synergistic kairomone. In the optimised method developed (Scheme 2.1), 3-hydroxybenzaldehyde in tetrahydrofuran (THF) using toluene as co-solvent is reacted with 2.6 equivalents of ethylmagnesium bromide affording the crystalline hydroxyphenol intermediate that was readily purified by re-crystallization from ethyl acetate. (Due to the poor solubility of the solid starting material in THF, it was necessary to dissolve this aldehyde in a minimum amount of hot toluene before adding the required amount of THF at ambient temperature.)

Catalytic hydrogenolysis of the resulting intermediate in methanol gave the target compound in nearly quantitative yield after vacuum distillation.



Scheme 2.1. Reaction conditions: *a*) EtMgBr, toluene-THF, 15–25°C; *b*) H_2/Pd-C, methanol, RT.

(±)-3-(1-Hydroxypropyl)phenol. Finely ground 3-hydroxybenzaldehyde (1250 g, 10.2 mol) was dissolved in warm anhydrous toluene (2.2 l). The solution was then allowed to cool to ca. 30°C, purged with dry argon gas and diluted with anhydrous THF (20 l). The efficiently stirred suspension was cooled to 10°C and a *Grignard*-solution prepared from ethyl bromide (1987 ml, 26.6 mol) and magnesium chips (648 g, 26.6 mol) in anhydrous THF (8.2 l) was added over the course of 3 h while carefully maintaining the reaction temperature between 15°C and 25°C using dry ice + water as cooling bath. The thick reaction mixture was stirred

and refluxed for 2 h, then cooled to 5°C, quenched with cold water (1.0 l), acidified with 5 M HCl solution (5.6 l). The phases were separated, the aqueous layer extracted with methyl *tert*-butyl ether (4 x 1.0 l). The combined organic phases were washed successively with of water, saturated $NaHCO_3$ and water (1.0–1.0 l), and dried over $MgSO_4$. After filtration, the solvent was evaporated at reduced pressure and the crude product re-crystallized in ethyl acetate (ca. 1.0 l) to give 1250 g of the intermediate hydroxy-phenol (80%) as a white solid.

Melting point: 106–107°C; lit: 107°C [23]; TLC: R_f = 0.19 (silica, toluene : methanol = 9:1 (volume by volume); for starting material R_f = 0.37. IR (KBr): ν 3400, 1590, 1480, 1270, 1090, 950, 890, 790, 702 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.95(t, *J* = 7.4 Hz, 3H), 1.77(m, 2H), 1.95(s, 1H), 4.56(br t, *J* = 6.5 Hz, 1H), 5.18(s, 1H), 6.66(m, 1H), 6.86(m, 1H), 7.20(m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.8, 146.5, 129.6, 118.4, 114.5, 112.8, 75.8, 31.7, 10.0.

3-n-*Propylphenol.* A solution of the above hydroxy derivative (381 g, 2.50 mol) in analytical grade methanol (2.0 l) was added to a pre-hydrogenated suspension of 10% Pd-on-carbon (28.0 g) in analytical grade methanol (1.3 l) while stirring. The reaction mixture was then hydrogenated at room temperature and atmospheric pressure (using a gas burette and reservoir) until gas absorption ceased (ca. 60 l during 12 h). The suspension was filtered under inert gas (nitrogen or argon) atmosphere, the filtrate concentrated at reduced pressure and the residue fractionated in vacuum. After a small forerun, 320 g (94%) the title phenol was collected as clear oil with a characteristic smell.

Bp: 93–95°C/2.3 mmHg; lit: 110°C at 10 mmHg [19]; n_D(at 25°C): 1.5236; Density: 0.9878 g/ml (at 24°C). ^1H NMR (400 MHz, $CDCl_3$): δ 0.93(t, *J* = 7.4 Hz, 3H), 1.63(m, 2H), 2.53(t, *J* = 7.4 Hz, 2H), 4.77(s, 1H), 6.64(m, 1H), 6.65(m, 1H), 6.75(m, 1H), 7.14(m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.2, 144.7, 129.4, 121.1, 115.4, 112.6, 37.8, 24.3, 13.8; MS (electron ionization): *m/*z 136 $[M]^+$ (45%), 121 (15%), 107 (100%), 77 (20%).

This short procedure has been successfully applied to the synthesis of other alkylated aromatics from the respective aromatic aldehydes (see Section 2.2).

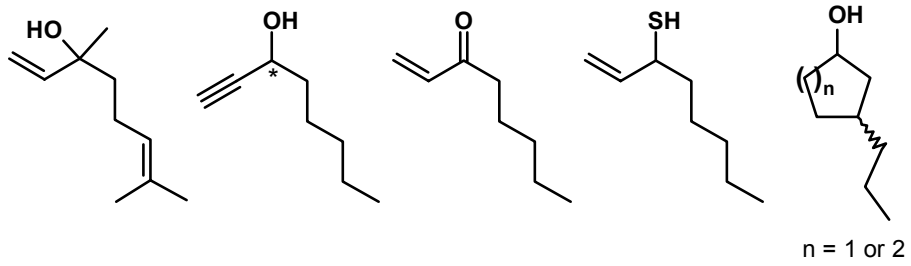
2.2. Design and synthesis of candidate attractants

Design of new tsetse attractants

For tsetse species of the *morsitans* group, the traps are usually baited either with natural odour sources (*e.g.*, cow urine) or with synthetic attractant components of host animal odours. Members of the *palpalis* and *fusca* groups do not respond satisfactorily to the presently used artificial bait systems. To find attractants for these latter "problem" species, various combinations of known kairomones were examined both in the laboratory and the field. During the CRP a series novel aromatic and aliphatic analogues of the natural kairomones were designed, synthesized and examined in the laboratory, hoping that some of them alone or in combination could be useful in field baits.

Structure design of the new analogues was based on several common drug design principles as follows: 1) replacing functionalities of natural kairomones by moieties having similar shape and electron distribution; 2) fixing conformations of flexible chains; 3) introducing additional functionalities. In the case of octenol, inexpensive precursors were explored as well. The structure of the analogues studied is shown in Fig. 2.1.

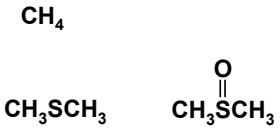
Aliphatics

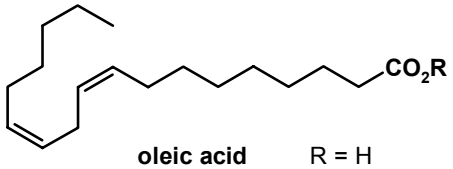


$CH_3(CH_2)_nCHO$ — **decanal** n = 8; **dodecanal** n = 10

$CH_3(CH_2)_nOCHO$ — **octyl formate** n = 7; **decyl formate** n = 9

$CH_3(CH_2)_nCN$ — **decanenitrile** n = 8; **dodecanenitrile** n = 10





oleic acid R = H
methyl oleate R = Me

Aromatics

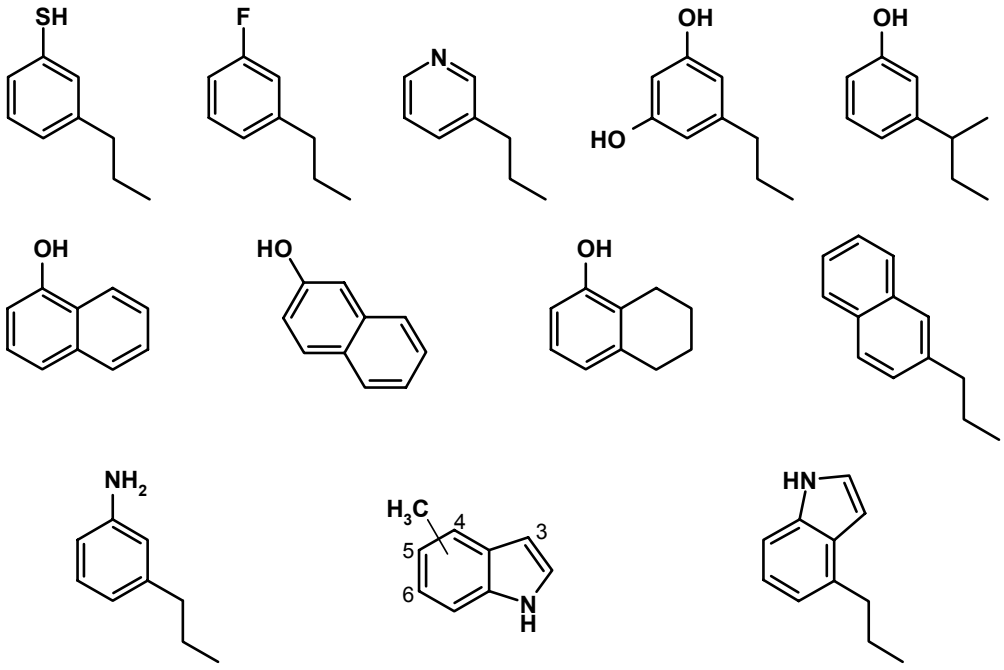
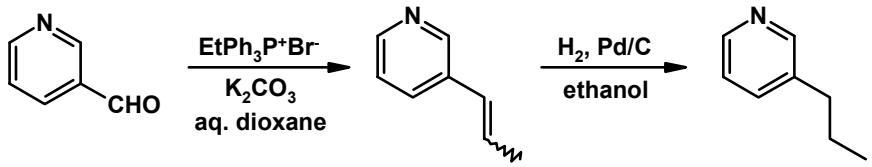


Figure 2.1. Chemical structure of the newly tested compounds.

Synthesis of new candidate tsetse attractants

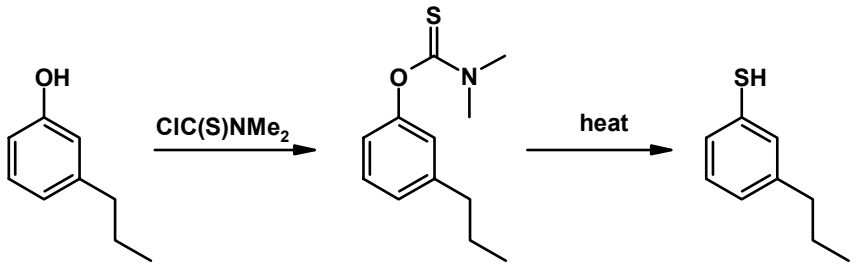
For propylphenol, either the side chain or the phenol moiety was replaced with different functionalities. The synthesis of aromatic compounds (±)-3-(2-butyl)phenol, 3-*n*-propylfluorobenzene and 2-*n*-propylnaphthalene was accomplished by the method developed for the large scale-synthesis of propylphenol (Scheme 2.1) by reacting the appropriate carbonyl compounds (3-hydroxybenzophenone, 3-fluorobenzaldehyde, 2-naphthaldehyde, respectively) with 2.5 equivalents of ethylmagnesium bromide using ether or THF as solvent for the *Grignard*-reaction. Hydrogenation of the resultant benzylic alcohol species gave the expected products in good yields.

3-*n*-Propylpyridine was synthesized in 50% overall yield by Pd-catalysed hydrogenation of 3-(1-propenyl)pyridine obtained from the *Wittig*-reaction of 3-pyridinecarboxaldehyde and ethyltriphenylphosphonium iodide by a solid-liquid two-phase method [25] (Scheme 2.2).



Scheme 2.2. Synthesis of 3-*n*-propylpyridine.

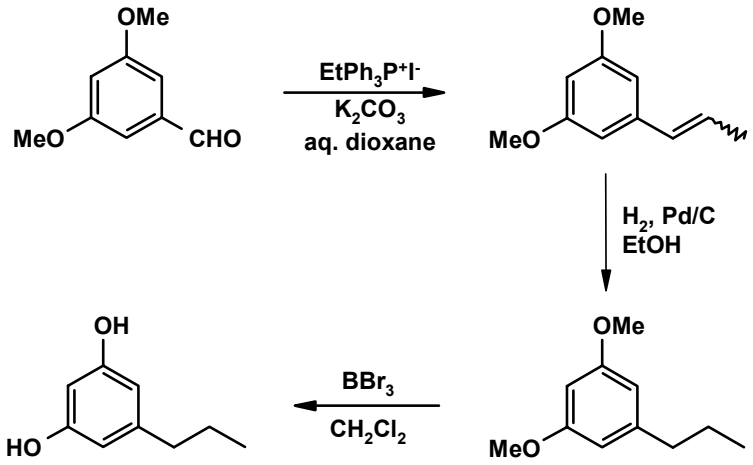
3-*n*-Propylthiophenol was prepared in 60% overall yield by thermal isomerization of *O*-(3-*n*-propylphenyl) *N*,*N*-dimethylthiocarbamate using known methodology [26] (Scheme 2.3).



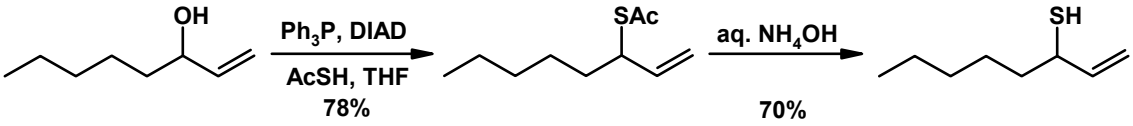
Scheme 2.3. Synthesis of 3-*n*-propylthiophenol.

5-*n*-Propylresorcinol (1,5-dihydroxy-3-*n*-propylbenzene or divarinol), a di-phenol analogue of propylphenol contains two phenolic hydroxyl groups both in *meta*-position to the propyl side chain. Its synthesis (Scheme 2.4) starts with the two-phase *Wittig*-reaction [27] of 3,5-dimethoxybenzaldehyde with ethyl-triphenyl-phosphonium iodide followed the catalytic hydrogenation of the intermediate alkene to afford 1,3-dimethoxy-5-*n*-propylbenzene. This ether was demethylated by boron tribromide [28] to the known title product, divarinol [29, 30].

Racemic 1-octen-3-thiol was prepared from the corresponding natural alcohol as shown in Scheme 2.5. The synthesis of this compound is based on the general method for the preparation of secondary thiols from the corresponding alcohols [31]. Mild alkaline hydrolysis of the thiolacetate, obtained using *Mitsunobu*-conditions, afforded the unpleasantly smelling thiol after chromatographic purification.



Scheme 2.4. Synthesis of 3-*n*-propylresorcinol.



Scheme 2.5. Synthesis of racemic 1-octen-3-thiol.

Comparison of the structures of the natural kairomones shows that the molecular models of octenol and propylphenol are fairly similar (Fig. 2.2). The calculated molecular volumes of octenol and propylphenol are ~544 $Å^3$and ~512 $Å^3$, respectively, while the surface areas for the two respective compounds are ~363 $Å^2$ and ~339 $Å^2$ (see Table 2.1). These data suggest that the same binding (receptor) site could accommodate both kairomones. It was decided to test this idea by designing conformationally rigid analogues of the two compounds. Two such cyclic analogues prepared are 3-*n*-propylcyclopentanol and 3-*n*-propylcyclohexanol having calculated surface areas of ~341 $Å^2$ and ~358 $Å^2$, respectively. These compounds possess two asymmetric carbon atoms and thus each exists as four sterically distinct molecules (diastereomers). The molecular models shown in Fig. 2.2 represent the *cis*-isomers of the cyclopentanol (B) and cyclohexanol (C) analogues in which the configuration of the alcoholic carbon atom (C-1) is *R*, corresponding to the one found in the natural octenol stereoisomer (A). Note that the orientation of the flexible side chains of the natural kairomones (*A* and *D*) allows the molecules to share the same receptor site. The two cycloalkanol analogues (*B* and *C*) can also be accommodated by the same putative binding (receptor) site.

3-*n*-Propylcyclopentanol [32] was prepared in 40% overall yield in two steps as a diastereomeric mixture (Scheme 2.6). First, CuI-catalyzed conjugate addition [33, 34] of *n*-propylmagnesium bromide to 2-cyclopenten-1-one in THF afforded (±)-3-*n*-

propylcyclopentanone. This ketone was then reduced by $LiAlH_4$ to give the final product as a *cis*/*trans* isomer mixture with the *cis* isomer predominating as established by ^1H NMR and gas chromatography. A stereoisomeric mixture of 3-*n*-propylcyclohexanol [35] was synthesized in a similar manner from 2-cyclohexen-1-one in 45% overall yield.

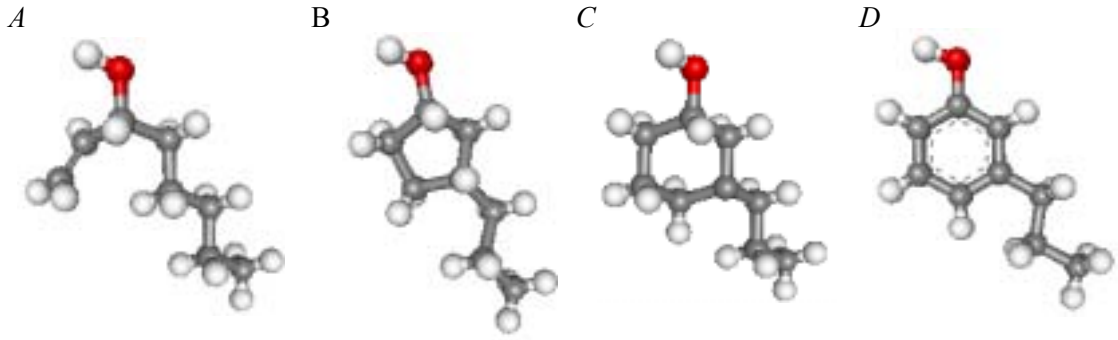
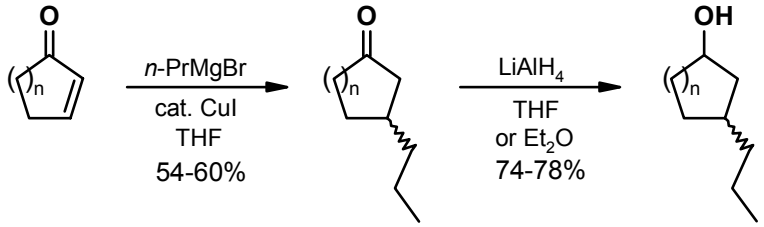


Figure 2.2. Structural similarity of stable conformers of natural kairomones (A and D) and their cycloalkanol analogues (B and C). A: (R)-1-octen-3-ol; B: (1R,cis)-3-n-propyl-cyclopentanol, C: (1R,cis)-3-n-propylcyclohexanol; and D: 3-propylphenol. Ball-and-stick molecular models were constructed by the molecular visualization program ViewerPro 4.2 software (Accelrys Inc.).



Scheme 2.6. Synthesis of isomeric mixtures of 3-*n*-propylcyclopentanol (n = 1) and 3-*n*-propyl-cyclohexanol (n = 2).

The details of the above syntheses and spectral data for the compounds were described in one of the previous working papers [36].

Isosteric analogues of phenol kairomones

The phenol-to-indole isosteric replacement is an established drug design technique that has proven to be useful in structure-biological activity relationship studies. Thus it was decided to submit to laboratory bioassay unsubstituted indole and the indole analogues of the common host odour components 3- and 4-methylcresol. For 3-methylcresol (or *m*-cresol), as it is shown in Fig. 2.3, there are actually two regio-isomeric indole analogues. For 4-methylindole (structure on the left) the methyl group is on the distal position of the ring system, while for 6-methylindole the methyl group is in a closer proximity of the indole NH group. Another indole derivative, a common tryptophan-metabolite present in animal feces as well as in plant volatiles, 3-methylindole (skatole) has already been found to elicit significant EAG activity, was retested in this series of experiments. These methylindoles are commercially available.

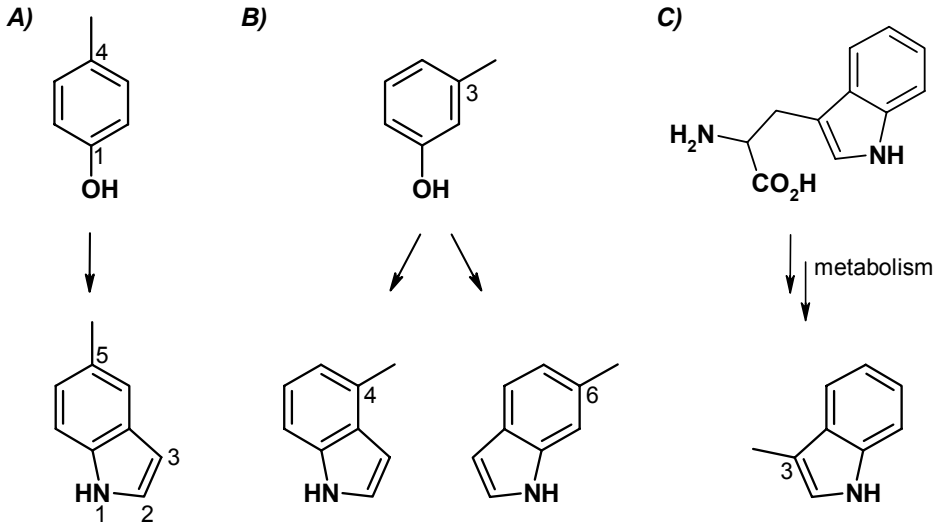
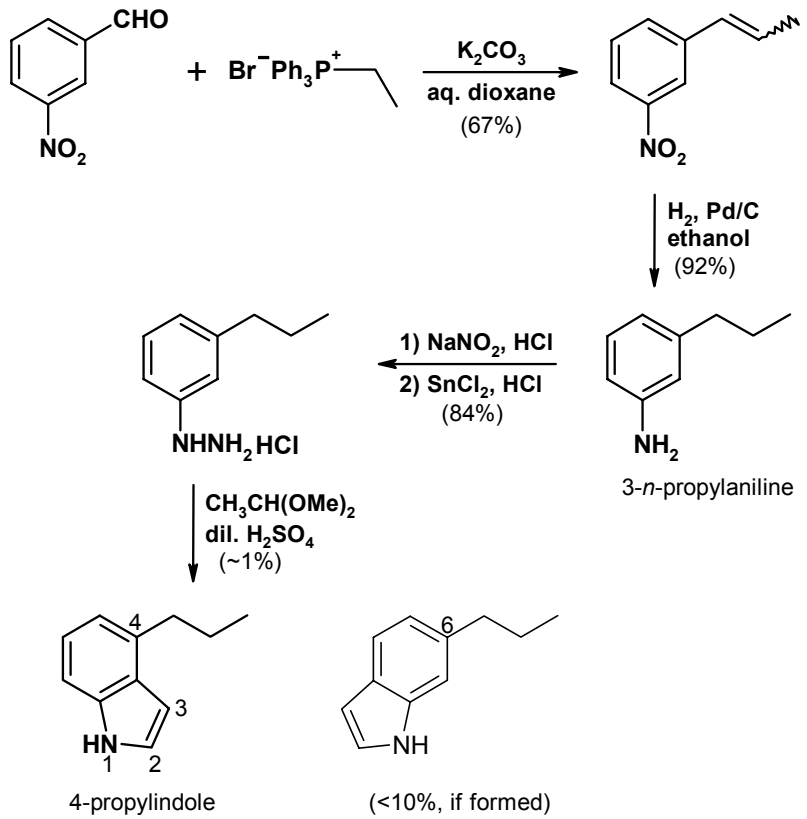


Figure 2.3. Structural analogy of A) p-cresol and 5-methylindole; B) m-cresol, 4-methylindole and 6-methylindole; C) biosynthesis of 3-methylindole (skatole).



Scheme 2.7. Synthesis of an isosteric indole analogue of propylphenol.

In connection with this study, the indole analogue(s) of 3-*n*-propylphenol were tested as well. Since this compound has not been described in the literature, a simple though low-yielding synthesis of this compound was developed (Scheme 2.7). The key step towards the synthetic intermediate 3-*n*-propylaniline, which can be considered also as an analogue of propylphenol, was the two-phase *Wittig*-reaction [27] used earlier for the preparation of other tsetse kairomone analogues. Note that the catalytic reduction provides both the saturated alkyl side chain and the amino group in one step. For cyclization of the hydrazine intermediate a modified *Fischer*-indole synthesis [37] was used giving the expected indole in low yield. Interestingly, only the 4-*n*-propylindole regioisomer was obtained.

Isosteric analogues of putative aldehyde kairomones

At an FAO/IAEA conference in Nairobi in September 1996, ICIPE researchers disclosed preliminary results on the isolation from the monitor lizard of two aldehydes, namely decanal and dodecanal, as potential tsetse kairomones. However, details on the biological activity (i.e., attractivity) of these substances have not been reported [38, 39]. It was decided to test both aldehydes and their respective isosteric formate and nitrile analogues, which are expected to be more stable in the field. Formates [40, 41] and nitriles [42, 43] have been shown to be active analogues of certain aldehyde semiochemicals.

In order to visualize the geometric and electronic similarities implicated in non-bonding interactions of the compounds with the receptor, decanal and its functional group analogues, decanitrile and octyl formate were compared by molecular modelling using *HyperChem*® Release 5.01 software (HyperCube Inc.) installed on an IBM clone with a Pentium 233 MHz processor. Geometry optimisation was done by MM+ force field molecular mechanics followed by semi-empirical energy minimisation using the AM1 method. Calculation of the electrostatic potential surfaces reveals that the shape and electron distribution of the aldehyde and its formate analogue are similar (Fig. 2.4). Due to the linear geometry of the –C≡N bond, the electrostatic potential contour of the nitrile is somewhat different from that of the other analogues although rotational flexibility allows this molecule to fit into the cavity of putative receptor.

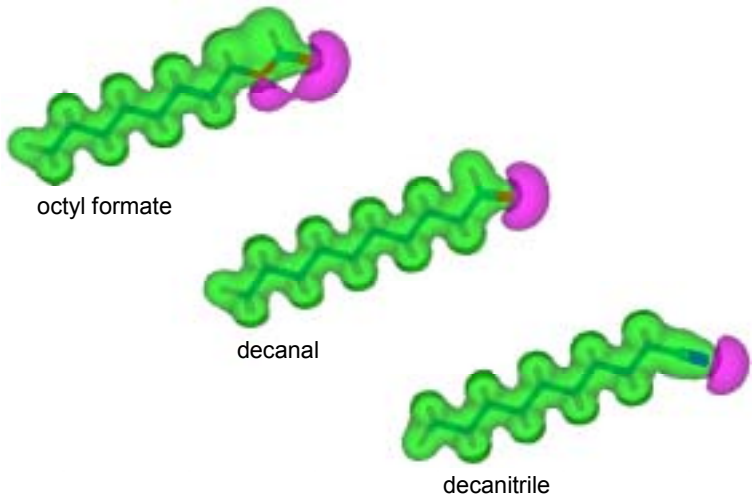


Figure 2.4. Electrostatic potential surfaces of decanal and its formate and nitrile analogues. Negative electrostatic potential values, indicating electron-rich areas, are in violet, positive values in green.

Based on the literature precedents and on the developed modeling study the C_{10} and C_{12} aldehydes, as putative kairomones, and their C_{10} and C_{12} nitrile, and C_8 and C_{10} formate analogues were included in the laboratory and field studies. Decanal, dodecanal, decanitrile and dodecanitrile were obtained from commercial sources. Octyl and decyl formates were synthesized on a hundred-gram scale by conventional esterification of 1-octanol and 1-decanol with an excess of formic acid as follows.

n-Octyl formate. A solution of freshly distilled 1-octanol (98.0 g, 0.752 mol) and 98% formic acid (69.1 g, 1.50 mol) was refluxed for 18 hr. Then the reaction mixture was poured onto saturated NaCl solution (30 ml) and the phases were separated. The organic layer was washed successively with saturated $NaHCO_3$ solution (2 × 30 ml), and water (30 ml), dried over $MgSO_4$, then fractionated to afford 84.0 g (70.5%) pure product as colourless oil. Bp.: 77^oC/15 mmHg; $n_D^{22} = 1.4178$.

n-Decyl formate. 133 g (95% yield) of this ester was obtained from 0.75 mol 1-decanol in a similar fashion. Bp: 167^oC/15 mmHg; $n_D^{22} = 1.4262$.

The ^1H NMR spectra of the products were in agreement with the expected structure (data not shown).

After the completion of the relevant studies under this CRP, ICIPE researchers described the composition of volatiles of the tsetse hosts buffalo and ox using gas chromatography coupled with electroantennographic detector (GC-EAD) [39]. They identified octanal, nonanal, decanal, and dodecanal that elicited electrophysiological responses from the antennae of *G. morsitans morsitans*. The results of a very recent interesting study on the analysis paracloacal gland secretion of the American crocodile, *Crocodylus acutus* [44], also deserve attention. The skin secretion consists of various terpenoid and long chain fatty alcohols and their esters. Interestingly, significant quantities of formates, derived from C_{13}-C_{18} alcohols, were found. Formates of C_9 to C_{28} fatty alcohols have also been identified from the preorbital gland of several African antelopes, including grysbok, steenbok, and oribi [45]. However, the ecological role, if there is any, of these exocrine secretion products has not been clarified yet. Nevertheless, these volatile compounds could be relevant as host odours for tsetse.

Synthesis of 1-octen-3-ol stereoisomers

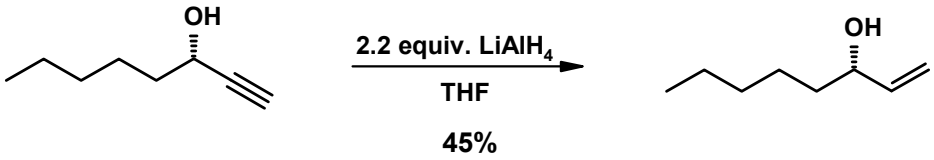
Of the volatiles emanating from cattle head 1-octen-3-ol was identified as a potent olfactory stimulant for tsetse [46]. This secondary alcohol contains an asymmetric centre at C-3 atom, thus exists in two stereoisomeric (enantiomeric) forms, the (*R*) and the (*S*) isomer. In their original study Hall et al. [46] demonstrated that foraging cattle emit the (*R*)-isomer (presumably produced by microorganisms from fodder). It must be noted that in mushrooms, the most abundant natural sources of (*R*)-1-octen-3-ol, which is also called *matsutake* alcohol, this characteristic flavour component is enzymatically produced by oxidative breakdown of linoleic acid, a common fatty acid. However, in the tropical plant *Phyllagathis rotundifolia* (Melastomataceae) glycosidically bound octenol, possessing (*S*)-configuration at C-3, has recently been reported [47].

Experiments with partially enriched (*R*)- and (*S*)-octenol isomers (86% and 66% enantiomeric excess, respectively), however, could not resolve the problem of the possible behavioural preference of tsetse for either of the stereoisomers and it was also reported that large doses of (racemic) octenol had repellent effect [48]. Nevertheless, cheap synthetic racemic octenol continues to be used in field baits. Behavioural studies, although repeatedly establishing the repellency of high doses of racemic mixture but not investigating the effect of the individual isomers, also continue to use "commercial octenol" [49, 50].

Racemic octenol appears to be a general attractant for many *Glossina* species, especially those belonging to *morsitans* group. This raises the need to study the behavioural effect of the two enantiomers individually to explain the failure or some conflicting results of experiments with the racemic mixture. Thus there is a need for both isomers of octenol in a pure (>98% *e.e.*)

form. (There are examples with chiral lepidopteran sex pheromones where the unnatural stereoisomer interferes with the biological activity of the natural pheromone. Also, some closely related species use different stereoisomers or distinct isomeric compositions of chiral compounds as pheromones.)

Earlier, octenol isomers were obtained by resolving racemic octenol, which is a lengthy and low-yielding procedure [51] and the resultant product is of inadequate enantiomeric purity (<90%). Multistep syntheses of octenol isomers also exist [52, 53]. The preparation of the enantiomerically pure isomers was done via a novel one-step route starting from commercially available (*R*)- and (*S*)-1-octyn-3-ols of high enantiomeric purity (>98% *e.e.*) (Scheme 2.8).



Scheme 2.8. Synthesis of (*S*)-1-octen-3-ol.

Reducing the propargylic alcohol into the corresponding vinyl alcohol on a hundred-milligram scale by an excess of $LiAlH_4$ in THF or, preferably, in ether gave, after purification by $AgNO_3$-impregnated chromatography to remove any unreacted terminal alkyne, the expected volatile alcohol in acceptable yields. Because the chiral centre is not involved in the reaction, the enantiomeric purity of the product should not be compromised (i.e., it should be >98% *e.e.*).

*(*S*)-1-Octen-3-ol.* A solution of (*S*)-1-octyn-3-ol (Aldrich) (0.60 g, 4.75 mmol) in anhydrous THF (4 ml) was added to an ice-cooled suspension of $LiAlH_4$ (0.40 g, 10.5 mmol) and anh. THF (16 ml). After stirring at room temperature for 14 h, the reaction mixture was cooled by an ice-bath, stirred vigorously, then water (0.40 ml), 1N aq. NaOH solution (0.40 ml) and water (1.2 ml) were successively added. After stirring for 30 min, the suspension was filtered, the filtrate concentrated in vacuum (<35^{o}C), and the residue purified by column chromatography on 3% $AgNO_3$-impregnated silica gel with hexane containing 0 to 5% ethyl acetate as eluant. Yield: 0.27 g (45%) of pure product as colourless oil with a mushroom-like odour.

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88(t, *J*=6.5Hz, 3H), 1.30–1.54(m, 8H), 2.0(s, OH), 4.10(m, 1H), 5.17(m, 2H), 5.87(m, 1H).

In a similar experiment with (*R*)-1-octyn-3-ol (Aldrich) the corresponding (*R*)-1-octen-3-ol was obtained in 40% yield.

It is noted that partial hydrogenation of the terminal C≡C bond to C=C bond using quinoline-poisoned *Lindlar*-catalyst ($Pd/BaSO_4$) in hexane was also attempted. After 2 hours ^{1}H NMR spectroscopy indicated partial hydrogenation and formation of the expected product (~50% conversion of the starting material), but after 4 hr the formation of the fully saturated octan-3-ol was observed. The saturation of the terminal alkene could not be prevented even in the presence of two equivalents of 1-hexene, which is often used to minimise the saturation of terminal alkenes. So this route was abandoned in favour to the one describe above.

Physicochemical and structural properties of known and candidate tsetse kairomones

In connection with structure-activity relationship (SAR) studies of the analogues prepared during this project an effort was undertaken to compile from the literature and based on the work done under this CRP a list with some basic physicochemical properties of tsetse kairomones and related synthetic compounds. These and, where exist, the *Chemical Abstract Service Registry Number*s are shown in Table 2.1. Additional physicochemical and molecular parameters that could be used in further, more refined SAR work are tabulated in Table 2.2. The hydrophobic (or lipophilicity) parameters (log P, i.e., logarithm of the measured partition coefficient of the substance for *n*-octanol/water system) were obtained from literature sources [54] when available. Otherwise, this and the other molecular parameters were calculated using the *ChemPlus* extension module (version 1.6) of *HyperChem* molecular modeling software. The calculation of the parameters was carried out as follows. The geometry of the molecules was first optimised (energy minimization) using molecular mechanics force field (MM+) method. Further energy minimization was done by quantum chemical calculations using the M1 semi-empirical method affording atomic charges of the molecule. Table 2.2 also shows the calculated refractivity, polarizability, the surface area and the volume of the molecules. Table 2.2 also shows the calculated charges on the phenolic or alcoholic oxygen atoms and on other relevant heteroatoms thought to be involved in interactions at the binding (receptor) site.

In the present work no attempt was made to analyse in molecular terms the structural characteristics responsible for the attractant properties of the known odours. Such an analysis appears futile because it is difficult to measure and quantify the complex nature of the behavioural responses in the field for large number of compounds. A cursory qualitative analysis of the data fails to reveal any simple correlation between either of the parameters and the known attractivity. It is also difficult to interpret the inactivity or inhibitory activity of some close phenolic analogues based merely on these values (for illustrative examples, such as 2-methoxyphenol, see Ref. [16]). Clearly, subtle structural changes affect the biological properties of the compounds profoundly and this is similar to what was observed for the well-studied insect sex pheromones. This indicates rather strict structural requirements for attractivity. Moreover, while many tsetse species respond, at least in electrophysiological experiments, to standard tsetse kairomones such as acetone, octenol, and certain phenols, important behavioural differences between species exist. Apparently, odours specific to a given host animal are significant for the species feeding on it (separate receptor evolved?) while reception of general tsetse attractants is also conserved. Phenols and octenol appear to be the general odours.

Compared to field experiments, for structure-behavioural activity relationship studies the laboratory investigation of well-defined behavioural responses readily observable in wind tunnel is more promising. Faster yet still simple are electroantennographic (EAG) studies using antennae of the given *Glossina* species, especially for screening a large number of candidate attractants ("high-throughput screening"). In fact, there have been several attempts to develop receptor models base on EAG responses of tsetse to analogues of phenols [55, 56, 57, 58] and octenol [59, 60, 61]. However, translation of the laboratory results (i.e., prediction of behavioural responses based on EAG responses) to attractivity in the field is not straightforward.

Table 2.1. Some physicochemical properties of known* and new candidate tsetse attractants and related compounds.

Compound	CAS Registry Number	Molecular Formula	Molecular Weight	Density g/ml	Refractivity index n_D/at ^oC	Boiling point ^oC/mmHg	Melting point ^oC
Acetone	67-64-1	C_3H_6O	58.08	0.791	1.3590/20	56	–94
Phenol*	108-95-2	C_6H_6O	94.11	1.071	1.5425/41	182	40–42
o-Cresol*	95-48-7	C_7H_8O	108.14	1.027	1.5361/20	191/760	32–34
m-Cresol*	108-39-4	C_7H_8O	108.14	1.034	1.5438/20	203/760	8–10
p-Cresol*	106-44-5	C_7H_8O	108.14	1.018	1.5312/20	202/760	32–34
2-Ethylphenol*	90-00-6	$C_8H_{10}O$	122.17	1.037	1.5360/20	196/760	–18
3-Ethylphenol*	620-17-7	$C_8H_{10}O$	122.17	1.001	1.5340/20	214/760	–4
4-Ethylphenol*	123-07-9	$C_8H_{10}O$	122.17		1.5328/20	218/760	42–45
2-*n*-Propylphenol*	644-35-9	$C_9H_{12}O$	136.19	0.989	1.5280/20	225/760	
3-*n*-Propylphenol*	621-27-2	$C_9H_{12}O$	136.19	0.988	1.5236/25	109/15	26
4-*n*-Propylphenol*	644-56-7	$C_9H_{12}O$	136.19	0.983	1.5230/20	232/760	22
2-*i*-Propylphenol*	88-69-7	$C_9H_{12}O$	136.19	1.012	1.5260/20	212/760	15–16
3-*i*-Propylphenol*	618-45-1	$C_9H_{12}O$	136.19	0.994	1.5250/20	228/760	25
4-*i*-Propylphenol*	99-89-8	$C_9H_{12}O$	136.19		1.5528/20	212/760	59–61
3-(2-Butyl)phenol	3522-86-9	$C_{10}H_{14}O$	150.22	0.975	1.5200/25	228/760	
2-Methoxyphenol*	90-05-1	$C_7H_8O_2$	124.14	1.129	1.5430/20	205/760	27–29
3-Methoxyphenol*	150-19-6	$C_7H_8O_2$	124.14	1.131	1.5520/20	114/5	<-17.5
4-Methoxyphenol*	150-76-5	$C_7H_8O_2$	124.14			243/760	55–57
5-*n*-Propylresorcinol	500-49-2	$C_9H_{12}O_2$	152.19			169/8	84–88
3-*n*-Propylthiophenol		$C_9H_{12}S$	152.25				
3-*n*-Propylfluorobenzene	28593-12-6	$C_9H_{11}F$	138.18				
3-*n*-Propylpyridine	4673-31-8	$C_8H_{11}N$	121.18	0.920	1.4949/25	185/760	
3-*n*-Propylaniline	2524-81-4	$C_9H_{13}N$	135.21		1.5408/25	107/2	
Indole	120-72-9	C_8H_6N	117.15			253/760	52–54
3-Methylindole (skatole)	83-34-1	C_9H_9N	131.18			265/755	95–96
4-Methylindole	16096-32-5	C_9H_9N	131.18	1.062	1.6060/20	267	5
5-Methylindole	614-96-0	C_9H_9N	131.18				60–62
6-Methylindole	3420-02-8	C_9H_9N	131.18	1.059	1.6070/20	112/5	
4-*n*-Propylindole		$C_{11}H_{13}N$	159.23				
Tetrahydro-1-naphthol	529-35-1	$C_{10}H_{12}O$	148.20			265/705	69–71
1-Naphthol	90-15-3	$C_{10}H_8O$	144.17			279/760	95–96
2-Naphthol	135-19-3	$C_{10}H_8O$	144.20			286/760	123
2-*n*-Propylnaphthalene	2027-19-2	$C_{13}H_{14}$	170.26				

*Naturally occurring cattle odours and related compounds as listed by Bursell et al. [16].

(continued)

Table 2.1 (continued)..

Compound	CAS Registry Number	Molecular Formula	Molecular Weight	Density g/ml	Refractivity index n_D/at ^oC	Boiling point ^oC/mmHg	Melting point ^oC
3-Methylindole (skatole)	83-34-1	C_9H_9N	131.18			265/755	95–96
rac. 1-Octen-3-ol	3391-86-4	$C_8H_{16}O$	128.21	0.830	1.4370/20	84-85/20	
(+)-1-Octen-3-ol	24587-53-9	$C_8H_{16}O$	128.21	0.830			
(–)-1-Octen-3-ol	3687-48-7	$C_8H_{16}O$	128.21	0.830			
rac. 1-Octen-3-thiol	61758-08-5	$C_8H_{16}S$	144.28				
rac. 1-Octyn-3-ol	818-72-4	$C_8H_{14}O$	126.20	0.864	1.4410/20	83/19	
3-*n*-Propylcyclopentanol	101567-67-3	$C_8H_{16}O$	128.21		1.4528/20	182/760	
3-*n*-Propylcyclohexanol	20558-10-5	$C_9H_{18}O$	142.24		1.4646/19	75-76/0.6	
Decanal	112-31-2	$C_{10}H_{20}O$	156.27	0.830	1.4280/20	208/760	
Dodecanal	112-54-9	$C_{12}H_{24}O$	184.32	0.835	1.4344/20	185/100	
Decanitrile	1975-78-6	$C_{10}H_{19}N$	153.27	0.818	1.4290/20	242/760	
Dodecanitrile	2437-25-4	$C_{12}H_{23}N$	181.32	0.827	1.4360/20	198/100	
Octyl formate	112-32-3	$C_9H_{18}O_2$	158.24	0.874	1.4178/22	77/15	–39
Decyl formate	5451-52-5	$C_{11}H_{22}O_2$	186.29	0.867	1.4262/22	167/15	

*Naturally occurring cattle odours and related compounds as listed by Bursell et al. [16].

Table 2.2. Compilation of some physicochemical and electronic properties of known and new tsetse attractants and related compounds.

Compound	Refractivity	Polarizability	Lipophilicity LogP		Surface area	Volume	Charge on *O*-atom
	calc., Å^3	calc., Å^3	meas.[a]	calc.[b]	calc., Å^2	calc., Å^3	AM1 method
Acetone	16.19	6.37	–0.24	0.38	210.48	276.87	–0.301
Phenol	27.75	11.07	1.46	1.76	252.08	353.92	–0.285
o-Cresol	32.79	12.91	1.95	2.23	275.43	401.52	–0.287
m-Cresol	32.79	12.91	1.96	2.23	278.56	406.48	–0.285
p-Cresol	32.79	12.91	1.94	2.23	280.56	406.90	–0.285
2-Ethylphenol	37.39	14.74	2.47	2.63	302.87	451.24	–0.288
3-Ethylphenol	37.39	14.74		2.63	305.36	457.32	–0.285
4-Ethylphenol	37.39	14.74	2.58	2.63	305.16	456.99	–0.285
2-*n*–Propylphenol	42.00	16.58	2.93	3.02	332.15	505.55	-0.289
3-*n*-Propylphenol	42.00	16.58		3.02	338.73	511.85	–0.285
4-*n*-Propylphenol	42.00	16.58	3.20	3.02	335.05	512.01	–0.285
2-*i*-Propylphenol	41.94	16.58	2.88	2.96	323.13	497.20	–0.290
3-*i*-Propylphenol	41.94	16.58		2.96	332.20	506.08	–0.285
4-*i*-Propylphenol	41.94	16.58	2.90	2.96	331.80	505.84	–0.285
3-(2-Butyl)phenol	46.54	18.41		3.35	355.76	550.76	–0.253
2-Methoxyphenol	34.22	13.54	1.32	1.51	291.26	429.14	–0.287
3-Methoxyphenol	34.22	13.54	1.58	1.51	294.27	431.69	–0.280
4-Methoxyphenol	34.22	13.54		1.51	293.65	431.69	–0.285
5-*n*-Propylresorcinol	43.69	17.21		2.74	344.82	533.69	–0.249
3-*n*-Propylthiophenol	48.41	18.94		3.26	359.32	548.00	–0.002
3-*n*-Propylfluorobenzene	40.52	15.85		3.45	328.41	498.49	0.137[c]
3-*n*-Propylpyridine	38.36	15.14		2.13	322.91	485.38	–0.113[d]
3-*n*-Propylaniline	45.00	17.29		2.52	341.96	523.26	–0.416[d]
Indole	37.14	15.70	2.14	1.82	283.67	415.35	–0.089[d]
3-Methylindole	42.19	17.54	2.60	2.29	306.71	466.40	–0.087[d]
4-Methylindole	42.19	17.54		2.29	309.74	465.06	–0.088[d]
5-Methylindole	42.19	17.54	2.68	2.29	312.44	468.20	–0.089[d]
6-Methylindole	42.19	17.54		2.29	309.70	468.42	–0.089[d]
4-*n*-Propylindole	51.39	21.21		3.08	362.49	567.70	–0.089[d]
Tetrahydro-1-naphthol	45.23	17.64		2.99	326.57	507.26	–0.288
1-Naphthol	44.20	18.34	2.84	2.76	306.60	473.62	–0.282
2-Naphthol	44.20	18.34	2.70	2.76	310.87	479.66	–0.283
2-*n*-Propylnaphthalene	56.75	23.21		4.31	384.40	612.74	not applicable

[a]Taken from Hansch et al. [54].

[b]Calculated values were obtained by the QSAR module of *HyperChem 5.01* program.

[c]Charge on the F-atom; [d]Charge on the N-atom; [e]Charge on the S-atom.

(continued)

Table 2.2. (continued).

Compound	Refractivity	Polarizability	Lipophilicity LogP		Surface area	Volume	Charge on *O*-atom
	calc., Å^3	calc., Å^3	meas.[a]	calc.[b]	calc., Å^2	calc., Å^3	AM1 method
rac. 1-Octen-3-ol	40.17	15.9		2.55	363.03	544.02	–0.330
rac. 1-Octen-3-thiol	46.30	18.26		3.13	381.52	579.18	–0.060[e]
*rac.*1-Octyn-3-ol	38.59	14.99		2.08	358.56	533.68	–0.324
3-*n*-Propylcyclopentanol	38.42	15.32		2.05	340.77	519.97	–0.328
3-*n*-Propylcyclohexanol	43.03	17.15		2.44	358.41	557.78	–0.331
Decanal	48.55	19.21		2.82	427.15	659.05	–0.291
Dodecanal	57.75	22.88		3.62	489.88	767.76	–0.291
Decanitrile	48.66	19.14		3.89	428.03	658.29	–0.051[d]
Dodecanitrile	57.86	22.81		4.68	490.42	767.22	–0.051[d]
Octyl formate	45.62	18.01		2.45	416.96	634.00	–0.297
Decyl formate	54.82	21.68		3.24	478.54	742.50	–0.297

[a]Taken from Hansch et al. [54].

[b]Calculated values were obtained by the QSAR module of *HyperChem* 5.01 program.

[c]Charge on the F-atom; [d]Charge on the N-atom; [e]Charge on the S-atom.

2.3. Studies on the dispenser formulations of known and novel tsetse attractants

Evaporation rates and degradation

The longevity of the polyethylene (PE) sachets loaded with the individual attractants used in the field was estimated under laboratory conditions. The sachets were made of commercial PE foil available in Hungary (thickness: 0.15 mm). The area available for release of the odours from the thermo-sealed sachet was 4 × 4 cm (total surface area: 32 cm^2) and ca. 1 ml of the test substance was measured into the sachets. Sealing was done using a conventional double-wire household thermo-sealer (*Severin* bag sealer): one of the heated wires is already covered with a thin protective thermo-stable plastic while the other, originally for cutting the foil, was also covered with a 3–4 mm wide baking paper to provide a second seal.

The sachets were aged at two temperatures, first at 20°C and then at 30°C. Duplicate sachets were used and weight measurements were done every 24 h at 0.1 mg accuracy (five significant digits; *Precissa 240A* electronic analytical balance). After the first six 24 h-period at 20°C(±1°C) in the laboratory, the sachets were placed into a thermostat (50-litre thermostat cabinet from Labor MIM, Budapest) set to 30°C(±1°C) and the top vent was open. Weight changes were followed for further three days.

The evaporation rate data calculated from the weight changes are given in Table 2.3. The data show that in this formulation octyl formate, the compound most "volatile" of the formulations, can be expected to last for about ten days in the field if air temperature does not exceed 30°C. Sachets filled with the other test compounds should, generally, last longer. None of the chemicals caused any apparent damage to the PE material.

The relatively high release rates for the synthetic formates manifested by the “wetting” of the outer surface of the sachets indicating that this ester readily infuses into the PE foil. This infusion was even more pronounced for pine essential oils, which thus could not be formulated in this type of sachet (see later). This observation is important because it indicates that an appropriate container should be selected for any newly identified and chemically different odour.

Table 2.3. Evaporation rate of tsetse kairomones and their analogues from polyethylene sachets

Compound	Evaporation rate at 20°C		Evaporation rate at 30°C		Relative change
	(mg/day)	(mg/h)	(mg/day)	(mg/h)	20→30°C
Octenol	3	0.13	12	0.52	4.0
Decanal	8	0.35	16	0.66	1.9
Dodecanal	15	0.61	40	1.65	2.7
Decyl formate	29	1.20	73	3.06	2.5
Octyl formate	42	1.75	86	3.60	2.1
Decanitrile	10	0.40	31	1.28	3.2
Dodecanitrile	6	0.25	12	0.52	2.1
	Average evaporation rate increase at Δ10°C: × ***2.6***				

Our data also show that the evaporation rate for octenol at 30°C corresponds to that recommended for the *morsitans* group [62] but is much lower than recently used for *G. brevipalpis* and *G. austeni* in South Africa where the optimal release rates doses) of octenol were found to be 2.3–9.1 mg/h [63].

In another experiment, larger PE sachet (5.5 cm × 6.5 cm; total surface area: 71.5 cm^2) separately filled with 4.5 ml of the candidate volatiles (aldehydes, formates or octenol) were found to have about 1.5–2 times higher release rates at 20°C, reflecting roughly the 2.3-fold increase of the surface area of the sachets (data not shown).

Gas chromatographic-mass spectrometric analysis of aged "1:4:8" mixture in polyethylene sachets

A laboratory study was conducted to establish the long-term fate of the conventional PE-sachet formulation of the propylphenol + octenol + *p*-cresol 1:4:8 (by weight) mixture. Note that at ambient temperatures *p*-cresol of a solid (for physicochemical data, see Table 2.1) and when measuring it either can be weighed as such or must be melted in a hot water bath or using a heat gun. *Caution!* Use protective gloves and eye-ware when handling large amounts of propylphenol, *p*-cresol and *m*-cresol. Also, all work with these materials should be carried out in a well ventilated hood to avoid inhalation of the noxious vapours of these phenols.

The experiment was carried out using regular 4 cm × 4 cm PE sachets made of 0.15 mm thick PE foil and filled with 4.0 ml of the 1:4:8 mixture. The sachets were sealed and hung in the laboratory for 30 weeks (10 January 2001 to 7 August 2001; 210 days) at ambient temperature (22 to 26°C). During the experiment the sachets were exposed to natural and a conventional light of the laboratory during working hours. The weight of the sachets was measured at the start, occasionally during and at the termination of the experiment.

The release rate of the formulation was found to be between 0.4 and 5.0 mg/h. The lower value was obtained for the first 70-day period when the average temperature was around 22°C. A higher release rate was observed during the summer when ambient daytime temperature was ~26°C. This latter release rate approximates that described in the *Training Manual for Tsetse Control Personnel* (page 20 in Ref. [62]) where the respective values for *p*-cresol, octenol and propylphenol are 1.5, 0.2 and 0.5 mg/h are given. (Note, however, that the last two numbers should be reversed in the *Manual*, to reflect the ratio of the ingredients, i.e. there should be more octenol than propylphenol!). In the experiments, 4 cm × 4 cm sachets were used and the temperature was 22–26°C, while the *Manual* presumably refers to a larger (5 cm × 4 cm) sachet resulting in a proportionally higher release. However, the *Manual* does not give the temperature at which the above values, required for optimal attractivity, were obtained. Higher release rates are to be observed at higher temperature. As it was demonstrated in the previous experiment with individual synthetic odours, a 10°C temperature increase results in a 2.6-fold average increase in the release rate of the compounds. It must be noted that the PE sachet's release rate depends not only on the temperature but also on the chemical nature of its content. For tsetse trapping polar compounds having phenolic or alcoholic function have been used so far. (For a discussion of the infusion into PE of various volatile flavour compounds, see [64]).

The gas chromatographic-mass spectrometric (GC-MS) analysis of fresh and aged 1:4:8 mixtures showed essentially identical profiles (Fig. 2.5) without any new product in the aged sample. The peak ratio for the initial 1:4:8 odour mixture was 1:5.9:13.2, relative to propylphenol, while for the aged sample it was 1:3.6:7.5 (note that the response of the mass

spectrometric detector is different for the three compounds thus the peak area ratio observed does not correspond to 1:4:8). This results indicates that *p*-cresol and octenol evaporated somewhat faster than propylphenol, i.e. that during the two-month period, the sachet was enriched in the least volatile propylphenol to some extent.

According to the GC-MS analysis of the mixtures using a VG ZAB 2SEQ instrument in electron impact mode, no newly formed substance could be detected (<2%) in the total ion chromatogram of the mass spectrometer. Consequently, this experiment indicates that there was essentially no degradation or other chemical transformation of the sachet's content.

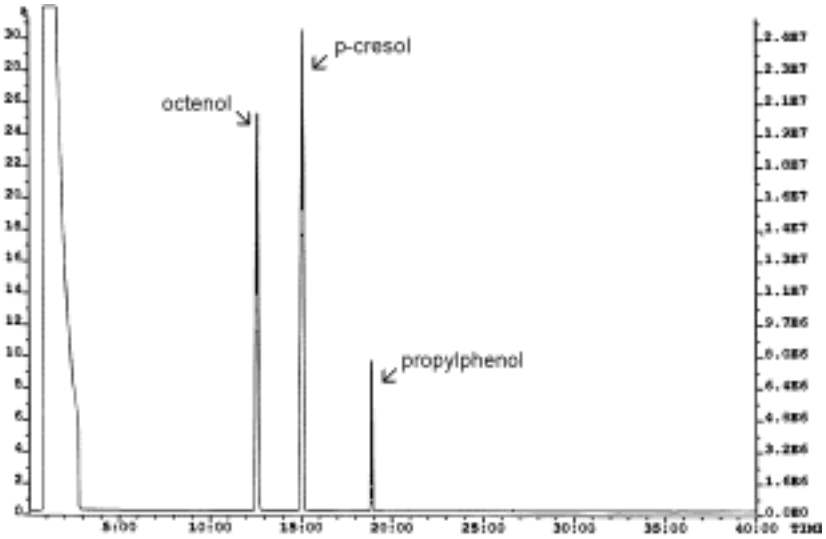


Figure 2.5. Gas chromatogram of laboratory aged 1:4:8 mixture of propylphenol + octenol + p-cresol. The samples were analysed by VG ZAB 2SEQ instrument (electron impact mode, total ion monitoring) using Rtx-5Amine capillary column (Restek Corp.; length: 30 m, id: 0.25 mm, film: 0.5 μm); initial temperature: 50°C for 5 min, ramp: 8°C/min, final temperature: 200°C).

Measurement of evaporation rates of two pine oils from polyethylene vials

In the laboratory of Dr. Patrick Guerin (University of Neuchâtel) it was recently discovered that several plant volatiles affect tsetse fly sensory physiology. Two commercially available natural terpene essential oil fractions, namely the pine needle oils of the Scots pine (*Pinus sylvestris* L.) and the Swiss mountain pine (*Pinus mugo* Turra var. *pumilio*, or *Pinus pumilionis*) induce EAG responses from antennae of *G. brevipalpis*. In order to conduct field studies to detect any behavioural responses to these natural product mixtures, technical information was needed for the formulation of these terpene-containing essential oils.

As mentioned before, the pine essential oils were found to diffuse readily through the PE sachet and soften it within minutes rendering such sachets inappropriate for dispensing these candidate attractant substances. Therefore, harder, capped polyethylene vials with a thicker wall were selected as dispensers. During the experiments none of the essential oils caused any apparent damage to the PE material of these vials. The longevity of these new attractant formulations was estimated under laboratory conditions. Commercial vials (Kartell, Italy, Art. No. 00731, size: 2.5 ml; wall thickness: 1 mm, total surface area available for evaporation,

including top and bottom of the cylindrical vial: 16 cm^2) were loaded with 1.5 ml of either *P. sylvestris* or *P. pumilionis* pine oils (1.3141 g and 1.3137 g, respectively). The essential oils used in this and subsequent field experiments were obtained from Aromex Ltd. (Budapest, Hungary). To estimate evaporation rates the loaded vials were aged at 25(±1.5)^oC. Duplicate vials were used for each oil and weight measurements of five significant digits (*Precissa 240A* electronic analytical balance) were done every 24 h. After 16 days, the vials were placed into a refrigerator (ca. –10^oC) and stored for gas chromatographic analysis. The results of the average net weight changes, calculated by subtracting the weight of the original empty vial from the total weight measured an intervals, were used to obtain release rates as shown in Table 2.4.

Table 2.4. Evaporation rate of candidate tsetse attractant pine oils (1.5 ml) from 2.5 ml polyethylene vials

Essential oil	Evaporation rate at 25^oC	
	mg/hour	mg/day
Pinus sylvestris	~1.9	45.3
Pinus pumilionis	~1.4	34.3

The data show that *P. sylvestris* oil is released at a somewhat higher rate than *P. pumilionis* oil. From these quantitative data it is expected that the particular vial containing 1.5 ml (~ 1.3 g) of the oils should last for about three weeks in the field at average temperatures around 25^oC.

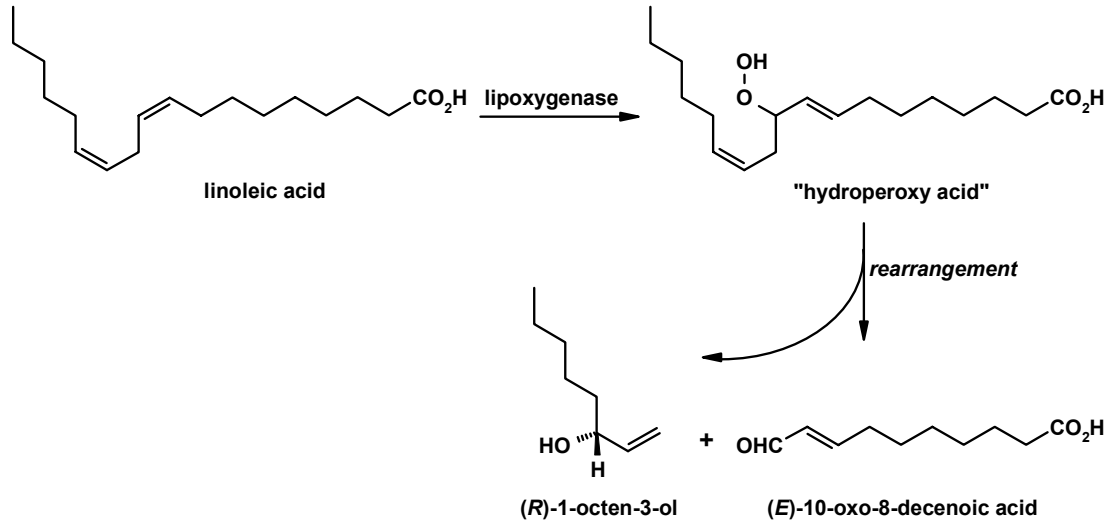
Several other studies [65, 66] as well as GC-MS analyses have shown that pine essential oils are distinct mixtures of over two dozens of different chemicals, mostly terpenoids. No attempt was made to analyse in detail the changes of the vial content during the experiment. (Cursory examination in the Budapest laboratory of the Solid Phase Micro Extraction (SPME) GC traces of the samples at the start and the end of the experiment no qualitative changes in the composition of either oil were seen but due to the complexity of the mixtures no quantitative evaluation was made.) However, it would be essential to monitor the change in the composition in more detail because it is important to know whether the bioactive constituents are released evenly and at the same rate as the other oil components because deviations could affect the *biological activity* "half-life" of the formulation (if, for example, the attractive components are released faster). Further studies in this respect are needed, especially when either of these oils finds application in tsetse control (see later).

2.4. Exploring alternative sources of known tsetse attractants

Currently synthetic octenol, which is a racemic mixture, is used in the field. Although this substance is readily available in bulk quantities, it is a relatively expensive component of several tsetse baits. Therefore it was decided to find alternative, preferably renewable sources for this important kairomone.

As discussed before, cattle release the *R*-stereoisomer of octenol. It is thought to be produced by microorganisms from fodder. However, the biochemical (metabolic) origin of octenol in cattle has not been studied. It is assumed that the biochemistry of octenol in cattle follows the pathway established for (*R*)-1-octen-3-ol (*matsutake* alcohol) in mushroom, where it is

produced enzymatically by oxidative breakdown of the common fatty acid, linoleic acid [(9*Z*,12*Z*)-9,12-octadecadienoic acid] [67, 68, 69]. The established biochemical transformations involved in the biosynthesis of (*R*)-1-octen-3-ol are shown in Scheme 2.9.



Scheme 2.9. The postulated biosynthesis of (*R*)-1-octen-3-ol from linoleic acid in ruminants follows that of in mushrooms.

It is also known that volatiles produced by oxidative and thermal decomposition of fatty meat and of vegetable cooking oils contain octenol in varying amounts. So it can be assumed that oxidative degradation of vegetable oils rich in linoleic acid glycerides would generate racemic octenol via a non-enzymatic oxidative route thus would provide this tsetse kairomone.

Linoleic acid is a major component of animal fats and milk and of the following vegetable oils [70] (content in %): maize (up to 60%), safflower (up to 80%), sesame (up to 50%), soya bean (up to 60%), pumpkin (up to 40%) sunflower oil (up to 70%), and cottonseed oil (up to 60%). These oils could be cheap sources of racemic octenol since literature data indicate that (auto)oxidation by air of lipids generates, along with other volatiles, octenol [71, 72]. The non-enzymatic oxidation follows a similar path to that shown above (Fig. 2.9) except that racemic octenol is formed.

Therefore the possible release of octenol in laboratory settings from methyl linoleate, a simple model ester of natural glycerides, was examined. The linoleate ester was exposed to oxygen gas in a closed glass vial at 20–24^{o}C for 6 days then the volatile decomposition products in the headspace were analysed using GC-MS analysis.

Solid-phase micro-extraction (SPME) used a Finnigan MAT GCQ instrument using Chrompack CP SIL 5 capillary column (length: 30 m, ID: 0.25 mm; film thickness: 0.25 μm) as described earlier [73]. Electronimpact (EI^+) mass spectra were taken at 70 eV ionization energy by full scan mode in 10-650 amu mass range with 0.5 s/scan velocity and acquisition threshold = 0. The temperatures of the ion source and of the transfer line were 200^{o}C and 195^{o}C, respectively. Detected components were identified by matching EI^+ mass spectra against the NIST library containing about 100,000 compounds. Samples were inserted into the

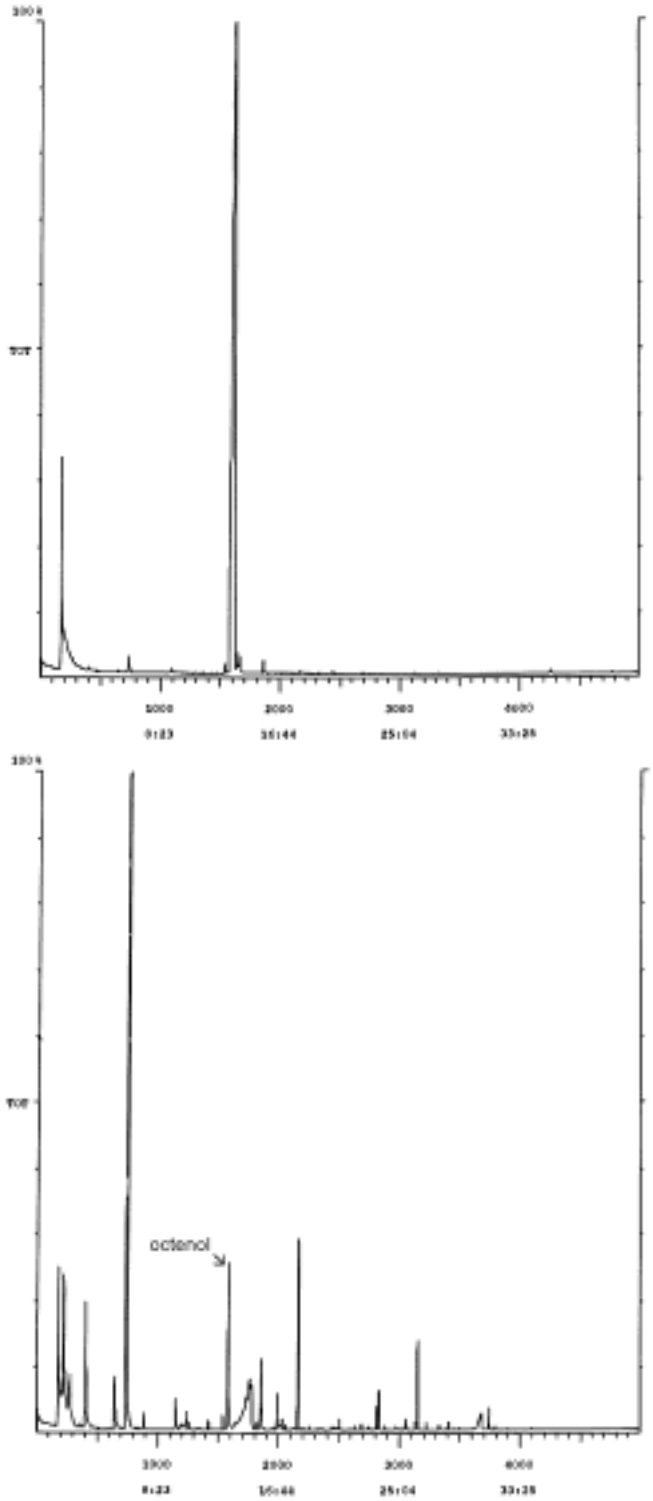


Figure 2.6 Chromatograms of solid phase micro-extraction GC-MS analysis of volatiles in headspace of octenol standard (upper) and O_2-exposed methyl linoleate (lower). Arrow indicates octenol, identified by its retention time and mass spectrum, as one of the oxidative degradation products of methyl linoleate.

injection port by HS-SPME method using 100 μm poly(dimethylsiloxane) fibre (SUPELCO). Optimal conditions were: 20 min sorption time at 40°C from a 3.5 ml closed glass vial containing 0.10 ml of test substrate. A narrow (0.75 mm ID) inlet liner was applied in order to detect fairly sharp and well-defined peaks. Desorption time was one min at 220°C. After every run the SPME fibre was conditioned for 20 min at 300°C in the injector of the GC followed by a blank run. Column temperature setting was programmed from 40°C (hold for 5 min), with 5°C/min increase rate up to 160°C (hold for 10 min), followed by a 20°C/min ramp to 210°C. The carrier gas was helium (35 cm/s linear velocity). Split injection was used.

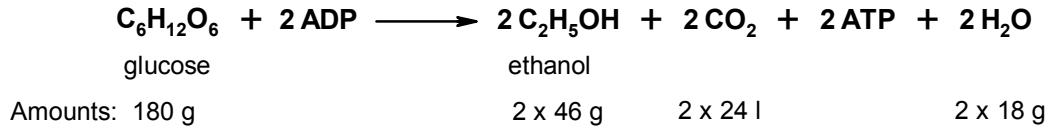
The results indicated the presence of over a dozen resolved volatiles (Fig. 2.6) (for experimental details, see [36]). Comparing the mass spectra of the octenol standard and that of the peak with the same retention time (13 min 34 s) from the linoleate sample kept under an oxygen atmosphere, octenol was identified in the linoleate headspace. Although this experiment used methyl linoleate as a model, the results indicate that this tsetse attractant, together with other volatiles, could be released upon aerial oxidation of vegetable oils rich in linoleic acid. Nevertheless, detailed field studies are required to establish the practical usefulness of the locally available vegetable oils as cheap octenol sources in the field.

Considerations of potential sources of carbon dioxide, a neglected kairomone

Carbon dioxide is a major stimulant and attractant component of cattle odour and it was estimated that an average steer emits 2.35 l/min CO_2 [48, 74]. On the other hand, for *G. morsitans morsitans*, artificially released CO_2 at doses of 2.5 to 15 l/min enhanced the catches of baits by two to six times [75]. Consequently, to be effective about 2 l/min CO_2 is required in the vicinity of traps.

For sake of simplicity the above amount of CO_2 corresponds to roughly 0.1 mol/min, which is 144 mol/day. Considering cheap carbonate salts as CO_2 sources, this calculation gives a daily requirement of ca. 12 kg $NaHCO_3$, or 15.2 kg anhydrous Na_2CO_3, or 14.1 kg $CaCO_3$ per trap. On a monthly basis, these amounts correspond to 360 – 450 kg salt. To these carbonates one should add an acid, either in solid or liquid form, for the slow liberation of the gaseous attractant. Obviously, this purely chemical approach is not only impractical but also unsustainable in large-scale tsetse trapping campaigns. Other, preferably renewable CO_2 sources should be sought.

Microorganisms have long been known to ferment carbohydrates and produce CO_2. Recently, specially formulated yeast, such as commercially available alginate-encapsulated yeast cell (see, for example, [76]), has been recommended to serve as a point source of CO_2. Nevertheless, the amount of sugar, serving as a feedstock according to the equation (Scheme 2.10), is still substantial and the fermentation process poses technical difficulties (e.g., providing oxygen and energy for the microorganism and regulating fermentation), which, however could be less formidable than problems of the inorganic chemistry mentioned.



Scheme 2.10. Aerobic alcoholic fermentation accompanied by respiration produces two mole ethanol and two mole of CO_2 from one mole glucose.

Another possibility, and perhaps more pertinent to the actual problem, is the use of living organisms inhabiting the area where tsetse is present. Potential CO_2-producing animals could be ants and termites. There are several studies in the literature of gas-production of arthropods. For example, recently CO_2-release data for the neotropical termite species, *Coptotermes formosanus* Shiraki, preferring high temperatures (28°C) and the temperate species *Reticulitermes flavipes* Kollar, surviving best at lower temperatures (22°C) was quantitatively analysed [77]. According to respirometric measurements, a single *C. formosanus* termite releases roughly 2 µl CO_2 per hour, corresponding to 0.033 µl/min. Accordingly at least 1 million termite, serving as "point sources", would release altogether ca. 33 ml CO_2 per minute which is still sixty-fold less than the dose established for a noticeable increase in tsetse trap captures. Nevertheless, it would be worthwhile to place traps near mounds built by subterranean termites.

Finally, it should also be mentioned that in the laboratory studies dichloromethane, which has been reported to be a mimic of CO_2 for corn rootworm larvae [78], is inactive in EAG studies for some *Glossina* species thus could be used as a solvent for EAG studies with tsetse (Patrick Guerin, personal communication).

2.5. Conclusions

- A method was developed and optimized for the large-scale synthesis of propylphenol and produced this kairomone on a kilogram scale.

- Based on the structure of know tsetse kairomones and molecular modelling experiments a series of novel aliphatic, cycloaliphatic and aromatic kairomone analogues were designed and prepared for laboratory and field studies.

- A simple method for the laboratory scale synthesis of the two stereoisomers of octenol of high enantiomeric purity was developed.

- The release rate of several experimental odours from polyethylene sachet and vial formulations was determined. Stability studies in the laboratory of the regular "1:4:8" sachet formulation established that the composition of the blend did not change significantly during 30 weeks.

- Based on literature data and the developed laboratory model studies, recommendations were made for detailed field-testing of linoleic acid-containing vegetable oils that, upon oxidative decomposition, could serve as inexpensive local octenol sources.

3. LABORATORY BIOASSAYS: ELECTROPHYSIOLOGICAL AND WIND TUNNEL EXPERIMENTS WITH KNOWN AND NEW CANDIDATE KAIROMONES

3.1. Electroantennographic studies with known and new candidate kairomones

Because field trapping experiments are laborious and time consuming appropriate laboratory studies could provide useful information on the olfactory sensitivity of tsetse flies to odours. Electroantennography (EAG) has been shown to be a rapid and powerful technique to pre-screen synthetic and natural compounds, as potential attractants. Because tsetse EAG responses have been shown to correlate with fly behaviour [46, 59], electrophysiology a promising laboratory method to examine the effect of candidate odours for less studied ("problem") tsetse species.

Recently, structure-activity relationship studies have been carried out with simple analogues of 1-octen-3-ol [60, 61]. Based on behavioural and EAG experiments with *G. morsitans morsitans* and *G. pallidipes* using phenolic host odour components, a binding site model has also been proposed [56]. Nevertheless, the role of the individual odour components is not quite clear and there is a need for improvement of cost-effective traps for several tsetse species. Experiments also indicate the presence of an unidentified attractant in ox odour [74]. Thus a "chemical approach" was taken to find new structural analogues of the presently known kairomones that could either be attractive by themselves or complement (synergise) the odours currently used in baits. The chemical structures of the analogues tested in electrophysiological experiments are shown in Fig. 2.1.

Materials and Methods

The chemical purity of the newly synthesized compounds was at least 95% as established by ^{1}H NMR and/or GC. Commercially obtained test substances were used as received and, in general had at least 95% purity, as stated by the supplier.

The EAG experiments were carried out in the Laboratory of Sensory Physiology, Institute of Zoology, University of Neuchâtel. Pupae of *G. brevipalpis* and G. *pallidipes* for their respective colonies were provided by the Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria, while pupae of *G. palpalis gambiensis* were obtained from CIRDES, Bobo-Dioulasso, Burkina Faso. After emergence, the flies were fed every second day on a silicone membrane with bovine blood, and held in a climate room.

In general, the methodology of the electrophysiological study was carried out as described earlier [79]. Briefly, for recordings from the antennae, flies (both males and females) were first anaesthetized with CO_2 then the head was cut off. The EAG potentials were recorded with glass capillary electrodes connected to a high impedance preamplifier. Both reference and recording electrodes were filled with 0.1 M KCl solution. The test chemicals were dissolved in dichloromethane and 10 µl aliquots of the test solutions (1 µg/µl) were applied to filter paper strips that were then enclosed in a 5 ml polypropylene syringe with the needle connected to a glass tube that blew purified and humid air (90% RH) over the antenna. One ml of the stimulus syringe volume was injected in 1 s into the main air stream (ca. 28 ml/s) flowing over the preparation. Controls were made using solvent only. The insects used were 3–5 days old. When needed, responses were normalized with respect to racemic octenol (100%) as follows: $[(R_t - R_c)/(R_o - R_c)] \times 100$, where R_t = response to the compound tested; R_o = response to octenol; R_c = response to the control (dichloromethane solvent).

In some cases electrophysiological responses were also analysed using gas chromatography-coupled electroantennographic detector (GC-EAD) technique using female antennae of *G. brevipalpis*. The GC column used was a Permabond-CW20M (Macherey-Nagel), the carrier gas was H_2, the temperature programme was: 40°C for 0 min, then 8°C/min to 230°C. One µl aliquots with 50 ng of compound were injected.

Results

The results of EAG studies with *G. brevipalpis* using a single dose of 10 µg of the test compound as described above are shown in Tables 3.1 and 3.2. (For chemical structures, see Fig. 2.1.) This species was most sensitive to octenol, which was used as a standard in the experiments.

Table 3.1. Normalized electroantennographic responses of *G. brevipalpis* to aliphatic compounds. Responses are normalized racemic octenol (n = 4, both sexes)

Compound	Mean EAG response (%)
Host odour components	
racemic Octenol	100
p-Cresol	91
Propylphenol	36
Candidate attractants	
Methyl sulfide	0
Dimethyl sulfoxide	0
Methane[a]	0
racemic Linalool	10
racemic 1-Octen-3-thiol	21[b]
3-n-Propylcyclopentanol	54
3-n-Propylcyclohexanol	25
Decanal	38
Dodecanal	13
Octyl formate	44
Decyl formate	53
Decanitrile	50
Dodecanitrile	19

[a]Methane was released from a cylinder at ca. 500 ppm concentration.

[b]After the assay with this thiol the antennae were not responsive to other odours.

Table 3.1 shows the results of the studies with naturally occurring kairomones and their aliphatic analogues. Methane, a microbial fermentation product emitted by pigs [80, 81, 82] was found to be essentially inactive in the EAG study. It was tested based on the assumption that methane gas may be the elusive attractant component of ox odour [74]. Methyl sulfide

and methyl sulfoxide were included for similar reasons and because of their structural similarity to acetone. Linalool, a common volatile terpene component of various plants, evoked much weaker responses than the structurally related octenol, which is a non-terpene allylic alcohol. The two alicyclic 3-*n*-propyl-cycloalkanols, each tested as a mixture of stereoisomers as obtained by synthesis, were EAG active indicating that combining the structural features of octenol and propylphenol (Fig. 2.2) might lead to novel attractants.

Decanal and dodecanal, two homologous aldehydes, proposed as kairomones of lizards [38] and recently of buffalo and ox [39] elicited some EAG responses. Their formate and nitrile mimics were somewhat more active in this assay. These laboratory results, predicting behavioural activity, strongly suggested the inclusion of these inexpensive compounds in field trapping studies.

The two alicyclic alcohols, each being a mixture of four possible stereoisomers, were studied by GC-EAD technique also. For 3-*n*-propylcyclopentanol, both the major (93%) and minor constituent (7%) of the sample was active. For the stereoisomeric mixture of 3-*n*-propylcyclohexanol, only the major component with a longer retention time (the *cis* isomer) of the sample solution was active. Representative GC-EAD traces for this latter compound are shown in Fig. 3.1.

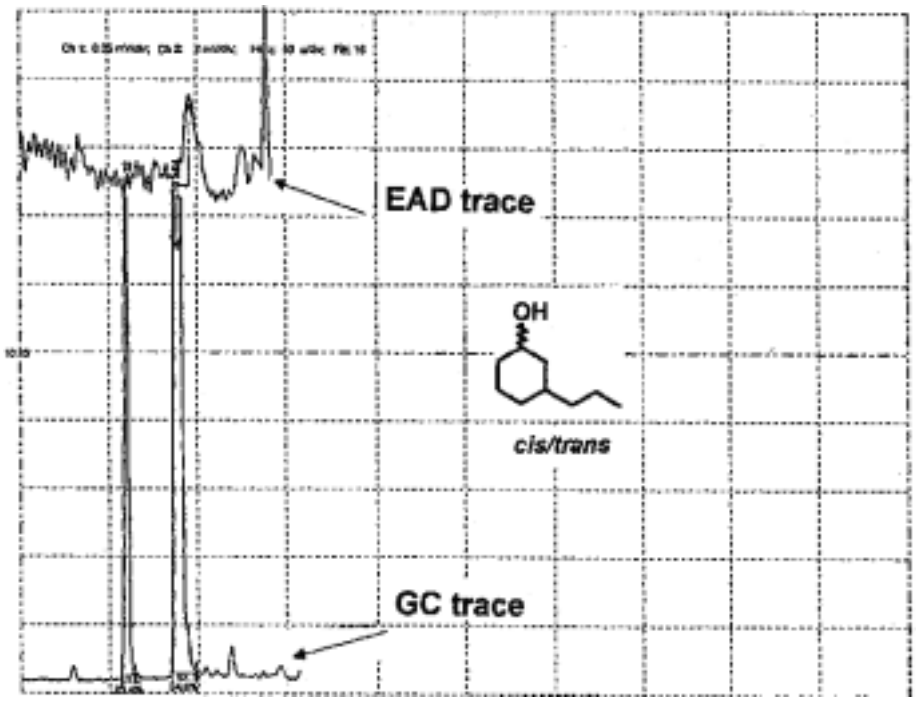


Figure 3.1. GC-EAD analysis of the diastereomeric mixture of 3-n-propylcyclohexanol using G. brevipalpis antenna as detector. Note that only the major (cis) isomer elicits electrophysiological response (EAD trace).

Table 3.2 shows the results of the studies with naturally occurring kairomones, the enantiomer pairs of octenol and its alkyne analogue as well as some other compounds. In this assay, no important difference was found between the synthetic stereoisomers of octenol. It is interesting that the reference (*R*)-enantiomer of octenol, obtained from a fragrance company, was somewhat more active in eliciting EAG responses. It is also noteworthy that both

commercially available 1-octyn-3-ol stereoisomers stimulate the antennae of this species. Earlier, Saini et al. [60] reported that 1-octen-3-one and racemic 1-octyn-3-ol evoked EAG responses comparable to racemic octenol for *G. morsitans morsitans*. It should be noted that 1-octen-3-one, as an α,β-unsaturated ketone, is capable of reacting with –SH or –NH nucleophiles at protein binding sites [83].

Table 3.2. Normalized electroantennographic responses of *G. brevipalpis* to various compounds using commercial (R)-1-octen-3-ol as standard (n = 4 of each sex)

Compound	Mean EAG response (%)
(*R*)-1-Octen-3-ol†	100
p-Cresol	91
Propylphenol	36
(*R*)-1-Octen-3-ol*	75
(*S*)-1-Octen-3-ol*	79
1-Octen-3-one	56
(*R*)-1-Octyn-3-ol	50
(*S*)-1-Octyn-3-ol	52
Skatole (3-methylindole)	45
Linoleic acid	25
Methyl linoleate	14

†Commercial (Robertet S.A., Grasse, France) product used as reference.
*Synthesized in this study.

Table 3.3. Normalized electroantennographic responses of *G. brevipalpis* to aromatic compounds. Responses are normalized to octenol. (n = 4, both sexes).

Compound	Mean EAG response (%)
Host odour components	
racemic Octenol	100
p-Cresol	92
Propylphenol	27
Kairomone analogues	
racemic 3-(2-Butyl)phenol	27*
3-*n*-Propylthiophenol	0
3-*n*-Propylfluorobenzene	0
3-*n*-Propylpyridine	2
1-Naphthol	0
2-Naphthol	0
5,6,7,8-Tetrahydro-2-naphthol	0
2-*n*-Propylnaphthalene	0
5-*n*-Propylresorcinol	21

*Activity is mostly due to trace contaminant. See text for explanation.

Among the newly tested odour candidates, skatole, a common tryptophan metabolite occurring in several plants and in animal excretions including faeces, also produced substantial EAG activity. Linoleic acid and its methyl ester, included as potential "slow-release" precursors of octenol, also elicited some EAG activity but whether this is due to possible degradation producing octenol has not been examined.

Table 3.3 shows the results of EAG studies with naturally occurring kairomones and some of their aromatic analogues. Only the branched chain 2-butylphenol derivative, previously reported to be weakly active in EAG tests with *G. pallidipes* [84], showed significant activity. Disappointingly, of the other analogues only the propylpyridine derivative showed measurable activity.

GC-EAD analysis allowed the separation of trace contaminants present in some synthetic compounds. In the case of 3-(2-butyl)phenol, the observed activity was found to be associated with a minor constituent. This contaminant is thought to be a known kairomone, 3-methylphenol (or *m*-cresol), a synthetic by-product arising from the reduction of residual starting material 3-hydroxybenzaldehyde contaminating the hydroxypropylphenol intermediate (see Scheme 2.1).

In further EAG studies, *G. pallidipes* antennae were used. Since indoles can be considered as bioisosteres of phenols, a series of methylindoles were tested in the laboratory using to see whether they could mimic cresols. The tryptophan metabolite 3-methylindole (skatole), occurring frequently in animal excretions as well as in plant volatiles, was already found EAG-active for *G. brevipalpis* antennae (see Table 3.2).

Results of dose–response studies with *G. pallidipes* antennae are shown in Figs. 3.2 and 3.3.

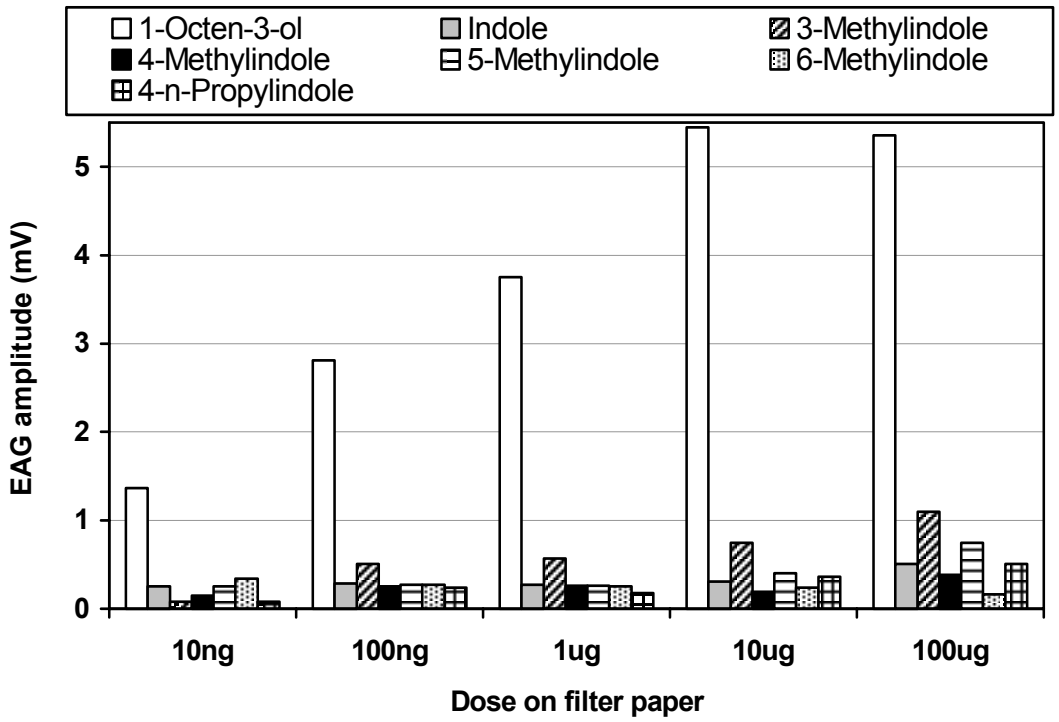


Figure 3.2. Dose dependence of EAG responses of G. pallidipes (3 females and 3 males, each 4–5 days old) antennae to various indoles. Dose range: 0.01 μg – 100 μg.

It was noted that antennae of *G. brevipalpis* were more sensitive to octenol than to phenolic kairomones and this compound was used as a reference standard in these experiments as well.

Figure 3.2 shows the dose-EAG response curves for a series of alkylindoles relative to octenol. Of the methylindoles tested, only 3-methylindole and, to some extent, 5-methylindole show EAG activity albeit the elicited dose-dependent responses are much weaker than that of the reference compound.

Figure 3.3 shows the dose-EAG response curves for octenol, propylphenol and 3-*n*-propylanilin, the latter being an aniline analogue of the phenolic kairomone. In this dose range the EAG responses to propylphenol for this species are about 10% to 15% that of octenol while propylaniline, even at the highest does tested, displayed only marginal activity.

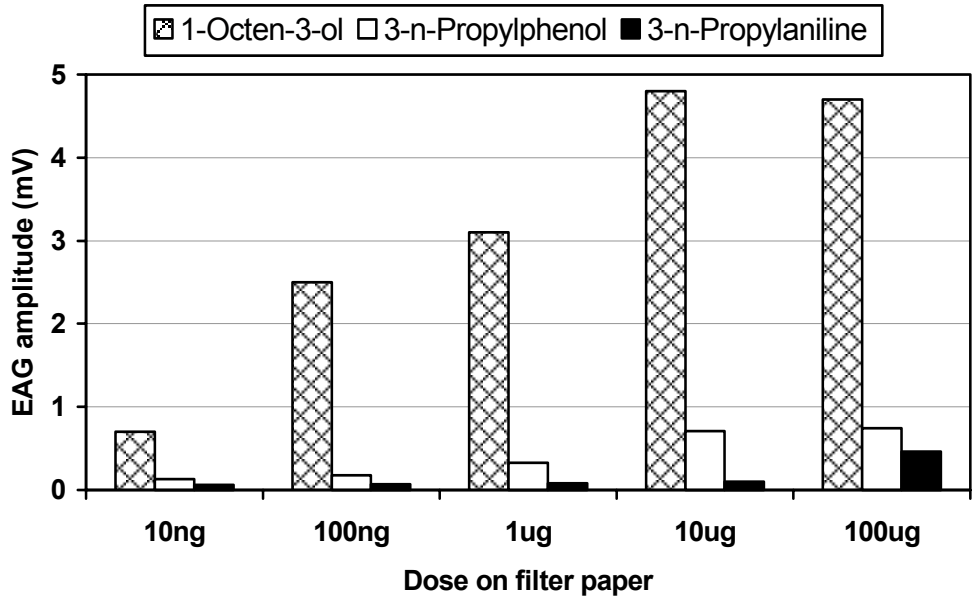


Figure 3.3. Dose dependence of EAG responses of G. pallidipes (3 females and 1 male; each 4–5 days old) antennae to different odours. Dose range: 0.01 μg – 100 μg.

Next the results of comparative EAG studies with *G. pallidipes* and *G. palpalis gambiensis* antennae are described. Pupae of *G. pallidipes* and *G. palpalis gambiensis* were provided to the Institute of Zoology, University of Neuchâtel, by the Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, and by CIRDES, Bobo-Dioulasso, respectively. After emergence, the flies were fed every second day on a silicone membrane with bovine blood, and held in a climate room.

The experiments were carried out at the Laboratory of Sensory Physiology, Institute of Zoology, University of Neuchâtel, as described above. Four flies were used per species, i.e. 2 per sex and 2 records were made from each fly, giving 8 antennograms per species.

The following materials were tested: the known tsetse attractants propylphenol, *p*-cresol, octenol and acetone (the components of POCA), the essential oils of *Pinus sylvestris* (Scots pine) (Aromex Ltd., Budapest, Hungary), and volatiles emanating from fresh leaves of *Pinus*

mugo var. *unicata* (Swiss mountain pine; collected in Switzerland). Dichloromethane (solvent) and blank filter paper were used as controls.

The EAG results, normalized to solvent control, are shown in Fig. 3.4. The known attractant octenol caused a better reaction for both species at all doses tested: amplitude increased with dose, and at 1 µg the increase was 5 times the control for *G. pallidipes* and 3 times for *G. palpalis gambiensis*.

The essential oil of *P. sylvestris* at low doses (<50 µg) elicited low EAG responses in both species. At the dose of 50 µg, the oil caused a 4 times greater response than the control for *G. palpalis* and twice that of the control for *G. pallidipes*.

With *p*-cresol, the increase of EAG response relative to solvent control was more than 4 times at all the doses tested for *G. pallidipes*. For *G. palpalis gambiensis* the highest index of increase was 2.5 at 1 µg.

The best dose for propylphenol was 100 ng for the two species, and the index of increase was 2.5 for *G. palpalis gambiensis* and above 4 for *G. pallidipes*. Higher or lower doses did not give such strong responses. Furthermore, the responses to octenol, *p*-cresol and propylphenol were invariably lower for *G. palpalis gambiensis* than for *G. pallidipes*. The overall trend was the same for both species with the exception of a decreased response for the highest dose of propylphenol.

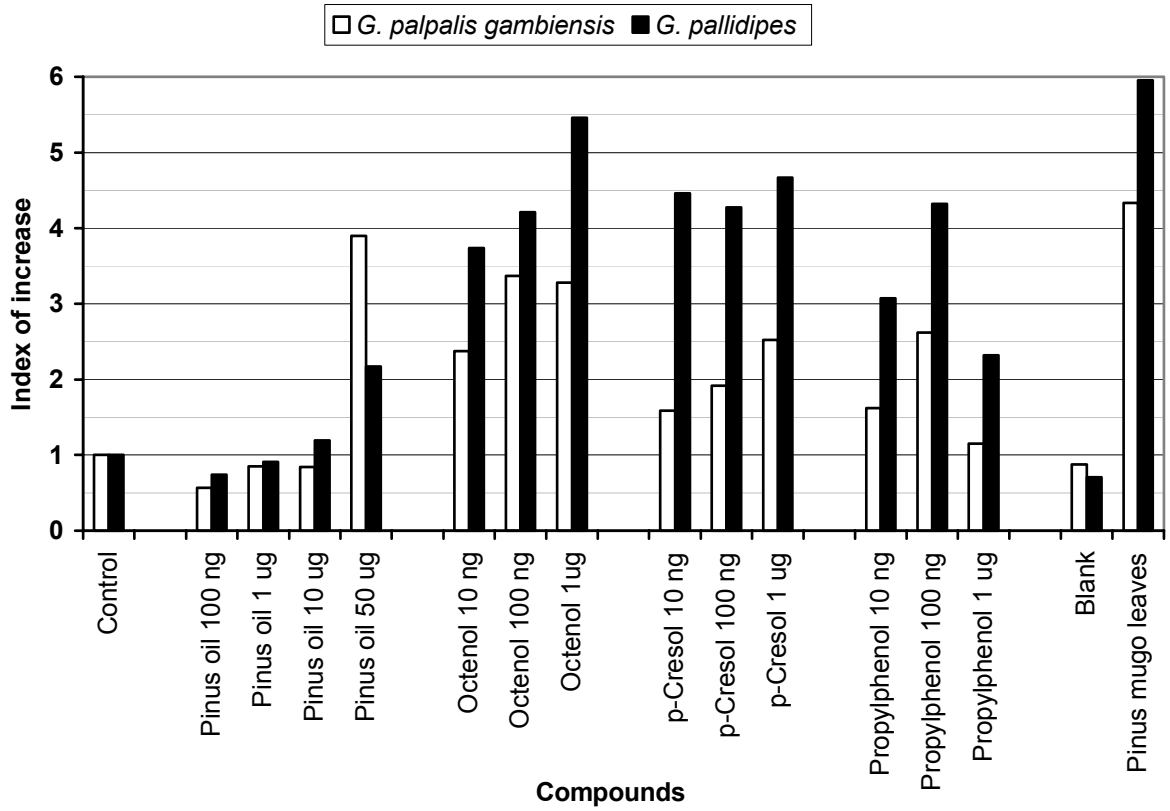


Figure 3.4. EAG responses of two Glossina species to various doses of different odours relative to dichloromethane solvent control.

Interestingly, leaf volatiles of the pine species *P. mugo unicata* evoked the best response of all treatments for both species. This demonstrates the significance of odour blends as this result can be explained by the multitude of compounds, mostly terpenoids, given off from the fresh leaves. It is possible that a hitherto unidentified olfactory stimulant component is responsible for the high EAG activity of the leaves.

In summary, the present EAG studies have demonstrated that antennae of *G. pallidipes* are sensitive to many compounds. Except for low doses of *P. sylvestris* oil, all odours tested gave higher EAG responses than the solvent control. This is significant, as *G. palpalis gambiensis* does not respond to the currently used tsetse attractants in the field.

3.2. Wind tunnel tests with known and new candidate kairomones

Materials and Methods

Wind tunnel studies were carried out in the Sensorial Physiology Laboratory of the Institute of Zoology, University of Neuchâtel. The work used two tsetse species, *G. pallidipes* and *G. palpalis gambiensis*. Pupae of the flies for their respective colonies were provided by the Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, and by CIRDES, Bobo-Dioulasso, respectively. After emergence, the flies were fed every second day on a silicone membrane with bovine blood, and held in a climate room under controlled climatic conditions that were same as for the wind tunnel (Fig. 3.5). For each trial, 5 tsetse (5 to 10 days old) starving for 2 days were placed in a cylindrical box (9 cm × 15 cm). Releases into the wind tunnel took place mainly from 9 to 11 am, and from 4 to 7 pm.

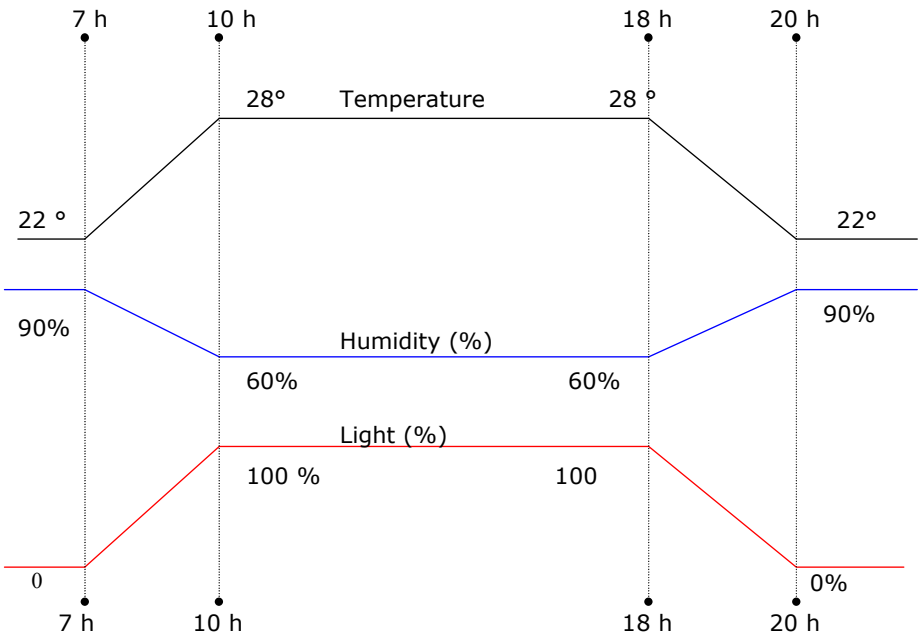


Figure 3.5. Environmental conditions of the wind tunnel.

The flight chamber is a 50 cm × 50 cm and 170 cm long tunnel. Charcoal air filters are present at the entrance and exit of the tunnel. A laminar flow of purified air into the tunnel is provided at a speed of 20 – 25 cm/s. To avoid any interference with the experimenters in the room, the glass tunnel was covered with paper with a slit to allow observations of the flies. Inside the tunnel a wire net tube (150 cm long and 30 cm in diameter) links the release box and the odour source and provides a support to the flies as they move towards the odour source.

The test bottle, holding the experimental and solvent control odours and the control communicate separately with the upwind end of the wind tunnel via vertical glass tubes (4 mm diameter). These glass tubes end in the middle of the connecting tube to the release box via a horizontal funnel covered with aluminium foil. This foil was perforated with little wholes to allow the passage of odours. Air that pushed the compounds from two bottles (experimental and solvent control) to the tunnel was first purified by a charcoal filter. Release of the filtered air is controlled by a computer via a solenoid. The frequency and duration of the delivery of the test odours were designed to last for three minutes.

The tested odours were acetone plus a 1:4:8 mixture of propylphenol + octenol + *p*-cresol (POCA) and *P. sylvestris* essential oil diluted as shown in Table 3.4.

Table 3.4. Treatments used and their doses for wind tunnel experiments

Compound	Solution concentration	Applied volume	Applied dose
Propylphenol (P)*	100 ng/µl	10 µl	1 µg
Octenol (O)*	100 ng/µl	40 µl	4 µg
p-Cresol (C)*	100 ng/µl	80 µl	8 µg
Acetone (A)*	10 µg/µl	100 µl	1 mg
Pinus sylvestris oil	1 µg/µl	50 µl	50 µg

*Components of POCA.

Except acetone that was diluted with water, all other compounds were dissolved in dichloromethane. Half an hour before odour release, the above treatments were applied to a piece of filter paper. After solvent evaporation, the paper was introduced into a gas-wash bottle for evaporation. The bottle was then connected to the vertical tube that conducted the odour to the upwind end of the wind tunnel. During each trial, the following fly reactions were recorded:

- fly activation (moving and wing vibration in the release box)
- short flights within the release box
- number of flies that left the release box
- number of flies that reached the odour source.

Results and discussion

The results of the wind tunnel experiments with the test odours are shown in Figs. 3.6 through 3.9. The solvent control (dichloromethane) activated only few flies of either species. The POCA blend highly stimulated *G. pallidipes* (about 90% of the flies) but *G. palpalis gambiensis* was less responsive (40 to 50% of the flies).

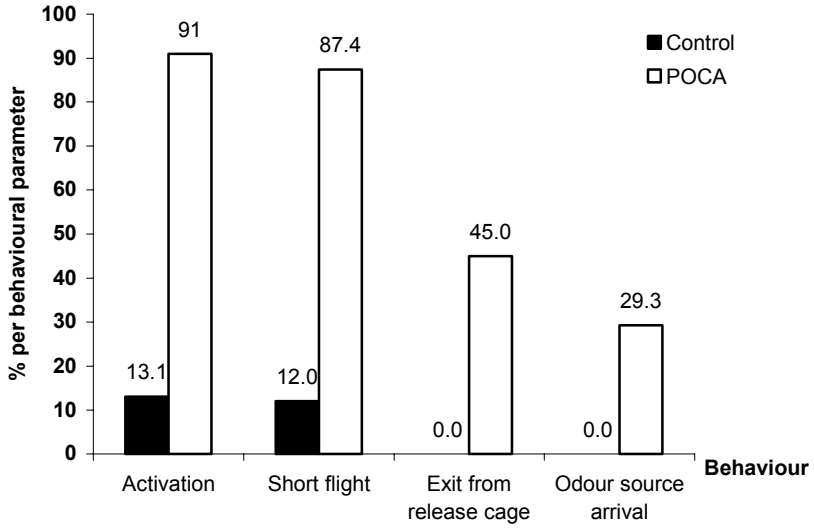


Figure 3.6. Behaviour of G. pallidipes in wind tunnel in the presence of propylphenol + octenol + p-cresol (1:4:8) plus acetone (POCA) (n = 191).

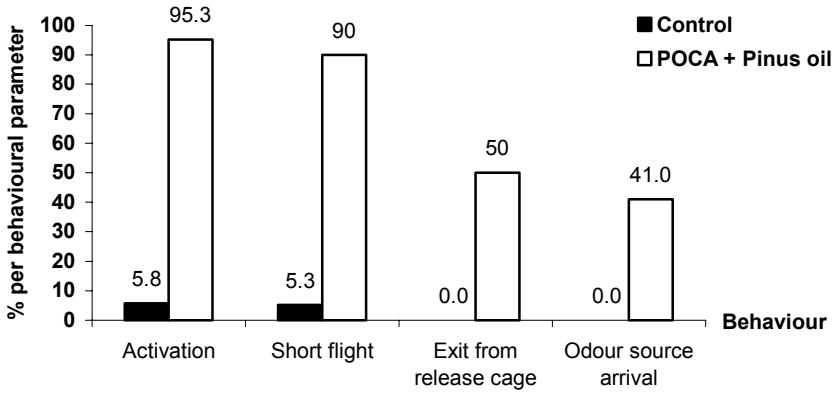


Figure 3.7. Behaviour of G. pallidipes in wind tunnel in the presence of propylphenol + octenol + p-cresol (1:4:8 blend) plus acetone (POCA) and P. sylvestris essential oil (n = 190).

Effects are more perceptible when one considers number of flies that left the release box and reached the odour source. For this criterion, no fly responded with the solvent control, whatever the species. With POCA, 45% of *G. pallidipes* left the release box and 29% reached the source whereas with the same mixture, 16 and 9% of *G. palpalis gambiensis,* respectively, left the release box and reached the odour source.

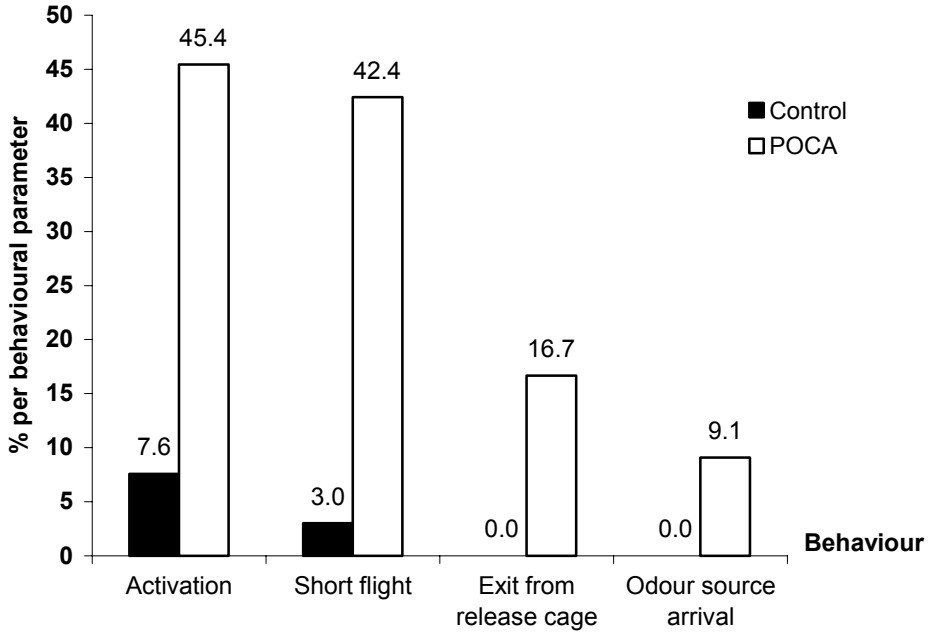


Figure 3.8. Behaviour of G. palpalis gambiensis in wind tunnel in the presence of propylphenol + octenol + p-cresol (1:4:8) plus acetone (POCA) (n = 66).

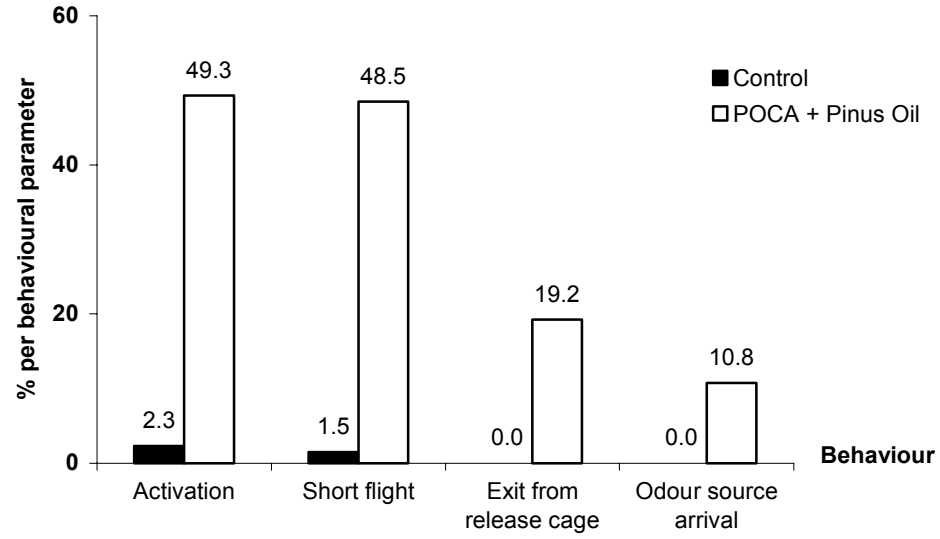


Figure 3.9. Behaviour of G. palpalis gambiensis in wind tunnel in the presence of propylphenol + octenol + p-cresol (1:4:8) plus acetone (POCA) and P. sylvestris oil (n = 130).

G. pallidipes was again more sensitive to the POCA + *P. sylvestris* oil blend, with 50% and 41% leaving the box and reaching the odour source, respectively, while only 19% and 10% of *G. palpalis gambiensis,* respectively, left the release box and reached the odour source.

With these results, one could argue that even if *G. pallidipes* is more sensitive to the odour treatments than *G. palpalis gambiensis*, the latter also responds to odours because for there was a clear difference between responses to solvent control and to the test odour blends.

In addition, it was noted that by adding, to the POCA blend *P. sylvestris* oil, a mixture of plant secondary compounds, responses increased for all behavioural parameters. It appears that the higher the number of attractant compounds is in the odour blend, the more perceptible the behavioural effects are.

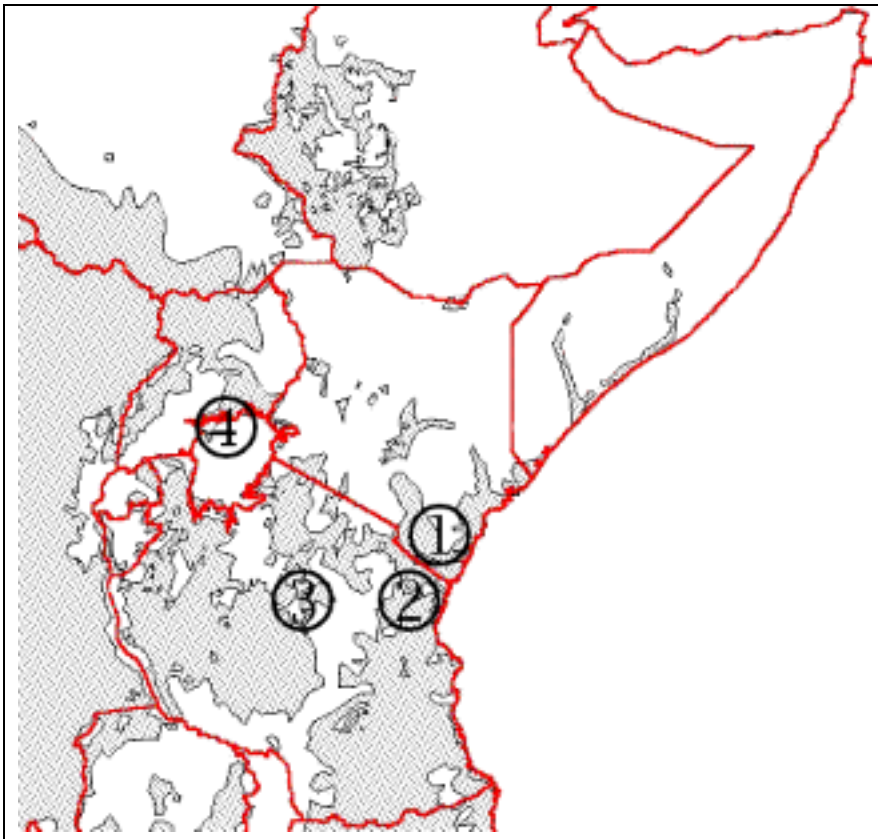
3.3. Conclusions

These results are all the more important since in the wind tunnel there were no visual stimuli as with the trap in the field. For all the behavioural parameters quantified, *G. pallidipes*, which was used as reference, as its behaviour was already studied at Neuchâtel earlier, was about two times more sensitive than *G. palpalis gambiensis*.. More screening efforts need to be directed towards the identification of optimal parameters (doses and blends of candidate kairomones) in order to achieve better results with *G. palpalis gambiensis* in the wind tunnel.

4. FIELD SCREENING: TRAPS AND ATTRACTANTS

Field experiments with various trap and target designs with and without candidate attractants were carried out in altogether five countries in East and West Africa.

4.1. Trapping Glossina *species in East Africa*



Kenya: ①,

Tanzania: ② and ③,

Uganda: ④.

(For details, see text.)

Shaded areas indicate tsetse-infested regions.

Figure 4.1. Location of experimental sites in East Africa.

4.1.1. Kenya

There is some controversy with regards to the classification of *Glossina austeni*: When Newstead originally described the species in 1912, he included it into the *palpalis* group. This has been corroborated by some molecular genetic analyses. It was also suggested to place *G. austeni* into a monotype subgenus *machadomyia*. Nevertheless, most researchers and tsetse field workers still include it in the *morsitans* group of tsetse flies that inhabit the savannah woodland in Africa. It is, however, considered to be a unique member of the group possessing some primitive characters. *G. austeni* is found in secondary shrub, thickets and islands of forests in Kenya, Tanzania, Mozambique, Swaziland, and South Africa. The fly has a scattered water edge distribution and does not occur more than 250 kilometres inland or 900 metres above the sea level and feeds mainly on suidae. Where livestock is held in *G. austeni* habitat the fly is a very effective transmitter of pathogenic trypanosomes and is one of the tsetse species with the highest vectorial capacity in the coastal region of Eastern Africa stretching from Somalia to South Africa.

In Kenya, *G. austeni* is found along the coastal belt often in a mixed population with *G. pallidipes* and *G. brevipalpis*. Isolated populations occur in the hinterland where the dense humid forest required by this species is interrupted by open savannah. The fly prevents productive livestock maintenance in grazing areas adjacent to watering source habitats and forest that it infests. The study of *G. austeni* has been restricted by lack of effective monitoring tools. Turner [85] relied solely on pupal searches for assessing distribution of *G. austeni* in Zanzibar. (Nb: when this CRP was initiated, the tsetse fly eradication operations on Zanzibar where still in progress.)

Development of bait technology for Glossina austeni *in Kenya – 1996.*

In an attempt to study the behaviour of the fly along the Kenyan coast, Owaga [86] used four different traps and, apparently owing to very low catches, expressed the records in percentage. Hall [87] and Madubunyi [88] developed various versions of sticky panels for *G. austeni*. It was demonstrated that over 80% of the fly of both sexes were caught at the bottom of the panels. Studies on the circumlocutory behaviour of the fly using electrified nets showed that of the flies caught 92% were of the grid covering the target and only 8% on either side of the target suggesting that that *G. austeni*, unlike *G. pallidipes* and *G. morsitans* does not circle the target but lands immediately on it. An improved sticky panel trap [89] has been the standard monitoring device in the SIT eradication programme on Unguja Island of Zanzibar [10].

Here a brief evaluation of the efficiency of sticky monopanels against conventional tsetse trap designs is presented, as well as a comparison of a fibreglass model similar to the monopanel type used for routine monitoring of *G. austeni* [89] during the Zanzibar tsetse eradication operations.

Methods and Materials

Traps and panels were compared 30 km along Voi-Taveta Road, in Taita Taveta District, Coast Province, Kenya, at the beginning of the dry season, immediately after the long rains, in an inland riverine *Acacia xanthophlea* habitat infested with *G. austeni* as the sole tsetse species present. Along the Bura River the habitat opens out and eventually ends at a dam. Biconical [90], Siamese [91], Nzi [92] traps and monopanels were compared in a 4 × 4 Latin square design experiment with three replicates, with fly collections and trap rotation every 24 hours.

The monopanels and the fibreglass models were made from material obtained from Henkel East Africa Company, and their colour and shape were the same as used in Zanzibar [89]. These were compared in a 2 × 2 Latin square experiment with three replicates over a period of six days. The panels were hung about 2 cm after the lower half was coated with a thin layer of Temoocid® adhesive (Kollant SPA, Italy). The flies were recovered every 2 hours, the temperature being read simultaneously between 06:00 h and 18:00 h. Data were analysed using Duncan multiple range test after log (x+1) transformation. Catch indices were calculated relative to biconical trap as control. Males and females were analysed separately.

Results

The sticky panels caught significantly (P<0.001) more males and females compared to the biconical, Siamese and Nzi traps (Table 4.1.1). The females had a catch index 20.65 compared to 13.90 for males, and 22.90 for both sexes taken together. The inland *G. austeni* population was composed of over 80% female population.

Table 4.1.1. Daily mean captures of *G. austeni* by various traps in a riverine environment at the Bura River in Taita Taveta District, Kenya, during the dry season in1996

Trap type	Males		Females		Total	
	Detrans mean	Catch index†	Detrans mean	Catch index†	Detrans mean	Catch index†
Biconical	0.10	1	0.26	1	0.33	1
Siamese	0.00	0.00	0.38	1.46	0.38	0.14
Nzi	0.00	0.00	0.16	0.62	0.16	0.48
Monopanel	1.39*	13.90*	5.37*	20.65*	7.67*	22.90*

†Catches relative to biconical trap.
*Significantly different relative to the biconical trap (P<0.001) for males, females and total captures.

The comparison of PVC and fibreglass monopanels showed that the fibreglass caught more males but less females (Table 4.1.2). When the sexes were pooled, the fibreglass monopanel had a slightly lower catch. The experiment was done during dry season when catches of the species are relatively low (see also [93]).

Table 4.1.2. Daily mean captures of *G. austeni* by PVC and fibreglass panels in a riverine environment at the Bura River in Taita Taveta District, Kenya, during the dry season in 1996

Trap type	Males		Females		Total	
	Detrans mean	Catch index†	Detrans mean	Catch index†	Detrans mean	Catch index†
PVC monopanel	0.22	1	2.28	1	2.50	1
Fibre glass panel	0.61	2.77	1.56	0.68	2.17	0.87

†Catches relative to PVC monopanel.

The diurnal activity pattern of *G. austeni*, with monitoring carried out at two-hour intervals, is shown in Fig. 4.2. The fly was active throughout the day between 06:00 h and 18:00 h. During this period, the average temperature varied between 19.8^{o}C and 28.7^{o}C at 06:00 h and 12:00 h, respectively. At the time of the experiment, the fly showed a morning activity peak between 08:00 h and 10:00 h and an afternoon activity peak between 16:00 h and 18:00 h. The observed activity pattern agrees with earlier observations [86]. The high catches also recorded early morning (06:00 h) suggest substantial activity between 18:00 h and 06:00 h.

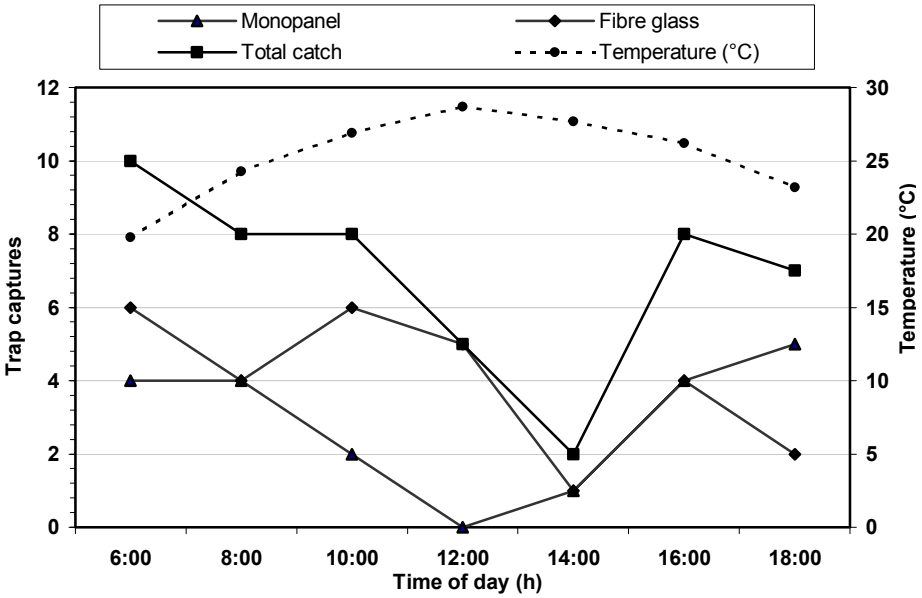


Figure 4.2. Dial activity of G. austeni as monitored by daily captures on two sticky traps in a riverine environment at the Bura River in Taita Taveta District, Kenya, during the dry season 1996.

In conclusion, the PVC monopanels caught significantly more *G. austeni* than either the biconical, Siamese or Nzi traps. The fibreglass monopanels catches in the preliminary experiment were comparable with those of PVC monopanel model used in Zanzibar to trap *G. austeni*. Capture data related to the diurnal activity of *G. austeni* showed that the fly was active throughout the day, with morning and afternoon peaks, although an apparent night activity of the fly was also recorded.

Comparing the reflectance or transmittance of fabrics, panels and sticky substances used for catching G. austeni

The light reflectance of PVC, fibreglass and blue fabric materials used for making traps was examined by spectroscopy. Moreover, Polybutylene 30 (Chemmoditys), Temoocid, Rentokill Fly adhesive and Rentokill Plastic Base/Fay Wrap adhesives used to make the trap or target surfaces sticky were analysed for light transmittance. Diffuse reflection for cloth and transmittance for netting and plastics were measured between 370 and 790 nm with a Li-Cor 1800 spectrometer (Lincoln, Nebraska, USA) equipped with an integrating sphere. A single layer of material was measured in triplicate against a black background for reflectance, while against a white background for transmittance at 1 nm resolution. Pressed $BaSO_4$ was used as a secondary working standard for the calibration of 100% reflectance. Repeatability of spectra was ±1% or better.

The sticky materials were also analysed the same way but with a sandwich approach, i.e. with a clear piece of fly roll on the other side. As insect vision differs from human vision in terms of enhanced sensitivity in the UV part of the spectrum, the mean values for reflectance or transmittance were summarised between 370 and 400 nm. The integrating sphere detector of the spectro-radiometer cannot measure reflectance quantitatively below 370 nm; however, none of the fabrics had substantial reflectance below this wavelength.

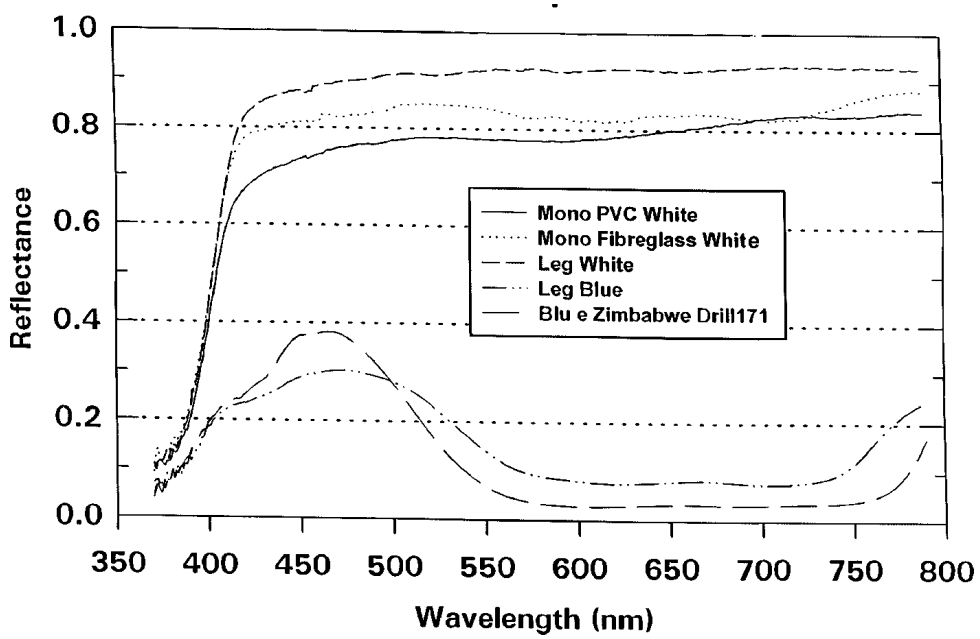


Fig. 4.3. Reflectance of trap and target materials used for G. austeni monitoring and control.

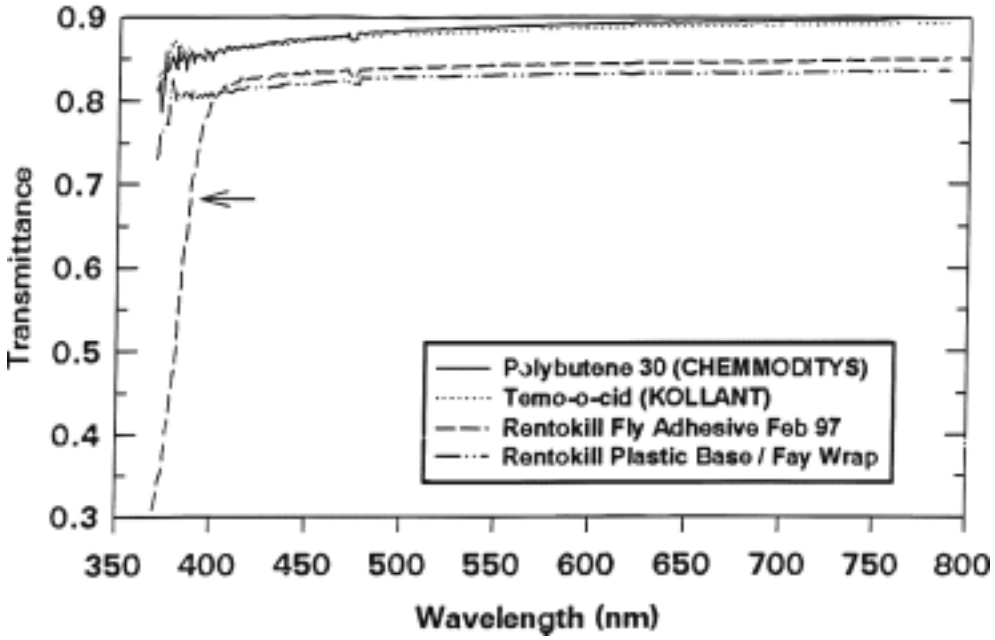


Fig. 4.4. Reflectance of adhesive materials used for G. austeni traps and targets.

The results of the measurements, shown in Fig. 4.3, indicate that white PVC and fibreglass panels have a similar reflectance, which is generally higher than that of blue fibreglass and of phthalogen blue cloth fabric. The reflectance of the blue colour shades, which attracted *G. austeni* in high numbers, was also similar.

As it is seen in Fig 4.4, Polybutylene and Temoocid glues, normally used to make panels sticky, had similar degrees of light transmittance. However, the Rentokill adhesive absorbs light in the UV range (below 400 nm as indicated by an arrow).

Development of bait technology for G. austeni *in Shimba Hills National Park, Kenya, - 1999 – 2001*

In February 1999, the attractiveness of various test compounds screened at that time by laboratory bioassays, was assessed in the field for *G. austeni.* The test was conducted in the Shimba Hills National Park, in the Rongo Mwagandi Forest, Kwale District, 30 km from the Indian Ocean. Large game including the elephant, buffalo, and various antelopes are found in the area. A modified leg panel (Fig. 4.5) made from a galvanised wire outline filled with 1000-gauge (0.15 mm) royal blue polyethylene was used. The lower half of the panel was smeared with Temoocid glue to hold the flies. Well-lit places in typical *G. austeni* habitat, 10–15 m inside the thicket, were selected as trapping sites. The habitat comprised a three-tier canopy with very tall trees, followed by a medium level of trees with shrubs at lowest level and virtually shielding off the direct sunlight. *G. pallidipes* and *G. brevipalpis* were also found in the same habitat and their catches were also recorded.

Fig. 4.5. Modified leg panel trap (for detailed description, see text). Note PE sachet, filled with odour, under trap.

The chemicals tested were decyl formate, octyl formate, decanal, octenol and dodecanal. The test chemicals (4.5 ml each) were sealed in 0.15 mm thick 5 cm × 6 cm PE sachets that dispensed the candidate odour attractants at the rate of 30 mg per day. Locally available vegetable products, namely olive oil, sunflower oil, coconut oil and linseed oil (see 2.4), dispensed from 5 ml glass vials capped with a punctured rubber septum, were similarly tested. The sachets and vials were suspended just above the ground, 30 cm from the sticky panels. Three separate 4 × 4 Latin square experiments were done, each with one replicate, with unbaited sticky panels as control. The sexes and tsetse fly species of the daily catches were recorded separately and the data subjected to analysis of variance after log (x+1) transformation.

The results obtained at the end of the rainy season of 1999 for *G. austeni* are shown in Tables 4.1.3 to 4.1.5. In general, trap catches were low in this study. The obtained data show that there was no statistically significant increase in the catches of either male or female *G. austeni*

by the sticky panels baited with the test odours. Some numerical differences should, however, be noted. Decanal that marginally increased male, but not female, catches of the species. Some catch increases were observed with decyl formate on both sexes: 1.57 for males and 1.37 for females. Octyl formate also increased catches by about threefold for males while decyl formate doubled the catches of females.

Table 4.1.3. Effect of odour baits on daily mean catches of *G. austeni* by modified leg panel traps in Shimba Hills National Park, during the end of the rainy season of 1999

Bait description	Males		Females	
	Detrans mean	Catch index	Detrans mean	Catch index
Unbaited panel	0.19	1	1.48	1
Decanal	0.41	2.19	1.22	0.83
Octyl formate	0.59	3.11	1.66	1.13
Decyl formate	0.30	1.57	2.02	2.02

Differences observed were statistically not significant.

In the other experiment with different odours, including octenol, dodecanal and olive oil (Table 4.1.4), none of the baits affected catches of the panel. Furthermore, of the various vegetable oils tested, coconut oil increased catches of male *G. austeni* by 3.43-fold (Table 4.1.5).

Table 4.1.4. Effect of odour baits on daily mean catches of *G. austeni* by modified leg panel traps in Shimba Hills National Park, during the end of the rainy season of 1999

Bait description	Males		Females	
	Detrans mean	Catch index	Detrans mean	Catch index
Unbaited panel	0.49	1	1.77	1
Octenol	0.25	0.51	0.89	0.50
Dodecanal	0.60	1.24	1.76	0.99
Olive oil	0.45	0.93	1.85	1.04

Differences observed were statistically not significant.

Table 4.1.5. Effect of odour baits on daily mean catches of *G. austeni* by modified leg panel traps in Shimba Hills National Park, during the end of the rainy season of 1999

Bait description	Males		Females	
	Detrans mean	Catch index	Detrans mean	Catch index
Unbaited panel	0.25	1	1.82	1
Coconut oil	0.86	3.34	1.03	0.56
Linseed oil	0.30	1.18	0.73	0.40
Sunflower oil	0.09	0.36	0.88	0.49

Differences observed were statistically not significant.

The results obtained for the two other *Glossina* species with decanal and the two formates are shown in Tables 4.1.6 and 4.1.7. The data indicate that these compounds did not significantly increase catches either of *G. pallidipes* or *G. brevipalpis* males and females. The panel baited with decanal showed only marginally increased captures of male but not of female *G. pallidipes*. No catches of *G. brevipalpis* could be recorded. However, some increases in catches were observed with the two formates on both sexes of the two species. For decyl formate, the catch indices were 1.57 for male and 1.68 for female *G. pallidipes,* respectively, and 3.28 for male *G. brevipalpis*. For octyl formate, the catch indices were 1.78 for male *G. pallidipes,* and 5.99 for male *G. brevipalpis*. However, no female *G. brevipalpis* was caught (Table 4.1.7).

Table 4.1.6. Effect of odour baits on daily mean catches of *G. pallidipes* by modified leg panel traps in Shimba Hills National Park, during the end of the rainy season of 1999

Bait description	Males		Females	
	Detrans mean	Catch index	Detrans mean	Catch index
Unbaited panel	1.41	1	4.05	1
Decanal	1.90	1.34	4.05	1.00
Octyl formate	2.51	1.78	4.09	1.01
Decyl formate	2.22	1.57	6.82	1.68

Differences observed were statistically not significant.

Table 4.1.7. Effect of odour baits on daily mean catches of *G. brevipalpis* by modified leg panel traps in Shimba Hills National Park, during the end of the rainy season of 1999

Bait description	Males		Females	
	Detrans means	Catch index	Detrans means	Catch index
Unbaited panel	0.09	1	0.00	-
Decanal	0.00	-	0.00	-
Octyl formate	0.54	5.99	0.00	-
Decyl formate	0.30	3.28	0.00	-

Differences observed were statistically not significant.

According to data in Tables 4.1.8 and 4.1.9, octenol, dodecanal and olive oil did not affect catches of *G. pallidipes*. However, octenol and olive oil did increase the catches for male *G. brevipalpis* by over threefold.

Table 4.1.8. Effect of odour baits on daily mean catches of *G. pallidipes* by modified leg panel traps in Shimba Hills National Park, during the end of the rainy season of 1999

Bait description	Males		Females	
	Detrans mean	Catch index	Detrans mean	Catch index
Unbaited panel	2.20	1	4.51	1
Octenol	1.88	0.85	4.14	0.92
Dodecanal	2.20	1.00	3.80	0.84
Olive oil	1.50	0.68	3.93	0.87

Differences observed were statistically not significant.

Table 4.1.9. Effect of odour baits on daily mean catches of *G. brevipalpis* by modified leg panel traps in Shimba Hills National Park, during the end of the rainy season of 1999

Bait description	Males		Females	
	Detrans mean	Catch index	Detrans mean	Catch index
Unbaited panel	0.19	1	0.00	-
Octenol	0.76	3.99	0.00	-
Dodecanal	0.19	1.00	0.00	-
Olive oil	0.61	3.22	0.00	-

Differences observed were statistically not significant.

Tables 4.1.10 and 4.1.11 show the responses of *G. pallidipes* and *G. brevipalpis* to sticky panels baited with coconut oil, refined linseed oil or sunflower oil. There was no significant influence of the tested oils on the catches of either species. The refined linseed oil showed an increase of catches of male *G. brevipalpis* but the numbers of the flies caught were extremely low. The numbers of the female *G. brevipalpis* caught were too low to be analysed.

Table 4.1.10. Effect of odour baits on daily mean catches of *G. pallidipes* by modified leg panel traps in Shimba Hills National Park, during the end of the rainy season of 1999

Bait description	Males		Females	
	Detrans mean	Catch index	Detrans mean	Catch index
Unbaited panel	1.03	1	1.44	1
Coconut oil	0.75	0.73	1.29	0.90
Linseed oil	0.74	0.72	1.21	0.84
Sunflower oil	0.83	0.81	1.81	1.26

Differences observed were statistically not significant.

Table 4.1.11. Effect of odour baits on daily mean catches of *G. brevipalpis* by modified leg panel traps in Shimba Hills National Park, during the end of the rainy season of 1999

Bait description	Males		Females	
	Detrans mean	Catch index	Detrans mean	Catch index
Unbaited panel	0.09	1	0.09	1
Coconut oil	0.09	1.00	0.00	-
Linseed oil	0.41	4.58	0.00	-
Sunflower oil	0.00	0.00	0.00	-

Differences observed were statistically not significant.

In conclusion, the study area used in 1999 had three tsetse fly species namely, *G. austeni*, *G. pallidipes* and *G. brevipalpis*. The presence of *G. pallidipes* was of importance for the test substances because it responds to known kairomones. Although the experiment was carried out in the dry season during the month of February, when the captured number of flies for the other two species was lower, the numbers were still sufficiently high to indicate whether the candidate attractants have any potential. Of the used odours, octyl and decyl formates resulted in higher though statistically not significant fly catch increases of both sexes of the three species. The increased captures by coconut oil for male *G. austeni* and female *G. pallidipes*, by dodecanal for female *G. pallidipes*, and by octenol, olive oil and linseed oil for male *G. brevipalpis* deserve further consideration.

Experiments in 2000

During June and December 2000, i.e. a month after the end of long and short rains, respectively, field trials took place in the Kenya coastal habitats in Mwatate, Taita Taveta district, and in Shimba Hills game park in the Rongo Mwagandi Forest (39°25' E, 4°15' S), near Kwale village, 40 km and 150 km, respectively, from the shores of the Indian Ocean,. The responses of *G. austeni, G. pallidipes* and *G. brevipalpis* to various chemicals alone and in combination as odour blends were tested using sticky panels. The panels were placed in dark dense thickets suitable for *G. austeni*, about 5 m from the forest edge, which practically eliminated direct sunlight exposure.

The following odour baits were tested: acetone, octenol, decanal, dodecanal, octyl formate, decyl formate, *P. sylvestris* oil, *P. pumilionis* oil, palm oil (from a local supermarket), isovaleric acid, and the "Zimbabwe-blend" (here called POCA) containing propylphenol, octenol and *p*-cresol (1:4:8 by weight) plus acetone. The first three components (the 1:4:8 mixture) was dispensed using PE sachets, the last component of POCA, i.e. acetone, was dispensed from a bottle. POMA refers the similar combination containing propylphenol, octenol and *m*-cresol (1:4:8 by weight) plus acetone.

The experiments were based on 24-hour catches in randomised Latin square designs with two replicates. Data from the field trials were analysed after log (x+1) detransformation. Where there was no difference between males and females, the data of both sexes are pooled.

The results obtained for *G. austeni* in June 2000 are shown in Tables 4.1.12 and 4.1.14. This was the first time during the studies under this CRP that *G. austeni* in the test area showed a significant response to odour. No differences were recorded between the sexes. Sticky panels baited with octenol and acetone doubled catches and represent a promising simple bait system for monitoring *G. austeni* (Table 4.1.12). None of the plant oils resulted in increased captures using the sticky panels.

Table 4.1.12. Effect of odour baits on daily mean catches of *G. austeni* by modified leg panel traps in Shimba Hills National Park, June 2000

Bait description	Detrans mean	Catch index
Unbaited panel	2.96	1
Pinus sylvestris oil	1.23	0.42
Pinus pumilionis oil	1.98	0.67
Palm oil	1.59	0.54
Octenol + acetone	6.35*	2.15

*Significantly different from the other treatments (P<0.001).

In the other set-up (Table 4.1.13), the POCA blend was found to more than double the catches of the panel trap. Moreover, for the combination of POCA + *P. sylvestris* essential oil, a catch index of 3.44 was observed.

Table 4.1.13. Effect of odour baits on daily mean catches of *G. austeni* (males and females) by modified leg panel traps in Shimba Hills National Park, June 2000

Bait description†	Detrans mean	Catch index
Unbaited panel	0.64	1
POCA	1.54	2.39
POMA	1.11	1.72
POCA + decanal	1.29	2.00
POCA + *Pinus pumilionis* oil	2.21	3.44

†POCA = propylphenol + octenol + *p*-cresol (1:4:8) in PE sachet plus acetone in bottle dispenser; POMA = propylphenol + octenol + *m*-cresol (1:4:8) in PE sachet with acetone in bottle dispenser.

It must be noted that Vreysen et al. [93] found that cow urine plus acetone was effective in the rainy season but not in the dry season. Brightwell and Dransfield [94] also reported that acetone + octenol + cow urine doubled catches of *G. austeni* in NG2F traps but did not state the season when the experiment was carried out. In their paper, they also refer to Vale's earlier preliminary experiment suggesting the attractivity of POCA to *G. austeni*.

The addition of other odours to POCA or POCA + *P. sylvestris* oil did not increase the catches in the experimental set-up (Table 4.1.14).

Table 4.1.14. Effect of odour baits on daily mean catches of *G. austeni* (males and females) by modified leg panel traps in Shimba Hills National Park, June 2000

Bait description†	Detrans mean	Catch index
Unbaited panel	0.896	1
POCA + *Pinus sylvestris* oil + octyl formate	0.835	0.93
POCA + *P. sylvestris* oil + decyl formate	0.841	0.94
POCA + decanal	1.107	1.24
POCA + octyl formate	0.762	0.85

†POCA = propylphenol + octenol + *p*-cresol (1:4:8) in PE sachet plus acetone in bottle dispenser.

The results for *G. pallidipes* and *G. brevipalpis* are shown in Tables 4.1.15 to 4.1.17. Data in Table 4.1.15 indicate that the leg panel baited with the octenol and acetone combination caught significantly more *G. pallidipes* than the unbaited control panel. *G. brevipalpis* flies were significantly repelled by the test odours used. The decreased catches of *G. brevipalpis* by traps baited with acetone + octenol stand in contrast to another experiment (Table 4.1.9) that suggested some attractancy of octenol baited traps. Moreover, a field study in South Africa [63] failed to record such a repellent effect on *G. brevipalpis*.

Table 4.1.15. Effect of odour baits on daily mean catches of males and females of *G. pallidipes* and *G. brevipalpis* by modified leg panel traps in Shimba Hills National Park, June 2000

Bait description	*G. pallidipes*		*G. brevipalpis*	
	Detrans mean	Catch index	Detrans mean	Catch index
Unbaited panel	7.451	1	1.069	1
Pinus sylvestris oil	4.431	0.59	0.231**	0.22
Pinus pumilionis oil	5.817	0.78	0.282**	0.26
Palm oil	2.712	0.36	0.374**	0.35
Octenol + acetone	53.79*	7.22*	0.196**	0.18

*Significantly different from other treatments (P<0.001).
**Significantly different from catches by the unbaited panel (P<0.001).

Table 4.1.16 shows that addition of some odour components to POCA can significantly increase catches of *G. pallidipes*. There was no difference between the effects of octyl and decyl formate and one can substitute for the other. Inclusion of *P. sylvestris* oil in the POCA and octyl or decyl formate combination doubled the catch. Interestingly, decanal alone can substitute for the *P. sylvestris* and formate blend. Moreover, addition of octyl formate to POCA increased *G. brevipalpis* catch though the overall number of flies caught was low and the results do not differ significantly.

Table 4.1.16. Effect of odour baits on daily mean catches of males and females of *G. pallidipes* G. *brevipalpis* by modified leg panel traps in Shimba Hills National Park, June 2000

Bait description[†]	*G. pallidipes*		*G. brevipalpis*	
	Detrans mean	Catch index	Detrans mean	Catch index
Unbaited panel	3.54	1	0.516	1
POCA + *P. sylvestris* oil +octyl formate	28.49*	8.05	0.319	0.62
POCA + *P. sylvestris* oil + decyl formate	33.28*	9.40	0.506	0.98
POCA + decanal	32.40*	9.15	0.603	1.17
POCA + octyl formate	14.87*	4.20	1.361	2.64

[†]POCA = propylphenol + octenol + *p*-cresol (1:4:8) in PE sachet plus acetone in bottle dispenser.
*Significantly different from unbaited control (P<0.001).

As it is shown in Table 4.1.17, POCA and the POCA + *P. pumilionis* oil blend attracted significantly more *G. pallidipes* than either POMA or the unbaited control. For *G. pallidipes* males the POCA + decanal blend was significantly more attractive than the control (P<0.05; data not shown); in an earlier replicate of this experiment a catch index of 9.15 (P<0.001) was obtained for both males and females of this species. The odours used in this test apparently do not influence captures of *G. brevipalpis*.

Table 4.1.17. Effect of odour baits on daily mean catches of males and females of *G. pallidipes* and *G. brevipalpis* by modified leg panel traps in Shimba Hills National Park, June 2000

Bait description†	*G. pallidipes*		*G. brevipalpis*	
	Detrans mean	Catch index	Detrans mean	Catch index
Unbaited panel	6.215	1	0.802	1
POCA	27.03*	4.35	0.550	0.69
POMA	9.645	1.55	0.650	0.81
POCA + decanal	17.57	2.83	0.828	1.03
POCA + *P. pumilionis* oil	31.25*	5.03	1.011	1.26

†POCA = propylphenol + octenol + *p*-cresol (1:4:8) in PE sachet with acetone in bottle dispenser; POMA = propylphenol + octenol + *m*-cresol (1:4:8) in PE sachet with acetone in bottle dispenser.
*Significantly different from other treatments ($P<0.001$).

Further experiments were conducted at Shimba Hills, near Kwale Village in the Rongo Mwagandi Forest (39°25' E, 4°15' S), to study the effect some individual odours and their combinations on the attractivity of the sticky panel trap to *G. austeni*, *G. pallidipes* and *G. brevipalpis*. In general, single odours used in this test resulted in low captures and no significant difference between the unbaited and baited traps could be observed (Table 4.1.18). For *G. brevipalpis*, however, decanal had notably higher catches. The short-chain aliphatic acid, isovaleric acid did not decrease catches in contrast to earlier works with other tsetse species [95, 95].

Table 4.1.18. Effect of single odour baits on daily mean catches of males and females of *G. austeni*, *G. pallidipes* and *G. brevipalpis* by modified leg panel traps in Shimba Hills National Park, Kwale Village, 2–7 December 2000

Bait description	*G. austeni*		*G. pallidipes*		*G. brevipalpis*	
	Detrans mean	Catch index	Detrans mean	Catch index	Detrans mean	Catch index
Unbaited panel	1.155	1	2.204	1	0.246	1
Pinus sylvestris oil	0.692	0.60	2.095	0.95	0.072	0.29
Decyl formate	0.762	0.66	2.405	1.09	0.196	0.80
Decanal	1.083	0.94	2.339	1.06	0.374	1.52
Isovaleric acid	1.155	1.00	1.926	0.87	0.196	0.80

Differences observed were statistically not significant.

In another experiment with odour combinations, selected odours were added to the POCA blend (Table 4.1.19). While none of the POCA-based odours affected catches of *G. austeni*, all odour-baits significantly increased catches of *G. pallidipes*. The combination of POCA with decanal or decyl formate lead to a further doubling of the number *G. pallidipes* captured. For *G. brevipalpis*, only the POCA + *P. sylvestris* oil and the POCA + decanal blends

increased *G. brevipalpis* catches significantly. The effect of these odours on the captures of this *fusca* species by simple trap is an important observation because only very recent studies have reported that *G. brevipalpis* is attracted to odours, such as cow urine and octenol in NG2F trap [94] and POCA or octenol + acetone in Nzi [92] or H traps [97].

Table 4.1.19. Effect of odour baits on daily mean catches of males and females of *G. austeni*, *G. pallidipes* and *G. brevipalpis* by modified leg panel traps in Shimba Hills National Park, Kwale Village, 2–7 December 2000

Bait description†	*G. austeni*		*G. pallidipes*		*G. brevipalpis*	
	Detrans mean	Catch index	Detrans mean‡	Catch index	Detrans mean‡	Catch index
Unbaited panel	0.813	1	2.013	1	0.516	1
POCA	0.473	0.58	9.636**	4.79	0.908	1.76
POCA + *P. sylvestris* oil	0.707	0.87	9.577**	4.76	2.319*	4.50
POCA + decyl formate	0.463	0.57	17.04**	8.47	1.785	3.46
POCA + decanal	0.644	0.79	17.70**	8.79	2.146*	4.16

†POCA = propylphenol + octenol + *p*-cresol (1:4:8) in PE sachet plus acetone in bottle dispenser.
‡Asterisks indicate significant differences from unbaited trap at the following levels of probability: * P<0.05; ** P<0.001.

Table 4.1.20. Effect of odour baits on daily mean catches of males and females of *G. austeni*, *G. pallidipes* and *G. brevipalpis* by modified leg panel traps in Shimba Hills National Park, Kwale Village, 2–7 December 2000

Bait description†	*G. austeni*		*G. pallidipes*		*G. brevipalpis*	
	Detrans mean‡	Catch index	Detrans mean‡	Catch index	Detrans mean‡	Catch index
Unbaited panel	0.374	1	2.317	1	0.374	1
POCA + isovaleric acid	1.310*	3.50	22.97**	9.91	4.335**	11.59
POCA + *P. sylvestris* oil + decyl formate + decanal	0.966	2.58	27.19**	11.74	2.542**	6.80
POCA + *P. sylvestris* oil + decyl formate + decanal + isovaleric acid	1.078	2.88	23.83**	10.28	0.919**	7.80
Acetone + octenol + *P. pumilionis*	0.319	0.85	9.837	4.25	1.155	3.09

†POCA = propylphenol + octenol + *p*-cresol (1:4:8) in PE sachet plus acetone in bottle dispenser.
‡Asterisks indicate significant differences from unbaited trap at the following levels of probability: * P<0.05; ** P<0.001.

Other odour combinations confirmed the importance of POCA as an important component of the bait (Table 4.1.20). For *G. austeni*, the POCA + isovaleric acid blend was significantly more effective than either components alone, especially for females (see Tables 4.1.18 and 4.1.19). It is notable that in this experiment the addition of *P. pumilionis* essential oil appears to have a detrimental effect on the otherwise attractive acetone + octenol blend (Table 4.1.14). POCA in combination with other test odours increased of catches of *G. pallidipes* by 10-fold and *P. pumilionis* oil neutralized the attractivity of acetone + octenol also for this species. Odour combinations containing POCA significantly increased catches of *G. brevipalpis* compared to the unbaited panel or the acetone + octenol + pine oil combination. The highest catch increase (>11-fold) *G. brevipalpis* was recorded for the POCA + isovaleric acid blend.

Comparison of laboratory and field results

It was also examined how results of laboratory studies (electrophysiological and wind tunnel tests) related to field trapping with *G. brevipalpis* and *G. pallidipes* using different odours and odour blends. Of course one also has to keep in mind that for field experiments with odours an appropriate standard trap or target design should already be available, which was not the case for all tsetse species. Ideally, odours should be tested at different dose rates.

EAG data presented in Tables 3.2 through 3.4 as well as Figs. 3.3 and 3.4 show that these two species perceive octenol, *p*-cresol and propylphenol, which are standard tsetse kairomones. Furthermore, for *G. brevipalpis*, significant electrophysiological responses were observed for several – but not all – new synthetic analogues of the conventional kairomones. For *G. brevipalpis* the strong EAG response to octenol translates to field attractivity (see, for example, Fig. 3.1 and Table 4.1.9, respectively). Of the synthetic analogues, octyl and decyl formates were EAG-active and also were attractive for *G. brevipalpis* males (Tables 3.1 and 4.1.7, respectively). For *G. pallidipes*, data from the limited number of EAG and field studies with single component odours (see Fig. 3.3 and Table 4.1.8, respectively) did not allow any meaningful comparison. However, the results of wind tunnel studies with the POCA blend indicated ("forecasted") the significant attractivity observed in the field (Tables 3.6 and 3.7 versus Tables 4.1.17 and 4.1.19).

4.1.2. Tanzania

Tanzania is infested with seven tsetse fly species that occur in more than 60% of the country [98]. Tsetse-transmitted trypanosomosis is severe for both humans and livestock in the country [99, 100, 101, 102, 103]. Although there have been control programmes to get rid of these vectors of trypanosomosis, so far insufficient work has been done in the country to develop effective traps and to identify attractive odours or odour blends for respective flies.

Glossina swynnertoni belongs to the *morsitans* or savannah group of tsetse flies. The biology of the species generally resembles that of *G. morsitans morsitans* [104]. *G. swynnertoni* generally occupies a very small part of Africa covering parts of northern Tanzania (Arusha region, parts of Dodoma, Singida, Shinyanga and Mara regions) and extends into south-western Kenya [104].

G. swynnertoni plays an important role as a vector of human and cattle trypanosomes in south-western Kenya and Tanzania. During the sleeping sickness epidemics of 1919–1921 in Maswa district, Tanzania, *G. swynnertoni* was incriminated as the main vector of the outbreak [103]. In Musoma District, Tanzania, *G. swynnertoni* was found to have higher trypanosome infection rate than *G. pallidipes* [100]. The relationship between *G. swynnertoni* and its hosts

at the Serengeti National Park also implicated this tsetse species as a vector of animal trypanosomes [101]. The majority of sleeping sickness cases in Tanzania are reported from Arusha, Kigoma and Kagera regions. The most important vectors of this human disease in these regions are *G. morsitans, G. pallidipes, G. swynnertoni* and *G. fuscipes* [99].

G. brevipalpis belongs to the *fusca* or forest group of tsetse flies. Its distribution covers the coastal belt of Kenya, spreading southwards to Tanzania, Mozambique and South Africa. In Tanzania, the fly spreads further inland and beyond into parts of the Democratic Republic of Congo [104]. *G. brevipalpis* is also the only tsetse fly species found on Mafia Island, Tanzania. Although in East Africa comparatively little is known about *G. brevipalpis,* it is a vector of trypanosomes to ruminants with infection rates comparable to those found in many other tsetse flies [105]. It poses a significant problem on Kenya coast and in Mozambique [94].

Field tests were conducted to screen already known traps in conjunction with odour baits for two species of tsetse flies, i.e. *G. swynnertoni* and *G. brevipalpis*, for which no attractants have been developed until very recently [94, 106]. Non-odour baited traps catch very few of these two species (see later). Trap catches of other species, namely *G. morsitans centralis* and *G. pallidipes*, were also analysed.

Materials and Methods

The study to evaluate conventional baits for *G. swynnertoni* was carried out at Sangaiwe in the Tarangire National Park ($35^0$45' E, $5^0$30' S) and Naitolya village (36^o03' E, 3^o40' S). (Naitolya was selected for electrocuting nets experiments that would not be allowed in the Tarangire National Park). Both sites are found in Arusha region and form a continuous *G. swynnertoni* belt. The habitat of *G. swynnertoni* is thorn savannah with acacia associated with *Combretum* and *Comiphora*, dotted irregularly over a grassy plain. Temperatures are normally high with low relative humidity. These conditions make the environment favourable to *G. swynnertoni* [107]. Other tsetse species found in the area include *G. morsitans* and *G. longipennis.* Common animals in the area include elephant, giraffe, antelopes, zebras, buffaloes, wildebeests and wild pigs.

The study area for *G. brevipalpis* was Mivumoni, coastal Tanga area (38^o45' E, 5^o30' S**)**. The habitat of this species is comprised of thick coastal forest [108]. The typical weather is hot and humid. Other tsetse flies found in the area are *G. pallidipes* and *G. austeni.* Animals found in the area are buffaloes, wild pigs, monkeys and antelopes. Occasionally, cattle from the neighbouring Mivumoni Livestock Multiplication Unit have become additional hosts.

Comparison of trap types

In 1999, nine trap types were compared at two locations over four days at Sangaiwe in Tarangire National Park for *G. swynnertoni* (September 1999) and at Mivumoni for *G. brevipalpis* (December 1999). The following traps were tested: F3 [109], Epsilon [110], biconical [111], NGU [112], pyramidal [113], Nzi [92], and other five new traps designed and constructed at Tsetse and Trypanosomiasis Research Institute (TTRI), Tanga, were tested for their relative effectiveness to the species at Sangaiwe. Amongst the traps, only S3 is documented to be effective in catching *G. swynnertoni*, however, the biconical trap has been in use for sampling this species in the country for many years [106].

The traps were randomly set in an open plain with isolated thorn acacia at 150–200 m intervals in an area where *G. swynnertoni* is abundant. Alongside with the traps, a 90 cm × 90 cm sticky black cloth target was set in the area to compare flies that are attracted to and land on the cloth with those that in addition enter the different traps.

The data in Table 4.1.21 indicate that the pyramidal trap was the best for *G. swynnertoni* followed by the S3 and the NGU trap. Catches of *G. brevipalpis* were too low to draw any conclusions.

Table 4.1.21. Mean fly catches for *G. swynnertoni* at Tarangire – Arusha, September 1999, and *G. brevipalpis* at Mivumoni – Tanga, December 1999

Trap type	*G. swynnertoni*	*G. brevipalpis*
S3	40.50	0.58
Biconical	15.00	0.53
Pyramidal	72.00	0.74
F3	17.00	0.37
Epsilon	26.00	0.26
NGU	35.00	Not tested
T1 (TTRI)	32.00	0.36
T2 (TTRI)	16.00	2.11
H	Not tested	0.47

Although the flies caught in the different trap designs were not statistically analysed, it can be summarized that the catches of *G. swynnertoni* in the various trap designs without odour baits were generally very low. Some traps caught no flies at all. The numbers of flies caught by the traps did not relate to the density of the local fly population, which was observed by recording the number of flies either landing on (but not entering) the trap or on moving objects including man or on the sticky black cloth. The sticky black cloth caught 178 flies in 24 hours time. The findings agree with the statement of Swynnerton [114], that *G. swynnertoni* is more refractory to entering traps than *G. morsitans*. However, a further assessment was made of four traps that were found slightly effective in the first trial. This included the biconical, S3, pyramidal, and Nzi traps. The study was conducted in a 4 × 4 Latin Square method at the Naitolya site. Catches of flies with these four traps were also very low (see Table 4.1.22). Statistical analysis of catches was made using the GV.xls programme, installed at the institute by Dr. Glyn A .Vale in 2000. The results of these analyses did not show significant differences in the number of flies caught by the different traps.

G. swynnertoni responds more readily to moving bodies like man, vehicles and hanging sticky panels than to stationary objects. In a preliminary experiment, a sticky panel mounted at the rear of a truck that drove a distance of about 2 kilometres caught 300 male and female flies, while a stationary sticky panel caught 40 male and female flies in 24 hours. (It is unknown whether, in addition to the movement of the vehicle, other parameters like the exhaust gas may have influenced the responses of the flies.) The observed beneficial effect of trap movement on fly captures, however, deserves further attention, for example by conducting tests with swinging traps.

Table 4.1.22. Daily mean catches of *G. swynnertoni* by traps without odour baits

Trap type	Known target species	Number of flies caught
Biconical	*G. palpalis*,* *G. brevipalpis**	7.5
S3	G. swynnertoni**	16.25
Pyramidal	G. *palpalis*,* *G. fuscipes**	10.25
Nzi	Tsetse and biting flies	15.75

*According to Ref. [62].
**According to Ref. [106].

Comparison of odours on trap catches

The relative effectiveness of odours on capturing the target fly species was tested using two different traps available at the time of the test: the S3 trap for *G. swynnertoni* and *G. morsitans centralis* (15–26 October 2000) and the H trap for *G. brevipalpis* and *G. pallidipes* (24 May – 6 June, 2001). The studies for *G. swynnertoni* and *G. brevipalpis* were carried out at Sangaiwe and Mivumoni, respectively. All field tests were done with five treatments fully randomised over five days in a 5 × 5 Latin square design. Trap catches were compared statistically using ANOVA.

Tests of conventional and new odour attractants on G. swynnertoni

The effects of various odour combinations on *G. swynnertoni* catches using the S3 trap are shown in Tables 4.1.23 to 4.1.25. In general, slightly more females were caught. Females caught in treatments (Table 4.1.23) were dissected to determine the age, physiological and reproductive status using the ovarian configuration method. No bias towards particular age classes could be detected. Most females trapped were unfed.

Table 4.1.23. Effect of odour baits on daily mean catches of male and female *G. swynnertoni* in S3 trap at Sangaiwe, Tarangire National Park (15–26 October 2000)

Bait description[†]	Total		Males		Females	
	Detrans Mean	Catch Index[‡]	Detrans Mean	Catch Index[‡]	Detrans Mean	Catch Index[‡]
Unbaited trap	13.02	1.00	4.53	1.00	8.33	1.00
POCA	19.78	1.52*	6.70	1.48	12.83	1.54*
POCA + decyl formate	16.15	1.24	4.69	1.03	10.80	1.30
POCA + octyl formate	21.66	1.66**	9.02	1.99**	12.16	1.46*
POCA + decanal	23.46	1.80***	8.67	1.91**	13.54	1.63**

[†]POCA = propylphenol + octenol + *p*-cresol (1:4:8) in PE sachet plus acetone in bottle dispenser.
[‡]Significance levels (difference relative to unbaited trap): * P<0.05; ** P<0.01; *** P<0.001.

Table 4.1.24. Effect of odour baits on daily mean catches of male and female *G. swynnertoni* in S3 trap at Sangaiwe, Tarangire National Park (15–26 October 2000)

Bait description[†]	Total		Males		Females	
	Detrans Mean	Catch Index	Detrans Mean	Catch Index	Detrans Mean	Catch Index[‡]
Trap without odour	11.91	1	4.40	1	7.19	1
POCA + *P. pumilionis* oil	19.03	1.60	8.04	1.83*	10.50	1.46
POCA + *P. sylvestris* oil	13.04	1.09	6.28	1.43	6.69	0.93
POMA	16.60	1.39	8.16	1.86*	8.52	1.18
Acetone	20.18	1.69*	7.93	1.80*	12.45	1.73*

[†]POCA = propylphenol + octenol + *p*-cresol (1:4:8) in PE sachet plus acetone in bottle dispenser; POMA = propylphenol + octenol + *m*-cresol (1:4:8) in PE sachet plus acetone in bottle dispenser.
*Significantly different relative to unbaited trap (P<0.05).

Table 4.1.25. Effect of odour baits on daily mean catches of male and female *G. swynnertoni* in S3 trap at Sangaiwe, Tarangire National Park (15–26 October 2000).

Bait description[†]	Total		Males		Females	
	Detrans Mean	Catch Index[‡]	Detrans Mean	Catch Index[‡]	Detrans Mean	Catch Index[‡]
Trap without odour	14.72	1	4.70	1	9.77	1
Octenol	21.49	1.46	6.92	1.47	13.50	1.38
P. pumilionis oil	21.05	1.43	9.13	1.94	11.40	1.17
P. sylvestris oil	20.66	1.40	8.78	1.87	11.36	1.16
POCA + *P. sylvestris* oil + decyl formate	23.86	1.62	9.01	1.91	14.55	1.49

[†]POCA = propylphenol + octenol + *p*-cresol (1:4:8) in PE sachet with acetone in bottle dispenser.
[‡]Differences observed were statistically not significant.

Table 4.1.26. Summary table for response of male and female *G. morsitans centralis* to the S3 trap baited with different odour baits at Sangaiwe, Tarangire National Park (15–26 October 2000).

Bait description[†]	Detrans Mean	Catch index[‡]
Unbaited trap	10.68	1
POCA	103.01***	9.64
POCA + decyl formate	105.60***	9.89
POCA + octyl formate	131.22***	12.28
POCA + decanal	115.28***	10.79
POCA + *P. pumilionis* oil	37.03***	5.32
POCA + *P. sylvestris* oil	25.95**	3.72
POMA	12.88	1.85
Acetone	12.66	1.82
Octenol	21.42	1.52
P. pumilionis oil	15.32	1.09
P. sylvestris oil	39.53**	2.80
POCA + *P. sylvestris* oil + decyl formate	84.71***	6.00

[†]POCA = propylphenol + octenol + *p*-cresol (1:4:8) in PE sachet plus acetone in bottle dispenser. POMA = propylphenol + octenol + *m*-cresol (1:4:8) in PE sachet plus acetone in bottle dispenser.
[‡]Significance levels (difference relative to unbaited trap): ** P<0.01; *** P<0.001.

Response of Glossina morsitans centralis *to the odours during the test*

The *G. morsitans centralis* infestation in the study area also permitted an assessment of their relative responses to the baits. A separate statistical analysis of trap catches for this species was performed using the GV.xls programme. Table 4.1.26 shows the results of the statistical analysis for *G. morsitans centralis* males and females pooled.

The two species of tsetse studied in the Tarangire National Park, i.e., *G. swynnertoni* and *G. morsitans centralis*, are very closely related. There is even evidence for some interspecific mating occurring. The two species were abundant at the trapping sites and they entered unbaited traps in equal numbers. POCA significantly increased trap catches over the trap alone for both species. Adding other odours to the POCA blend tends to increase catches further: decanal and octyl formate added to POCA affected both *G. swynnertoni* and *G. morsitans centralis,* whereas the essential oils of *P. sylvestris* and *P. pumilionis* added to the POCA mixture resulted in increased trap catches for *G. morsitans centralis*. Among the volatiles added individually to the traps, only acetone significantly attracted *G. swynnertoni*, whereas the essential oil of *P. sylvestris* significantly increased *G. morsitans centralis* catches.

Response of G. brevipalpis *and* G. pallidipes *to odours*

For *G. brevipalpis* studied at Mivumoni the best treatments added to the H trap were POCA + decanal and POCA + the essential oil of *P. sylvestris* ($P<0.05$) (Table 4.1.27). It is equally important to note that adding acetone, POCA, POCA + *P. pumilionis* oil, POCA + octyl formate, and POCA + *P. sylvestris* oil + decyl formate more than doubled catches, although the differences recorded were not statistically significant.

For *G. pallidipes*, also studied at this site, acetone alone as well as all blends containing POCA increased catches significantly.

Table 4.1.27. Mean catches of male and female *G. brevipalpis* and *G. pallidipes* to odour baits in H traps, Mivumoni, 24 May – 6 June, 2001

Bait description[†]	*G. brevipalpis* (males + females)		*G. pallidipes* (males + females)	
	Detrans mean	Catch index	Detrans mean	Catch index
Unbaited trap	0.10	1.00	2.14	1.00
POCA	0.41	4.32	6.92**[‡]	3.24
POCA + decyl formate	0.19	1.97	8.15***	3.81
POCA + octyl formate	0.30	3.16	10.85***	5.08
POCA + decanal	0.60*	6.25	8.88***	4.15
POCA + *P. pumilionis* oil	0.50	5.24	10.16***	4.76
POCA + *P. sylvestris* oil	0.70*	7.28	9.66***	4.52
Acetone	0.51	5.35	4.91*[‡]	2.30
Octenol	0.33	3.49	2.90	1.36
P. pumilionis oil	0.23	2.40	2.33	1.09
P. sylvestris oil	0.00	0.00	3.50	1.64
POCA + *P. sylvestris* oil + decyl formate	0.38	3.97	7.86**	3.68

[†]POCA refers to propylphenol : octenol : *p*-cresol (1:4:8) plus acetone.
[‡]The effect of POCA plus acetone was significant only for females.
Significance of differences, if any (relative to unbaited trap): * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Comparison of laboratory and field results

As mentioned earlier both *G. brevipalpis* and *G. pallidipes* respond to several natural and synthetic odours in the laboratory. Data shown in Table 4.1.27 indicate that experimental results obtained with POCA in the wind tunnel (Figs. 3.6 and 3.7) predict field attractivity of this blend for each species provided that the odour blend is used in combination with an effective trap design.

Conclusions

Some of the tested odours attract *G. swynnertoni.* This is evidenced by significant catch increases of flies in traps that were not effective without these odours or odour blends. This means that some of the identified odours / odour blends are efficient attractants inducing a searching behaviour that makes the flies find and enter the trap.

Some odours being studied are highly effective in attracting *G. morsitans centralis.*

G. brevipalpis show little response to the H trap without an attractant odour. The use of some odours significantly increased catches of this species.

G. pallidipes responds to acetone and also to POCA combined with other odours but does not respond to octenol and to the two pine essential oils tested. This behaviour is similar to that of *G. morsitans centralis*.

4.1.3. Uganda

Glossina fuscipes fuscipes, a member of the *palpalis* or riverine group of tsetse flies, inhabits riverbanks and lakeshores in close ecological association with reptiles, wild ruminants and birds. *G. fuscipes fuscipes* occupies approximately two thirds of the tsetse-infected areas of Uganda. This species is an opportunistic feeder and has been able to adjust to new hosts. This behaviour has made it a dangerous vector of both human and animal trypanosomosis. At Lake Victoria, the major host of the species is the monitor lizard, *Varanus niloticus niloticus* [115].

The biconical trap is the main device currently used to survey and control the *palpalis* group of tsetse flies [62]. In Uganda, the pyramidal trap has also been widely used as a survey and control tool for *G. fuscipes fuscipes*. Much of the recent control has been achieved through the use of insecticide impregnated pyramidal traps [116].

This trap was part of the integrated effort used to contain the sleeping sickness epidemic in 1987–1988 in the country. However, the main drawback in the control of *G. fuscipes fuscipes* using pyramidal traps has been the large number of traps required per km^2. Unlike some areas of Africa where as few as four odour baited targets/km^2 have been used successfully for controlling savannah tsetse species, bigger numbers of pyramidal traps are required for intervention against *G. fuscipes fuscipes*. Depending on vegetation density, control in some areas requires between 25 and 30 permanently placed and serviced (regularly clearing the surrounding vegetation, etc.) traps/km^2.

The need for this large number of traps is due to the thick vegetation preventing the traps to be visible for the flies over sufficiently long distance. The identification and use of effective odour baits is hoped to compensate for this and improve trap efficiency for this riverine species. Chorley's early observation that olfactory cues are important in the host-location of *G. fuscipes fuscipes* [117, 118] was corroborated recently [38, 119, 120] but the chemical

components responsible for attraction remain to be clarified. Therefore it was decided to examine several known odours and new odour candidates to find appropriate bait composition to improve the pyramidal trap for *G. fuscipes fuscipes*. Captures of this species by the pyramidal trap and other known trap types were also compared.

General methods and materials

The studies were conducted on Buvuma Islands, Lake Victoria, Mukono district. The tsetse species in the area is *G. fuscipes fuscipes*. The experimental sites were identified in the hinterland of the island. The vegetation in the area is made of evergreen thickets and shrubs resulting from the regenerating forest. Human activity is very minimal with only small-scale charcoal burning and some subsistence agriculture with cassava, bananas and maize as a base. Once trap sites were identified, the surrounding vegetation was cleared to increase visibility and to allow light falling over the top of the trap.

The experiments conducted followed a completely randomised Latin Square design. Traps were set at 9.00 a.m. for a period of 24 hr, thus covering the two activity peaks of the fly during the day. Then the traps were transferred to the next randomly identified site. The different bait/trap combinations were statistically compared using ANOVA.

Field evaluation of different combinations of candidate attractants for Glossina fuscipes fuscipes

All studies were conducted in the villages of Lukoma, Galatiya and Wabivu on Buvuma Islands, Lake Victoria, during the dry season. The vegetation in the area is riparian forest, with *Acacia* species dominant along at the shoreline. Monitor lizards and monkeys are the commonest wild animals. The experimental sites set at intervals of 100 –150 metres along the shoreline, where high tsetse populations are found. For each experiment a 4 × 4 Latin square design was used in one replicate.

The effectiveness of several known kairomones and new odours, alone or in combination, for tsetse was tested with pyramidal traps [116] with top cages for fly collection. The synthetic odours were packed in 4 cm × 4 cm PE sachets filled with 3–4 ml of the test samples. Acetone was dispensed from 200 ml glass bottles with a 2–3 mm diameter hole in the cover. Vegetable oil (TAMU) and corm oil (Elianto vegetable cooking oil) were purchased from local market and were dispensed from ca. 5 cm × 5 cm PE sachets. Pine essential oils were dispensed from 2.5 ml capped cylindrical PE vials. The sachets and vials were tied with a wire hanging from a black flap of the trap. The acetone bottle was inserted halfway into a hole dug one foot from the trap. *G. fuscipes fuscipes* males and females collected from the traps on consecutive days were counted separately.

Results

The results of the experiments conducted in 1998 and 1999 on Buvuma Island, Lake Victoria, are shown in Tables 4.1.28 to 4.1.33.

Table 4.1.28 shows the effect of various two-component odour blends on catches of *G. fuscipes fuscipes* by pyramidal traps. The binary mixtures of the putative lizard kairomone aldehydes and their formate analogues did not influence trap catches. The mixture of the two nitrile analogues halved the captures by the pyramidal trap indicating that the combination of these compounds may act as a repellent to *G. fuscipes fuscipes*.

Table 4.1.28. Daily mean catches of male and female *G. fuscipes fuscipes* by pyramidal traps baited with experimental odours, Buvuma Island, Lake Victoria, March 1998. Catch indices were calculated relative to unbaited pyramidal trap

Bait description	Detrans mean	Catch index
Unbaited trap	46.42	1.00
Decanal + dodecanal (1:1)	38.56	0.83
Octyl formate + decyl formate (1:1)	45.28	0.98
Decanitrile + dodecanitrile (1:1)	24.70	0.53*

*Significantly different relative to unbaited trap and other odours ($P<0.05$).

In Table 4.1.29 catch indices for some single component odour baits and two vegetable oils are shown. Clearly, none of the experimental odours increased catches of the pyramidal trap.

Table 4.1.29. Daily mean catches of male and female *G. fuscipes fuscipes* by pyramidal traps baited with experimental odours, Buvuma Island, Lake Victoria, March – April 1999. Catch indices were calculated relative to unbaited trap. (Differences observed were statistically not significant.

Bait	Catch index
Unbaited trap	1.00
Octenol	0.89
Decanal	0.82[†]
Dodecanal	0.96[†]
Decanitrile	1.06
Octyl formate	1.28[†]
Decyl formate	0.85
Corn oil (Elianto)	0.94
Vegetable oil (TAMU)	1.10

[†]These compounds were included in two separate tests carried out on different days. The two different experimental set-ups did not catch a significantly different number of flies if compared to the control (unbaited trap).

The results of subsequent experiments are shown in Table 4.1.30. The blend of propylphenol + octenol + *p*-cresol and acetone (POCA), an efficient attractant for several savannah species, did not enhance the attractivity of the pyramidal trap. New synthetic odours also proved ineffective. Neither single odours components such as acetone and octenol nor the blend of propylphenol + octenol + *m*-cresol and acetone (POMA) were effective (Table 4.1.31).

Table 4.1.30. Daily mean catches of male and female *G. fuscipes fuscipes* by pyramidal traps baited with experimental odours, Buvuma Island, Lake Victoria, 28 March – 31 March 2001. Catch indices were calculated relative to unbaited trap. (Differences observed were statistically not significant.)

Bait description[†]	Detrans mean	Catch index
Unbaited trap	63.94	1.00
POCA	69.50	1.09
POCA + octyl formate	43.02	0.67
POCA + decyl formate	45.19	0.71

[†]POCA = propylphenol + octenol + *p*-cresol (1:4:8) in PE sachet plus acetone in bottle dispenser.

In another experiment, however, the essential oil of the pine *Pinus pumilionis* increased catches over twofold (Table 4.1.32). A detailed analysis of the data by sexes indicated that there was a significant difference ($P<0.05$) for male and female catches with traps baited with this essential oil giving catch increases of 1.87 and 2.79, respectively, for the sexes. When *P. pumilionis* oil was used in combination with the POCA blend, no catch increase was observed (Table 4.1.33).

Table 4.1.31. Daily mean catches of male and female *G. fuscipes fuscipes* by pyramidal traps baited with experimental odours, Buvuma Island, Lake Victoria, 18 April – 21 April 2001. Catch indices were calculated relative to unbaited pyramidal trap. (Differences observed were statistically not significant.)

Bait description[†]	Detrans mean	Catch index
Unbaited trap	97.75	1.00
Acetone	103.82	1.06
Octenol	105.57	1.08
POMA	116.98	1.20

[†]POMA = propylphenol + octenol + *m*-cresol (1:4:8) in PE sachet plus acetone in bottle dispenser.

Table 4.1.32. Daily mean catches of male and female *G. fuscipes fuscipes* by pyramidal traps baited with experimental odours, Buvuma Island, Lake Victoria, 18 April – 21 April 2001. Catch indices were calculated relative to unbaited pyramidal trap

Bait description[†]	Detrans mean	Catch index
Unbaited trap	36.06	1.00
Pinus pumilionis oil	85.09	2.36*
Pinus sylvestris oil	26.04	0.72*
POCA + *P. sylvestris* oil + decyl formate	48.69	1.35

[†]POCA = propylphenol + octenol + *p*-cresol (1:4:8) in PE sachet plus acetone in bottle dispenser.

*Significantly different relative to the pyramidal trap ($P<0.05$).

Table 4.1.33. Daily mean catches of male and female *G. fuscipes fuscipes* by pyramidal traps baited with experimental odours, Buvuma Island, Lake Victoria, 28 March – 31 March 2001. Catch indices were calculated relative to unbaited pyramidal trap. (Differences observed were statistically not significant.)

Bait description[†]	Detrans mean	Catch index
Unbaited trap	51.01	1.00
POCA + decanal	71.57	1.40
POCA+ *Pinus pumilionis* oil	57.38	1.12
POCA + *Pinus sylvestris* oil	56.95	1.12

[†]POCA = propylphenol + octenol + *p*-cresol (1:4:8) in PE sachet plus acetone in bottle dispenser.

In summary, experiments in the dry season of 2000 showed that among the new odours, only the essential oil of *P. pumilionis*, which is a mixture of various terpenes derived from the Swiss mountain pine, increased catches of the pyramidal trap by 2.36-fold with females more responsive than males. The study also established that the blend of acetone and the 1:4:8

mixture of propylphenol + octenol + *p*-cresol (or *m*-cresol) do not enhance the capture rate of *G. fuscipes fuscipes* in the pyramidal trap.

Studies on the effect of odours and taps on G. fuscipes fuscipes *using electrified grids*

The development of electrified nets or grids provided a very useful tool for studying tsetse behaviour in response to visual and olfactory stimuli [121, 122, 123]. The placement of an electrified grid in the close vicinity of traps allows an adequate assessment of the close-range attraction of the trap and to differentiate between flies that are trapped and the ones that are attracted but do not enter the trap.

The present study used electrified grids to evaluate the attractiveness of the pyramidal trap baited either with octenol, a potent attractant component of ox head volatiles, or octyl formate, a potential attractant analogue of decanal, which is a purported kairomone from monitor lizards [120] (but see [38]). The experiment was carried out according to a fully randomised 3 × 3 Latin square design using two replicates.

The study was conducted on Buvuma Island in Lake Victoria in January 2000 with mean daily temperature of 28^{o}C. The square-shaped (1 m × 1m) electrocuting nets used were powered by 50 kV output spark boxes connected to 12 V lead-acid batteries. The electrified grids were set a few centimetres apart from the pyramidal trap and faced the lakeshore. Electrocuted flies were collected on a corrugated aluminum sheets located under the grid and coated with a non-drip insect trapping adhesive for subsequent counting.

The results of the experiments are shown in Table 4.1.34. Catch indices, relative to the unbaited trap, for flies caught in the cages of the pyramidal trap and electrocuted by the grid were analysed separately for the two sexes. There was no significant difference between the odours and the control regarding the total number of flies attracted (electrocuted in the grid) or trapped (in the pyramidal trap). Also, no difference was found for the number of males attracted or trapped by either odour bait. However, two synthetic odours significantly increased the number of females attracted: octenol and decyl formate were, respectively, 3.76- and 4.17-fold more attractive (electrocuted, but no trap entering) than the unbaited control.

Table 4.1.34. Daily mean catches of male and female *G. fuscipes fuscipes* by electrified grid and pyramidal trap combinations baited with experimental odours, Buvuma Island, Lake Victoria, January 2000. Catch indices were calculated relative to unbaited trap (two replicates)

Bait description	Trap cage catch index			Grid catch index			Cage + Grid Total
	Males	Females	Total	Males	Females	Total	
Unbaited trap	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Octenol	1.15	1.20	1.17	0.51	4.17*	1.67	1.42
Decyl formate	0.98	0.94	0.94	0.68	3.76*	1.60	1.30

*Significantly different relative to the unbaited grid with pyramidal trap and to male catches ($P<0.05$).

The results of these studies show that the ox odour octenol and two formate analogues of a potential natural kairomonal aldehyde do not significantly increase captures of *G. fuscipes fuscipes* by pyramidal traps. It is most interesting, however, that both formates significantly

enhanced the attractivity of the trap towards females as demonstrated by the increases of *G. fuscipes fuscipes* females electrocuted at the grid. A corresponding increase of female trap entry into the trap as not recorded. It has been shown for other tsetse species that a proportion of flies that approach odour baited traps fly round them and just stay in the vicinity of the trap for a while without entering the trap [96, 122]. Mohamed-Ahmed [38] reported a twofold catch increase of *G. fuscipes fuscipes* females when lizard urine was placed in the vicinity of electrified grids but no explanation for the different behaviour of the sexes was given. Although it is encouraging to know that the principle of kairomones functions for female *G. fuscipes fuscipes*, further studies on attractants are needed for this species.

Comparative field evaluation of pyramidal, biconical, NGU, and H traps for catching Glossina fuscipes fuscipes *in Uganda*

As the previous experiment indicated a problem with the entering behaviour of *G. fuscipes fuscipes* into pyramidal traps, studies on different traps were conducted on Buvuma Islands in Lake Victoria in March 2001. The traps used were the biconical trap [90], pyramidal trap modified with a top cage, the NGU trap [112] and the newly developed H trap [96]. All traps were made from the same colours of blue, black and netting material. The performance of these traps to catch *G. fuscipes fuscipes* was assessed in the absence of odour attractants.

Field tests were laid out in a completely randomised 4 × 4 Latin square design with the biconical trap as the control using one replicate. Traps were set up in the morning at 8.00 a.m. and data collected 24 h later then the traps were rearranged. Catches for the two sexes were recorded separately and trap comparisons were made using ANOVA.

The results shown in Table 4.1.35 indicate that the H trap was significantly less effective for *G. fuscipes fuscipes* than the other three traps. The NGU trap caught also less flies but the difference was not statistically significant. No differences were observed between catches of males and females.

Table 4.1.35. The indices of increase, relative to the biconical trap, for daily catches of males and females *G. fuscipes fuscipes*. Buvuma Island, Lake Victoria, 28 March – 31 March 2001

Trap type	Detrans mean	Index of increase
Biconical	117.00	1.00
H trap	1.727	0.01*
Pyramidal	92.535	0.79
NGU	53.004	0.45

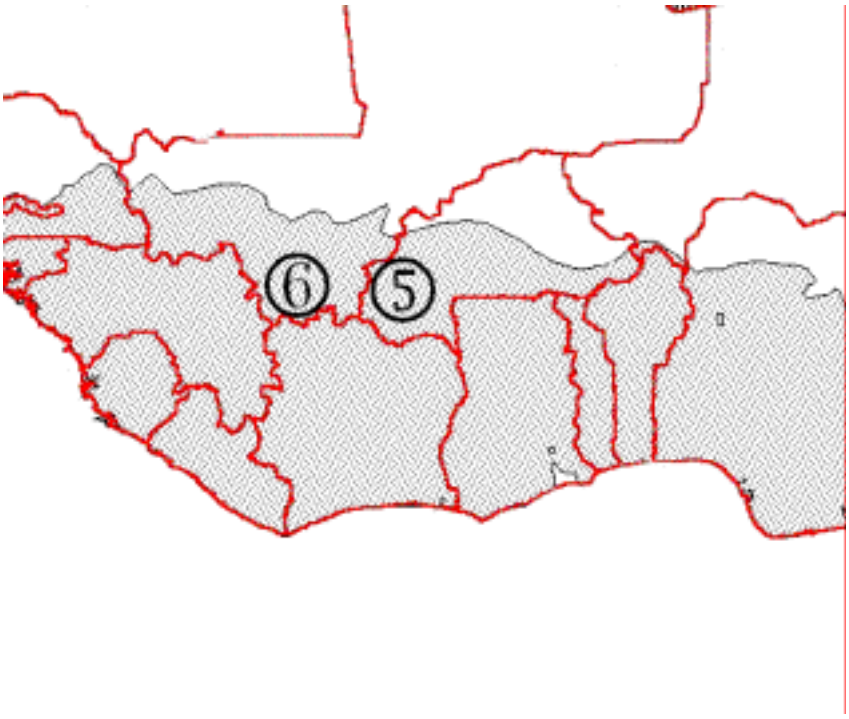
*Significantly different relative to the biconical trap ($P<0.05$).

Discussion

Among tsetse, the principle for distinguishing hosts from other features of the environment such as trees may involve an adaptive process that may bring along a preference for shapes and sizes of objects that resemble the natural hosts. For example *G. morsitans morsitans* and *G. pallidipes* feed mainly on suids and bovids and only rarely on primates, and they have a preference for horizontal, compact trap shapes. On the other hand, *G. palpalis* takes a significant number of feeds from man [124] and is more attracted to upright trap shapes. The

poor performance of the horizontally elongated H trap suggests that *G. fuscipes fuscipes* may be attracted to differently shaped objects. Although the identification of kairomones that induce trap approaching behaviour is an encouraging finding, the lacking trap entry behaviour underlines that additional work is needed both on trap design and odour attractants for *G. fuscipes fuscipes*.

4.2. Trapping Glossina *species in West Africa*



Burkina Faso: ⑤,

Mali: ⑥.

(For details, see text.)

Shaded areas indicate tsetse-infested regions.

Figure 4.5. Location of experimental sites in West Africa.

4.2.1. Burkina Faso

Development of attractants for Glossina palpalis gambiensis *and* Glossina tachinoides

In West Africa two main riverine species, *Glossina palpalis gambiensis* and *Glossina tachinoides* occur sympatrically. There are some attractants for *G. tachinoides* [125, 126], but this is not the case for *G. palpalis gambiensis*. The Challier-Laveissière biconical trap [90] and the Vavoua monoconical trap [127] have been used to catch these two species relatively efficiently, but there is still need for further improvements of the trapping systems.

Analysis of blood meals from different *Glossina* spp. show that riverine *Glossina* take most of their meals from reptiles, particularly monitor lizard [128]. This indicates the existence of compounds in reptiles that attract these tsetse fly species. In order to understand what may attract riverine tsetse species, several laboratory and field studies were conducted between 1996 and 2001.

Experiments with monitor lizard in a stable

First, the attractivity of immobile and mobile monitor lizards for *G. palpalis gambiensis* was compared. A 0.5 kg monitor lizard (*Varanus niloticus*) was put in a blue nylon net and then laid immobile in the middle of a 3.2 × 4.4 × 2 m^3 fly proof stable. The net mesh was large enough to allow flies to pass through. For every trial, 50 two-day-old teneral *G. palpalis gambiensis* from the Centre International de Récherche Developpement sur l'Elevage en zone Subhumide (CIRDES) insectaries were released over two hours, thereafter all flies were caught and separated as either engorged and unfed flies. After this first test the immobile monitor lizard was released from the nylon net to enable its free movement in the room. Another batch of 50 flies were released and, two hours later recaptured to count again the number of fed and unfed flies. These two 2-h tests were repeated ten times.

The results, shown in Fig. 4.6, indicate that practically, no fly took blood from the immobile monitor lizard and, on average, only 1% fed on it, while 39% of the flies had fed on the mobile lizard.

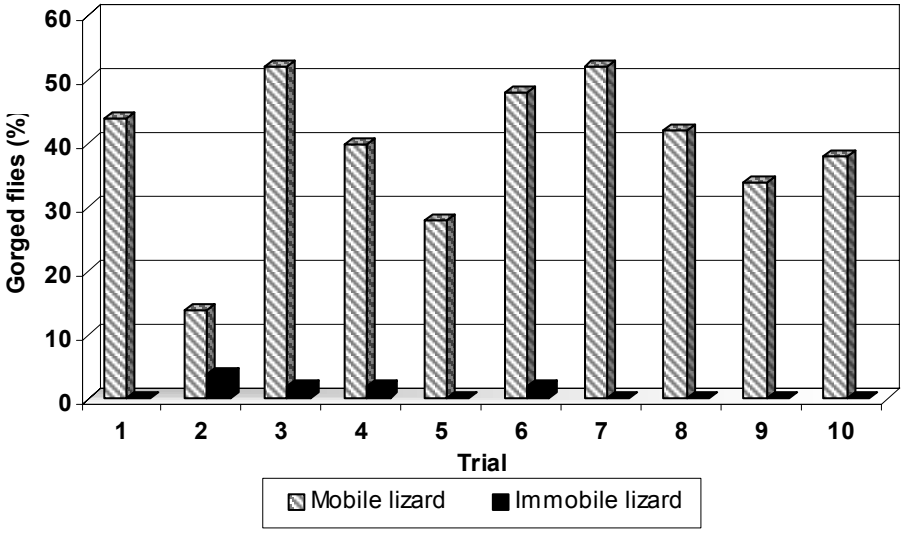


Figure 4.6. Feeding behaviour of G. palpalis gambiensis on monitor lizard.

Discussion

The species of the described studies belong to the *palpalis* group inhabiting riverine vegetation and have been observed to rely mostly on reptiles, including crocodiles and monitor lizards, as host. Challier [129] and Laveissière [130] reported that some 54 % of *G. tachinoides* fed on reptiles, whereas Küpper et al. [131] observed that flies of this species prefer ruminants and hippopotamus while only 19% feed on monitor lizards. *G. palpalis gambiensis* was found to prefer varans as hosts [128]. The understanding what host odour may be involved in the recognition and finding of these reptile hosts is important for the identification of improved trap/bait system for riverine tsetse species in West Africa.

Visible but immobile monitor lizards did not attract *G. palpalis gambiensis*. It should be noted that flies were not dissected for engorgement determination, so the ones that were only lightly engorged were counted as unfed. Because 39% of the flies did feed on moving monitor lizard, it is evident that they were attracted by the reptile. As the monitor lizard moved in the room the distance to the flies changed, which was not the case with the immobile lizard, which may have influenced the experiments. Nevertheless, previous studies with the *morsitans* group also have shown that responses to host odours of tsetse of are activated by host movements [132].

Field trials with attractants

Experiments in Kourouma with monitor lizards. This work was carried out in April and May in 1996 at Kourouma, a village located about 100 km from Bobo–Dioulasso, North–West Burkina Faso. Biconical traps were set up along the Dougnana River that was bordered by forest gallery. The adjacent vegetation was woody savannah. The experiments compared in a Latin square design, the empty trap (control) with two other traps containing monitor lizards (*V. niloticus*), about 0.5 kg each. At the beginning, the lizards were put individually in a silk bag and hung up inside the trap for 10 hours, from 7:00 am to 5:00 pm. For the subsequent experiments, the silk bag was replaced with a black and white nylon net because it was suspected that the silk bag retained too much odour.

Tables 4.2.1 and 4.2.2 show the results of the effect of lizards in traps on catching *G. palpalis gambiensis*. When the lizard was contained in a silk bag no differences were recorded between traps (Table 4.2.1).

Table 4.2.1. Comparison of empty (control) and experimental traps containing a monitor lizard in a silk bag, Kourouma, 9–13 April 1996. Total captures of *G. palpalis gambiensis* do not differ significantly ($P < 0.05$)

Day	**Trap**					
	Empty control		Lizard I		Lizard II	
	Males	Females	Males	Females	Males	Females
1	7	8	14	12	3	10
2	3	9	6	11	12	13
3	11	14	4	11	5	12
Total per sex	21	31	24	34	20	35
Total per trap	52		58		55	

Table 4.2.2. Comparison of empty (control) traps and traps containing a lizard in a net; Kourouma, 20–25 May 1996. Total captures *G. palpalis gambiensis* do not differ significantly ($P < 0.05$)

Days	Site I				Site II			
	Control I		Lizard I		Control II		Lizard II	
	Male	Female	Male	Female	Male	Female	Male	Female
1	1	1	5	0	0	0	0	3
2	4	3	0	0	1	2	0	0
3	0	1	2	0	0	0	0	1
4	0	0	0	0	0	0	2	0
Total per sex	5	5	7	0	1	2	2	4
Total per trap	10		7		3		6	

The results in Table 4.2.2 were obtained by comparing traps containing a monitor lizard in a net with empty traps simultaneously at two sites. The experiments were repeated twice. Again, no differences were found between traps with or without monitor lizards.

Experiments in Samorogouan with monitor lizards. This work was carried out along the Pindia River, near the village of Tenasso, Kénédougou Province. Due to the proximity of the village, the site is frequented by people and animals. Although agricultural activities had caused a degradation of both soil and vegetation, there are still some intact forest areas. The study was conducted from December 1996 to January 1997. In separate tests with monoconical and biconical traps the effect of absence (control) and presence of a monitor lizard in a trap on tsetse captures was assessed following a 2 × 2 Latin square design. The test was repeated 8 times. Blood meal smears of the gut of captured flies were collected on filter paper for determination of food source using enzyme-linked immunoassay (ELISA) [133, 134]. The ELISA used animal-specific antisera developed at the GTZ/BgVV Service-Laboratory (Berlin, Germany). First, samples were tested for blood of human, ruminant, suids, domestic pig, warthog, bushpig, monitor lizard, and avian origin. Samples identified as of ruminant origin were differentiated in a second test for cow, sheep, goat, buffalo, bushbuck, duiker, kob, and hartebest blood. Samples that gave no positive ELISA-response were tested again in a third series for dog, elephant, donkey, fowl, hippopotamus, crocodile, rat, and lion blood.

Results

In all replicates, the monoconical traps caught only *G. palpalis gambiensis*. In total 440 flies were trapped during the 2 week test period (more than 27 flies per trap per day). With biconical traps, altogether 223 flies were caught (mean catches of 14 per trap per day). Irrespectively of the type of the trap, more males were captured (67.4%) (Table 4.2.3).

Table 4.2.3. Daily mean captures (± SEM) of *G. palpalis gambiensis* without or with a monitor lizard in traps, Samorogouan, 17–21 December, 1996 and 7–11 January 1997 (n = 8).

Sex	Monoconical trap (Site I)			Biconical trap (Site II)		
	empty	with lizard	ratio	empty	with lizard	Ratio
Males	9.88±6.08	8.5±6.14	0.86	3.38±2.33	5.25±4.13	1.55
Females	21.38±17.11	15.25±7.74	0.71	10.38±7.37	8.88±9.26	0.86
Total	31.25±21.66	23.75±12.29	0.76	13.75±9.22	14.13±12.18	1.03

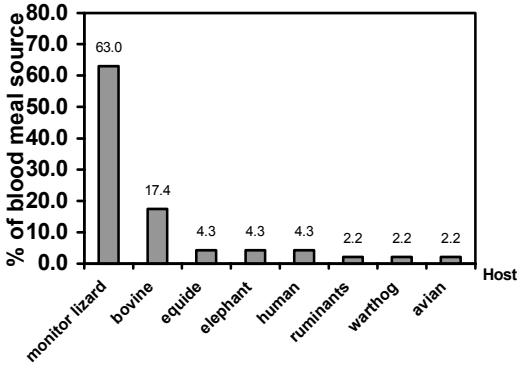


Figure 4.7. Host preference of G. palpalis gambiensis as determined by ELISA from blood of trapped flies, Samorogouan, December 1996 – January 1997 (n=46).

ELISA analysis of the blood meals showed that from 46 identified meals, most flies had fed on monitors lizards, followed by cattle (Fig. 4.7).

Discussion of experiments with monitor lizards

The experiments in Kourouma were conducted during the dry season, and the relative low numbers of captures observed (Tables 4.2.1 and 4.2.2) are accountable to environmental conditions. This must also be the reason why no *G. tachinoides* was caught (during dry cold season, the usually recorded apparent density for this species is 4 flies per trap per day).

The high density of *G. palpalis gambiensis* in Samorogouan indicates their abundance in the area during the dry cold season. Differences recorded for the two trap types may be influenced by site-related and other parameters.

For both traps, monoconical and biconical, the presence of an essentially immobile monitor lizard in the trap does not seem to influence *G. palpalis gambiensis* behaviour and trapping efficiency. In the case of the monoconical trap the empty controls caught more flies than the experimental traps with immobile monitor lizard.

Immobile monitor lizards apparently do not attract *G. palpalis gambiensis*, as their presence has no positive influence on trap captures. This seems to contradict the finding that reptiles, particularly the small monitor lizard, which can easily hide and may frequently not visible for the flies, provide an important component of the *G. palpalis gambiensis* blood meal (Fig. 4.7), particularly from lizard.

The experimental conditions could have also influenced the results. For example: a) the thickness of the fabric used to make the trap may have reduced the diffusion of the reptile odours; b) the lizard was not clearly visible to the flies, and it was immobile in the trap, while trials in laboratory (stable) showed that mobile lizard attract more tsetse flies than immobile ones. Also, tsetse could be attracted by lizards but the flies may not enter the trap for some reason. This issue could be assessed by using electrified grids.

Comparison of trap types

In order to find the optimal trap for further field experiments, four trap types were compared at two locations from October to November 1997 for *G. palpalis gambiensis* along the Mouhoun River in Padema District, Western Burkina Faso, using 4 × 4 Latin square design. Trap catches were compared statistically using ANOVA. The results are shown in Table 4.2.4.

Table 4.2.4. Mean catches of *G. palpalis gambiensis* fly (both sexes) by various traps in Padema District, October – November 1997 (n = 26). The catch indices are not significantly different from unity

Trap type	Flies captured (males + females)	
	Mean	Catch Index
Biconical	13.06	1
Monoconical	21.54	1.65
Ecran Gouteux (Screen trap)	9.00	0.69
Nzi	2.24*	0.17

It must be noted that the biconical and the monoconical traps are relatively small in size compared to the two other traps. The results show that the monoconical trap is the best even the difference is not significant. (This could explain why many others trials done in the same location or elsewhere showed frequently the biconical as the best trap for *G. palpalis gambiensis*.) However, for Tabanids, the Nzi and the screen traps proved to be the best (data not shown).

Experiments with attractants in Burkina Faso in 1997 and 2001

A part of the work in 2001 was undertaken simultaneously at two sites from 27 February to 1 July. One site was Solenzo, Banwa Province, in the west part of Burkina Faso, about 200 km from Bobo-Dioulasso; the other was along the banks of the Mouhoun River in the village of Montionkuy, also Banwa Province. Biconical and monoconical (Vavoua) traps were compared at both sites using a Latin square design. In addition, the attractivity of four odours was compared using unbaited traps as control, also applying a Latin square design random placement of the traps. The different odours included the established odour blend attractant used for many savannah species, i.e. a mixture of propylphenol, octenol, *p*-cresol (4.0 ml of their 1:4:8 blend formulated in PE sachets) plus acetone (in a 50 ml glass bottle glass bottle with a plastic cap with a 0.7 mm diameter hole) (POCA); *P. sylvestris* essential oil (1.5 ml in 2.5 ml PE vials); and decyl formate (3.8 ml in PE sachets) as well as their combinations. Except for the acetone bottle that was set on the ground under the trap, the sachets and vials were attached to the fabric of the trap. The distance between each trap was about 500 m. The traps and odours were set up at about 7 am and fully randomized rotation took place every 24 h over 15 days.

The effects of odours on the captures by monoconical and biconical traps are shown in Tables 4.2.5 and 4.2.6. Only two tsetse species, *G. palpalis gambiensis* and *G. tachinoides* were captured in the two trap types.

During the three repetitions, the daily average capture was about 37 flies per trap with a domination of *G. tachinoides* (more than 56%) over *G. palpalis gambiensis*. On average, the male to female sex ratio was about 1:2 for each species (Table 4.2.5). Treatments containing the POCA blend caught more than the unbaited traps of both species (Table 4.2.5). For *G. palpalis gambiensis*, the inclusion of POCA blend increased the trap catch by more than 1.6, although the difference was not significant. However, traps with POCA caught significantly ($P<0.03$) more *G. tachinoides* than unbaited ones. Inclusion of other odour candidates did not improve captures rates.

Table 4.2.5. Attractivity of various odours on captures of two *Glossina* species in biconical trap in Montionkuy, Banwa Province, 27 February – 1 July 2001 (n = 15)

Bait description†	*G. palpalis gambiensis*			*G. tachinoides*		
	Daily mean captures		Catch Index	Daily mean captures		Catch Index
	Male/Female	Total		Male/Female	Total	
Unbaited trap	4.86/6.33	11.19	1	4.13/7.93	12.06	1
POCA	7.33/14.0	21.33	1.90	9.73/17.4	27.13*	2.25
P. sylvestris oil	3.66/7.26	10.92	0.98	4.93/9.53	14.46	1.20
POCA + *P. sylvestris* oil	6.66/11.6	18.26	1.63	7.8/18.06	25.86*	2.14
POCA + *P. sylvestris* oil +decyl formate	7.73/10.8	18.53	1.66	11.66/14.46	26.17*	2.17

†POCA = propylphenol + octenol + *p*-cresol (1:4:8) in PE sachet plus acetone in a bottle dispenser.

*Significantly different from unbaited control (ANOVA, $P<0.05$).

For *G. palpalis gambiensis*, the biconical trap with the POCA blend trapped more flies than unbaited controls but the differences were not always significant. With monoconical traps, all the treatments caught less than the control.

In case of *G. tachinoides*, the POCA odour blend in many cases doubled the trap catches and the differences were significant. With regards to the monoconical trap only the POCA + *P. sylvestris* essential oil blend increased catches significantly (>2.7-fold).

In trials with monoconical traps, the average apparent density recorded was 18 flies/trap/day. In contrast to the captures with biconical traps, *G. palpalis gambiensis* was the predominant species in this case (>54%). The sex ratio was similar to the one observed with biconical traps. For *G. tachinoides*, only the POCA + *P. sylvestris* oil odour blend resulted in significantly increased captures rates (Table 4.2.6).

The predominance of *G. tachinoides* during the test could be linked to the fact that the trials lasted through the early phase of the rainy season when this species is more abundant. During preliminary surveys in January, *G. palpalis gambiensis* was the predominant species. *G. tachinoides* seems to be sensitive to the odour blends as they all caught more flies than the unbaited control, irrespective of the trap. This confirms earlier reports [134, 135] on the response of *G. tachinoides* to attractants. In addition, it appears that a combination of propylphenol, octenol, *p*-cresol and acetone (POCA), the odour blend developed originally for the *morsitans* group species, also increases trap catches of species belonging to the *palpalis* group of tsetse flies.

Table 4.2.6. Attractivity of various odours in Vavoua monoconical trap to *Glossina* species in Montionkuy, Banwa Province, 27 February – 1 July 2001 (n = 15)

Bait description[†]	*G. palpalis gambiensis*			*G. tachinoides*		
	Daily mean captures		Catch Index	Daily mean captures		Catch Index
	Male/Female	Total		Male/Female	Total	
Unbaited trap	3.53/8.46	11.99	1	1.66/3.66	5.32	1
POCA	2.66/5.0	7.66	0.64	3.33/4.86	8.19	1.54
P. sylvestris oil	4.06/5.13	9.19	0.77	2.93/3.13	6.06	1.14
POCA + *P. sylvestris* oil	4.4/7.43	11.93	0.99	4.53/9.93	14.46*	2.72
POCA + *P. sylvestris* oil + decyl formate	3.2/5.36	8.56	0.71	3.60/3.60	7.20	1.35

[†]POCA = propylphenol + octenol + *p*-cresol (1:4:8) in PE sachet plus acetone in a bottle dispenser.
*Significantly different from unbaited control (ANOVA, P<0.05).

Comparison of laboratory and field results

With *G. palpalis gambiensis*, positive reactions were obtained with the biconical traps containing POCA alone or with a combination of POCA and other odours. In the wind tunnel the initial flying behaviour of this species was effectively stimulated by POCA (see Figs. 3.8 and 3.9 in Chapter 3.2) but the effect of this odour bait on attraction in the field was not significantly different from that of unbaited control traps. The relatively low numbers of flies landing on the odour source (<10%) in the wind tunnel appears to predict this field behaviour.

Conclusion

The different experiments described above show how difficult it is to develop an attractant for *G. palpalis gambiensis*.

Trials with monitor lizards in a stable revealed that tsetse reactions depend on lizard mobility. This is confirmed by fields trials where immobile lizards had no effect on trap catch. *G. palpalis gambiensis* responds better to biconical traps containing the POCA mixture. This is also supported by wind tunnel data showing that almost 50% of the flies were attracted by this odour composition. The behavioural responses in the wind tunnel were slightly improved by the inclusion of the essential oil of *P. sylvestris*.

G. pallidipes was used as a positive control for these experiments in the wind tunnel, and *G. palpalis gambiensis* showed a similar response. Also, the EAG responses of the species to different odours were very similar. This suggests that optimisation of an attractant for *G. palpalis gambiensis* should continue along the times of what has been undertaken for other better researched *Glossina* species.

The results form laboratory (electrophysiological and wind tunnel) test and field experiments confirm that the work under this CRP identified promising attractant blends for the investigated West African riverine species.

4.2.2. Mali

There are three *Glossina* species in Mali: the riverine species *G. palpalis gambiensis* and *G. tachinoides*, and the savannah group species *G. morsitans submorsitans*, all of which are vectors of trypanosomosis.

In addition to these tsetse flies, other Diptera are also present that, for example in situations of high trypanosomosis, may serve as mechanical vectors. The range of these species includes Tabanidae (*Ancala*, *Atylotus*, *Chrysops*, *Haematopota* and *Tabanus* species) as well as various stable flies (Muscidae: Stomoxyinae).

Most of the experiments under this CRP were carried out at the Madina-Diassa Ranch (7 ^{o} 40' – 7^{o}50' E, 10^{o}40' – 10^{o}50' N), having an area of ca. 18,000 ha and partially bordered by the Baoulé River. The average elevation is 400 m. The climate in the region is of the Sudanese-Guinean type with a rainy season from May to October and a dry season from November to April.

The characteristic savannah vegetation consists of *Isoberlinia doka*, *Uapaca somo* and *Monotes kerstingii*. Of the evergreen and semi-deciduous trees that form a forest belt along the Baoulé River *Pterocarpus santalinoides* and *Cynometra vogelii* are characteristic for the habitat. Wild animals are abundant in the area and the most common ones are monkeys, kob, warthog, buffalo, crocodile, varan, and hippopotamus.

The efficient use of traps, alone or in combination with insecticides, to defeat African trypanosomosis requires a massive communal participation [135]. This would be beneficial from the development of manageable, relatively cheap and durable odour baits. Previous studies were mainly based on the very volatile acetone in high release rate formulations, which needed replacement every 2 to 3 days. It is necessary to find other odours, either single components or odour blends, and formulations that last for months, preferable for three months, which is the required time for re-impregnation of traps and screens with the

insecticide, e.g., deltamethrin. The selection of the most effective trap design is also important.

In comparison with attractants for East African tsetse species, relatively few studies have been carried out with West African *Glossina* species [126]. In Burkina Faso, blue/black targets have been used for the above three *Glossina* species [136]. For *G. morsitans submorsitans*, acetone and octenol were found attractive [137]. For *G. tachinoides*, ox urine, the mixtures of its phenolic components and octenol [125, 126, 137, 138, 139, 140], as well as octenol and acetone [141] have been found attractive. No effective odour bait has been reported for *G. palpalis gambiensis*.

There are only very limited data on the attractiveness of odour baits for tsetse species in Mali. In order to identify odour baits for use in vector control in Mali, various known attractant chemicals and odour blends, as well as some selected new synthetic analogues were studied. In addition to the biconical trap [111], a cheap and widely used trap design, other newly developed trap types were evaluated.

Comparison of trap types

In 2000, the relative attractivity of several unbaited traps was compared. The results shown in Table 4.2.7 indicate that the biconical trap was the best for all *Glossina* species involved in this test.

Table 4.2.7. Catch indices of different traps, relative to biconical trap, for three *Glossina* species caught in the Sudanese-Guinean zone, Madina-Diassa Ranch, dry season, 2000

Trap type	Catch index for		
	G. m. submorsitans	*G. p. gambiensis*	*G. tachinoides*
Biconical	1	1	1
Monoconical (Vavoua)	0.50	0.44 / (1.16[†])	0.42
Cubical (F3)	0.24	0.17	0.10
Pyramidal	0.53	0.24	0.14
Nzi	0.20	0.18	-
H	0.57	0.08	-
Malaise	-	0.13	-

[†]There was a 1.16 increase rate in the Sudanese zone near Bamako but the difference is not significant from the control biconical trap.

Experiments with odours and odour blends in 1996 and 1998

In 1996 and 1998, the tests were conducted during the dry and rainy seasons at the Madina-Diassa Ranch. The attractiveness of the following odours were studied in woodland savannah and gallery forest using both Challier-Laveissière biconical and Vavoua monoconical traps: *m*-cresol, octenol, *m*-cresol + octenol (1:3 ratio), *m*-cresol + octenol (2:2 ratio), octenol + cow urine, cow urine, and acetone + octenol + cow urine.

The odours (4 ml) were dispensed from PE sachets. Acetone was released form a 250 ml plastic bottle with a 1.0 mm hole on the cover. Cow urine was obtained locally one day before the test and was dispensed from a "pot a confiture" (250 ml). The traps were set up 200 m apart in the gallery forest along the bank of the river Baoulé, and 500 m apart in the woodland savannah. The experiments were done according to a 6 × 6 Latin square design with daily randomized rotations. Two replicates were performed in all studies.

Trapping studies in 1996

The results for *G. morsitans submorsitans* are shown in Tables 4.2.8 and 4.2.9. For each trap types the octenol + cow urine odour blend was the best both in the dry and in the rainy seasons. For the biconical trap the attractivity of the trap increased over four-fold by the inclusion of this odour mixture in the trap, while for the monoconical trap this increase was 2.60 and 2.80 for the dry and rainy seasons, respectively. The data suggest that phenolic odours, either the synthetic *m*-cresol or those in fermenting cow urine, on their own are not as effective as their combination with octenol.

Table 4.2.8. Daily mean captures per trap of *G. morsitans submorsitans* species in biconical traps during the dry (13 March – 4 April) and the rainy (17 July – 9 August) seasons in woodland savannah at Madina-Diassa Ranch, Mali, in 1996

Bait description	Dry season	Catch index	Rainy season	Catch index
Unbaited trap	14.54	1	26.45	1
m-Cresol	23.83*	1.64	39.12	1.48
m-Cresol + octenol (1:3)	31.87*	2.19	48.66*	1.84
m-Cresol + octenol (2:2)	43.04*	2.96	42.25	1.60
Octenol + cow urine	67.29*	4.63	107.4*	4.06
Cow urine	22.66*	1.56	42.20	1.60

*Significantly different from control (ANOVA, $P<0.05$).

Table 4.2.9. Daily mean captures per trap of *G. morsitans submorsitans* in monoconical traps during the dry (13 March – 4 April) and the rainy (17 July – 9 August) seasons in woodland savannah at Madina-Diassa Ranch, Mali, in 1996

Bait description	Dry season	Catch index	Rainy season	Catch index
Unbaited trap	12.00	1	16.66	1
m-Cresol	9.50	0.79	18.20	1.09
m-Cresol + octenol (1:3)	15.45	1.29	26.95*	1.62
m-Cresol + octenol (2:2)	24.08*	2.01	29.33*	1.76
Octenol + cow urine	31.25*	2.60	46.66*	2.80
Cow urine	9.20	0.77	17.41	1.04

*Significantly different from control (ANOVA, $P<0.05$).

Catches of different *Glossina* species by these odours in two different traps were also compared in a gallery forest during the dry and rainy seasons. The results are shown in Tables 4.2.10 and 4.2.11. The data indicate that the combination of octenol either with cow urine or *m*-cresol is the most attractive to *G. morsitans submorsitans* and *G. tachinoides*, although some seasonal differences can be noted. The tested odours failed to increase catches of the unbaited traps for *G. palpalis gambiensis*.

Table 4.2.10. Daily mean captures per trap of three *Glossina* species in biconical traps in gallery forest at Madina-Diassa Ranch, Mali, during the dry (13 March – 4 April, 1996) and rainy seasons (17 July – 9 August, 1996)

Bait description	*G. m. submorsitans*		*G. tachinoides*		*G. p. gambiensis*	
	Season					
	dry	rainy	dry	rainy	Dry	rainy
Unbaited trap	2.95	0.37	4.04	4.50	13.62	8.16
m-Cresol	4.95*	2.29*	9.20	7.54	7.79	4.79
m-Cresol + octenol (1:3)	6.87*	1.16	7.04	7.75	8.54	4.70
m-Cresol + octenol (2:2)	9.37*	1.54	7.50	8.75*	8.37	5.20
Octenol + cow urine	20.58*	3.87*	10.08*	8.87*	10.08	7.29
Cow urine	6.04	0.83	8.08	5.54	11.66	5.58

*Significantly different from control (ANOVA, P<0.05).

Table 4.2.11. Daily mean captures per trap of three *Glossina* species in monoconical traps gallery forest at the River Baoulé, Mali, during the dry (13 March – 4 April, 1996) and rainy (17 July – 9 August, 1996) seasons

Bait description	*G. m. submorsitans*		*G. tachinoides*		*G. p. gambiensis*	
	Season					
	dry	rainy	dry	rainy	Dry	rainy
Unbaited trap	2.58	0.75	6.95	3.66	12.70	3.12
m-Cresol	2.66	1.04	8.53	4.70	11.66	3.62
m-Cresol + octenol (1:3)	5.16	1.54	8.62	4.79	14.08	3.37
m-Cresol + octenol (2:2)	6.04	1.08	8.58	4.70	14.41	3.62
Octenol + cow urine	14.54*	4.50*	10.00	6.50	12.25	3.87
Cow urine	2.70	0.45	7.16	4.08	10.29	4.37

*Significantly different from control (ANOVA, P<0.05).

The results of the experiments carried out in 1996, as summarized in Table 4.2.12, indicate that the biconical trap performs better for all three *Glossina* species, although the differences for the *palpalis* group are statistically not significant. It is also notable that, with the exception of cow urine, all odours tested substantially increase trap catches of *G. morsitans submorsitans*. The most effective odour combination is octenol + cow urine, which increases catches of the *morsitans* species even for the otherwise relatively ineffective monoconical trap by over 5.6-fold in both seasons. As mentioned before, *G. tachinoides* are trapped best during the dry season by biconical traps using octenol + cow urine odour combination (catch index = 2.33).

Table 4.2.12. Summary of index of increases, relative to control traps without odour, of daily mean captures of three different *Glossina* species (males + females) in a gallery forest in different traps with various odours in experiments in 1996

Bait description	*Glossina* species	Biconical		Monoconical	
		Season		Season	
		Dry	Rainy	Dry	Rainy
m-Cresol	*G. p. gambiensis*	1.17	1.45	0.91	1.16
	G. tachinoides	2.27	1.67	1.23	1.28
	G. m. submorsitans	1.67	6.11*	1.03	1.38
m-Cresol	*G. p. gambiensis*	1.28	1.43	1.10	1.08
+ octenol (1:3)	*G. tachinoides*	1.74	1.72	1.23	1.30
	G. m. submorsitans	2.32*	3.88*	2.00*	2.05
m-Cresol	*G. p. gambiensis*	1.26	1.58	1.13	1.16
+ octenol (2:2)	*G. tachinoides*	1.85	1.94	1.28	1.28
	G. m. submorsitans	3.16*	4.11*	2.33*	1.44
Octenol + cow	*G. p. gambiensis*	1.52	2.21*	0.96	1.24
urine	*G. tachinoides*	2.49*	1.97	1.43	1.77
	G. m. submorsitans	6.95*	10.33*	5.62*	6.00*
Cow urine	*G. p. gambiensis*	1.76	1.69	0.80	1.40
	G. tachinoides	2.00	1.23	1.02	1.11
	G. m. submorsitans	2.04	2.22	1.04	0.61

*Significantly different from unbaited control (ANOVA, $P<0.05$).

Trapping of Tabanids and Stomoxys *species*

During the studies in 1996, a substantial number of Tabanid and *Stomoxys* species, both potential mechanical vectors of *Trypanosoma vivax* and *Trypanosoma evansi*, were caught in the two types of traps containing the attractants discussed above. Table 4.2.13 provides a summary of these data.

For Tabanidae, the monoconical trap performed generally better than the biconical trap. Adding octenol + cow urine to Vavoua traps increased captures by 8.6-fold, while for biconical traps this combination of odours increased captures by 15-fold over the control although the absolute numbers were still low. The most frequently occurring tabanid species in the traps were *Ancala*, *Atylotus*, *Chrysops*, *Haematopota* and *Tabanus*. For *Stomoxys* species only the monoconical trap caught flies and cow urine, especially in combination with octenol, appeared to be the best attractant in this study.

Table 4.2.13. Total captures of Tabanidae and *Stomoxys* flies by two trap types baited with various attractants during the dry and rainy seasons, 13 March – 4 April and 17 July – 9 August, respectively, in 1996.

Bait description	Tabanids	Stomoxes
Biconical (Challier-Laveissière) trap		
Unbaited control	3	0
m-Cresol	6	0
m-Cresol + octenol (1:3)	17*	0
m-Cresol + octenol (2:2)	13*	0
Octenol + cow urine	45*	1
Cow urine	10	1
Monoconical (Vavoua) trap		
Unbaited control	55	18
m-Cresol	68	13
m-Cresol + octenol (1:3)	132*	28
m-Cresol + octenol (2:2)	193*	25
Octenol + cow urine	475*	44
Cow urine	57	38

*Significantly different from unbaited control (ANOVA, $P<0.05$).

Comparison of trap prices

The estimated costs of traps and odours are as follows:

Biconical	=	US$ 16.00
Monoconical	=	US$ 12.00
Pyramidal	=	US$ 11.00
Cubical	=	US$ 30.00
Acetone (250 ml)	=	US$ 1.00
Octenol sachet (4 ml)	=	US$ 1.20
m-Cresol sachet (4 ml)	=	US$ 0.20

The cubical trap is expensive, not practical and not efficient against *Glossina* species and is attacked by animals and chewed by termites. The pyramidal trap is relatively cheap, because of the wooden stick it requires, but its installation is not simple and it easily loses its shape from the slightest wind.

The biconical and monoconical traps, however, are both economical and fairly effective although the latter is less effective in capturing tsetse flies. Of these two, the use of monoconical traps can be recommended for tsetse control and the biconical traps for the estimation of apparent densities and reinforcement barriers in Mali. It was also established that the slow release formulation used (4 ml of octenol and *m*-cresol mixture in PE sachets) remained attractive for about three months, which is the required time for re-impregnation of control materials with insecticide.

Trapping studies in the dry season of 1998

Based on the results of the studies in 1996, the experiments were repeated at the same location during the dry season in 1998 with some modifications: *m*-cresol, proven to be ineffective in the previous tests, was now omitted while acetone was included in combination with the best attractant mixture, octenol + cow urine. The experimental set-up was the same as used in 1996. The results of the studies for the two different trap types are shown in Tables 4.2.14 and 4.2.15.

In the woody savannah experimental site *G. morsitans submorsitans* was the only tsetse species in traps (Table 4.2.14). Confirming the results obtained in 1996, the octenol + cow urine blend significantly increased traps catches by 2.5-fold and by 1.9-fold for the biconical and Vavoua traps, respectively. The addition of acetone to this blend resulted in a slight (2.87-fold) but significant increase over the unbaited biconical control trap. For the Vavoua trap, acetone had no added effect on attraction.

Table 4.2.14. Daily mean captures per trap of *G. morsitans submorsitans* in two different traps in woodland savannah at Madina-Diassa Ranch, Mali, during the dry season (13 March – 5 April, 1998)

Bait description	Biconical trap	Catch index	Monoconical trap	Catch index
Unbaited trap	16.37	1	8.45	1
Octenol	16.58	1.01	9.70	1.14
m-Cresol + octenol (1:3)	27.33	1.66	10.04	1.18
m-Cresol + octenol (2:2)	28.91	1.76*	8.95	1.05
Octenol + cow urine	41.16	2.51*	16.08	1.90*
Acetone + octenol + cow urine	47.00	2.87*	13.75	1.62*

*Significantly different from control (ANOVA, $P<0.05$).

Trapping studies were carried out simultaneously in a gallery forest during the dry season, and the results for the three *Glossina* species captured are shown in Table 4.2.15. As before, the odour combinations containing octenol + cow urine were the most attractive for the savannas species *G. morsitans submorsitans* and the riverine species *G. tachinoides*. For the biconical traps, the inclusion of acetone did not significantly improved captures. For the monoconical trap, only the savannah species responded to odours in this study and the maximum increase of 3.46-fold was observed for the acetone + octenol + cow urine blend.

Table 4.2.15. Summary of catch indices, relative to control traps without odour, of daily mean captures of three different *Glossina* species (males + females) in a gallery forest in different traps with various odours during the dry season (13 March – 5 April, 1998)

Bait description	*Glossina* species	Catch index	
		Biconical trap	Monoconical trap
Unbaited trap	*G. palpalis gambiensis*	1 (2.33)†	1 (4.54)†
	G. tachinoides	1 (3.33)†	1 (5.70)†
	G. m. submorsitans	1 (2.45)†	1 (2.50)†
Octenol	*G. p. gambiensis*	0.64*	0.80
	G. tachinoides	0.51	0.78
	G. m. submorsitans	1.32	1.78*
m-Cresol + octenol (1:3)	*G. p. gambiensis*	0.94	1.03
	G. tachinoides	1.57	1.25
	G. m. submorsitans	2.25	1.78*
m-Cresol + octenol (2:2)	*G. p. gambiensis*	0.64	0.84
	G. tachinoides	2.05	1.34
	G. m. submorsitans	1.98	1.58*
Octenol + cow urine	*G. p. gambiensis*	1.20	0.70
	G. tachinoides	2.58*	1.48
	G. m. submorsitans	4.49*	2.25*
Acetone + octenol + cow urine	*G. p. gambiensis*	1.05	1.00
	G. tachinoides	2.65*	1.64
	G. m. submorsitans	5.98*	3.46*

†Absolute mean captures per day per trap (shown for unbaited trap only).
*Significantly different from control (ANOVA, $P<0.05$).

Trapping Tabanid and Stomoxys *species*

In the dry season of 1998, similarly to the experiments in 1996, a substantial number of tabanid flies were caught especially by monoconical traps baited with octenol + cow urine. *Stomoxys* species were also found in this type of traps but none of the odours increased captures (data not shown).

Trapping studies in the rainy season of 1998

Based on the laboratory studies under this CRP, indicating that several aldehydes and their bioisosteric analogues elicit EAG responses, a series of these compounds were included in field experiments. These experiments were conducted again at the Madina-Diassa Ranch experimental sites, from 21 October to 13 November 1998. The effects of six new candidate odours and of octenol on the attractivity of biconical and monoconical traps erected in woodland savannah and gallery forest were studied. The following odours were tested: decanal, dodecanal, decanitrile, dodecanitrile, octyl formate, decyl formate, and octenol.

The odours were released from PE sachets containing separately 1 ml each of the new odours and 4 ml octenol. Three replicates were used in an 8 × 8 Latin square design.

Trap capture data are summarized in Table 4.2.16. It can be seen that none of the single odours influenced trap captures. In some traps *G. tachinoides* were found but their total number was 4 for the biconical trap and 10 for the monoconical trap (data not shown).

Table 4.2.16. Relative captures of *G. morsitans submorsitans* in two different traps in woodland savannah at Madina-Diassa Ranch, Mali, during the rainy season (21 October – 13 November, 1998). Differences observed were statistically not significant (ANOVA, P>0.05)

Bait description	Catch index	
	Biconical trap	Monoconical trap
Unbaited trap	1 (20.62)†	1 (11.12)†
Octenol	1.13	1.74
Decanal	1.54	1.06
Dodecanal	0.93	1.59
Decanitrile	1.11	1.20
Dodecanitrile	1.08	1.56
Octyl formate	0.95	1.47
Decyl formate	1.32	1.11

†Absolute mean captures per day per trap (shown for unbaited traps only).

Table 4.2.17. Summary of indices of increases, relative to control traps without odour, of daily mean captures of three different *Glossina* species (males + females) in a gallery forest in different traps with various synthetic odours during the rainy season (21 October – 13 November, 1998).

Bait description	*Glossina* species	Catch index	
		Biconical trap	Monoconical trap
Unbaited trap	*G. p. gambiensis*	1 (2.04)†	1 (0.83)†
	G. tachinoides	1 (9.20)†	1 (2.45)†
	G. m. submorsitans	1 (7.79)†	1 (2.66)†
Octenol	*G. p. gambiensis*	0.93	0.80
	G. tachinoides	0.82	1.13
	G. m. submorsitans	2.10*	1.68*
Decanal	*G. p. gambiensis*	1.10	0.85
	G. tachinoides	0.70	0.81
	G. m. submorsitans	1.24	1.12
Dodecanal	*G. p. gambiensis*	1.06	0.90
	G. tachinoides	0.62	1.13
	G. m. submorsitans	1.03	0.93
Decanitrile	*G. p. gambiensis*	0.69	0.80
	G. tachinoides	0.51*	1.10
	G. m. submorsitans	1.15	1.14
Dodecanitrile	*G. p. gambiensis*	1.28	1.05
	G. tachinoides	0.71	1.05
	G. m. submorsitans	0.81	1.07
Octyl formate	*G. p. gambiensis*	1.69	1.20
	G. tachinoides	0.90	1.50
	G. m. submorsitans	1.03	0.93
Decyl formate	*G. p. gambiensis*	0.97	1.60
	G. tachinoides	0.66	1.16
	G. m. submorsitans	0.97	1.14

†Absolute mean captures per day per trap (shown for unbaited traps only).
*Significantly different from control (ANOVA, P<0.05).

Trapping studies were carried out simultaneously in a gallery forest during the rainy season and the results for the three *Glossina* species caught are shown in Table 4.2.17. The only compound that increased captures of both trap types was octenol but its attractivity was restricted to the savannah species, *G. morsitans submorsitans*, which is similar to the results noted at the same location in the dry season earlier that year (see Table 4.2.15). A marked effect of octenol was observed for males of this species for which captures by monoconical traps increased 2.56-fold (data not shown). It is interesting to note that decanitrile decreased catches of *G. tachinoides*, though this was observed for the biconical trap only.

Trapping of Tabanids and Stomoxys *species*

In the rainy season of 1998, similarly to the experiments in 1996, a substantial number of tabanid and *Stomoxys* flies were caught (data not shown) especially by monoconical traps containing octenol that increased trap catches of tabanids by 2.3-fold and *Stomoxys* species by 2.89-fold. Furthermore, the new odour candidates dodecanal, dodecanitrile and octyl formate increased catches of *Stomoxys* species by 2.36-, 1.96- and 1.96-fold, respectively.

Additional studies with various odours and odour combinations

Following preliminary experiments at Madina-Diassa Ranch in two biotopes in 1992, further studies were conducted again at the same location (savannah woodland and gallery forest) during dry and rainy seasons. In these experiments, the attractivity of various known odours, alone or in mixture, in biconical and monoconical traps were compared in 6 × 6 or 8 × 8 Latin square design. In later experiments several new odours were also included. Two to four replicates were used. The results of the 1992 experiments are shown in Table 4.2.18, while those in 1996 and 1999 are shown in Tables 4.2.19 to 4.2.22.

Table 4.2.18. Summary of catch increases, relative to control traps without odour, of daily mean captures of three *Glossina* species (males + females) in a gallery forest in biconical traps baited with various odours in the dry and rainy seasons (March and July–August, respectively, of 1992). A 5 × 5 Latin square design was used in four replicates during 20 days

Bait description	*Glossina* species	Season	
		Dry	Rainy
Unbaited trap	*G. p. gambiensis*	1 (2.50)[†]	1 (2.60)[†]
	G. tachinoides	1 (0.85)[†]	1 (3.05)[†]
	G. m. submorsitans	1 (4.00)[†]	1 (1.90)[†]
Acetone + octenol	*G. p. gambiensis*	1.20	0.46
	G. tachinoides	0.53	0.79
	G. m. submorsitans	2.58*	8.00*
Acetone + *m*-cresol + octenol (2:2)	*G. p. gambiensis*	1.84	0.88
	G. tachinoides	2.35*	2.67*
	G. m. submorsitans	1.28	5.92*
m-Cresol + octenol (2:2)	*G. p. gambiensis*	1.28	0.88
	G. tachinoides	2.47*	2.51*
	G. m. submorsitans	1.62	3.89*
Acetone + octenol + cow urine	*G. p. gambiensis*	2.68*	1.29
	G. tachinoides	2.23*	3.34*
	G. m. submorsitans	2.92*	7.95*

[†]Absolute mean captures per day per trap (shown for unbaited traps only).
*Significantly different from unbaited control (ANOVA, $P<0.05$).

Table 4.2.19. Summary of catch indices, relative to control traps without odour, of daily mean captures of three *Glossina* species (males + females) in a gallery forest in biconical and monoconical traps baited with various odours in the dry season (13 March – 5 April, 1996). Four replicates were used

Bait description	*Glossina* species	Trap type	
		biconical	monoconical
Unbaited trap	*G. p. gambiensis*	1 (6.62)†	1 (12.70)†
	G. tachinoides	1 (4.04)†	1 (6.95)†
	G. m. submorsitans	1 (2.95)†	1 (2.58)†
m-Cresol	*G. p. gambiensis*	1.17	0.91
	G. tachinoides	2.27	1.23
	G. m. submorsitans	1.67	1.03
m-Cresol + octenol (1:3)	*G. p. gambiensis*	1.28	1.13
	G. tachinoides	1.74	1.28
	G. m. submorsitans	2.32*	2.33*
m-Cresol + octenol (2:2)	*G. p. gambiensis*	1.26	1.13
	G. tachinoides	1.85	1.28
	G. m. submorsitans	3.16*	2.33*
Octenol + cow urine	*G. p. gambiensis*	1.52	0.96
	G. tachinoides	2.49*	1.43
	G. m. submorsitans	9.95*	5.62*
Cow urine	*G. p. gambiensis*	1.76	0.80
	G. tachinoides	2.00	1.02
	G. m. submorsitans	2.04	1.04

†Absolute mean captures per day per trap (shown for unbaited traps only).
*Significantly different from unbaited control (ANOVA, $P<0.05$).

Table 4.2.20. Summary of catch indices, relative to control traps without odour, of daily mean captures of three *Glossina* species (males + females) in a gallery forest in biconical and monoconical traps baited with various odours in the rainy season (17 July – 9 August, 1996). Four replicates were used.

Bait description	*Glossina* species	Trap type	
		biconical	monoconical
Unbaited trap	*G. p. gambiensis*	1 (3.29)†	1 (0.75)†
	G. tachinoides	1 (4.50)†	1 (3.66)†
	G. m. submorsitans	1 (0.37)†	1 (3.12)†
m-Cresol	*G. p. gambiensis*	1.45	1.16
	G. tachinoides	1.67	1.28
	G. m. submorsitans	6.11*	1.38
m-Cresol + octenol (1:3)	*G. p. gambiensis*	1.43	1.08
	G. tachinoides	1.72	1.30
	G. m. submorsitans	3.88*	2.05
m-Cresol + octenol (2:2)	*G. p. gambiensis*	1.58	1.16
	G. tachinoides	1.94	1.28
	G. m. submorsitans	4.11*	1.44
Octenol + cow urine	*G. p. gambiensis*	2.21*	1.24
	G. tachinoides	1.97	1.77
	G. m. submorsitans	10.33*	6.00*
Cow urine	*G. p. gambiensis*	1.69	1.40
	G. tachinoides	1.23	1.11
	G. m. submorsitans	2.22	0.61

†Absolute mean captures per day per trap (shown for unbaited traps only).
*Significantly different from unbaited control (ANOVA, $P<0.05$).

Table 4.2.21. Summary of catch indices, relative to control traps without odour, of daily mean captures of three *Glossina* species (males + females) in a gallery forest in biconical and monoconical traps baited with various odours in the dry season (5 – 20 December 1999). Two replicates.

Bait description	*Glossina* species	Trap type	
		biconical	monoconical
Unbaited control	*G. p. gambiensis*	1 (0.62)†	1 (0.87)†
	G. tachinoides	1 (0.68)†	1 (1.75)†
	G. m. submorsitans	1 (0.93)†	1 (0.80)†
Acetone + octenol + *m*-cresol	*G. p. gambiensis*	3.40*	1.21
	G. tachinoides	7.00*	2.03*
	G. m. submorsitans	3.73	4.77*
Acetone + octenol + decanal	*G. p. gambiensis*	1.70	0.64
	G. tachinoides	3.45	0.67
	G. m. submorsitans	2.46	4.00*
Acetone + octenol + dodecanal	*G. p. gambiensis*	1.70	2.00*
	G. tachinoides	2.54	0.82
	G. m. submorsitans	1.66	3.66*
Acetone + octenol + octyl formate	*G. p. gambiensis*	0.90	0.57
	G. tachinoides	1.63	0.35
	G. m. submorsitans	2.00	3.88*
Acetone + octenol + decyl formate	*G. p. gambiensis*	1.10	0.92
	G. tachinoides	1.81	0.53
	G. m. submorsitans	2.46	3.55*
Pinus sylvestris oil	*G. p. gambiensis*	1.50	1.00
	G. tachinoides	4.18*	1.64
	G. m. submorsitans	1.53	1.44
Pinus pumilionis oil	*G. p. gambiensis*	1.20	1.14
	G. tachinoides	2.72	0.92
	G. m. submorsitans	1.00	0.44

†Absolute mean captures per day per trap (shown for unbaited traps only).
*Significantly different from unbaited control (ANOVA, $P<0.05$).

Table 4.2.22. Summary of catch indices, relative to control traps without odour, of daily mean captures of three *Glossina* species (males + females) in a gallery forest in biconical and monoconical traps baited with various odours in the dry season (19 February – 6 March 2001). Two replicates. (The differences observed were statistically not significant at P>0.05).

Bait description[†]	*Glossina* species	Trap type	
		biconical	monoconical
Unbaited trap	*G. p. gambiensis*	1 (4.87)[‡]	1 (5.75)[‡]
	G. tachinoides	1 (3.62)[‡]	1 (4.43)[‡]
	G. m. submorsitans	1 (4.31)[‡]	1 (3.87)[‡]
Acetone + octenol + *p*-cresol	*G. p. gambiensis*	1.16	0.75
	G. tachinoides	1.55	1.21
	G. m. submorsitans	0.71	0.48
POCA	*G. p. gambiensis*	0.91	0.56
	G. tachinoides	1.25	1.15
	G. m. submorsitans	1.15	0.91
POMA	*G. p. gambiensis*	0.56	0.52
	G. tachinoides	0.96	1.05
	G. m. submorsitans	1.47	1.04
Acetone + octenol + *p*-cresol + octyl formate + decyl formate	*G. p. gambiensis*	1.03	0.71
	G. tachinoides	1.56	1.26
	G. m. submorsitans	0.79	0.62
Acetone + octenol + *m*-cresol + octyl formate + decyl formate	*G. p. gambiensis*	0.76	0.25
	G. tachinoides	0.98	0.59
	G. m. submorsitans	0.59	0.45
Acetone + octenol + *p*-cresol + *Pinus sylvestris* oil	*G. p. gambiensis*	1.14	0.55
	G. tachinoides	1.51	1.32
	G. m. submorsitans	0.69	0.53
POCA + octyl formate + decyl formate + *Pinus sylvestris* oil	*G. p. gambiensis*	0.92	0.52
	G. tachinoides	1.86	0.57
	G. m. submorsitans	1.40	0.62

[†]POCA is a 1:4:8 mixture of propylphenol, octenol and *p*-cresol (in sachet) and acetone (in separate bottle dispenser); POMA is a 1:4:8 mixture of propylphenol, octenol and *m*-cresol (in sachet) and acetone (in separate bottle dispenser).
[‡]Absolute mean captures per day per trap (shown for unbaited traps only).

Conclusion

During the multiyear studies in Mali, the acetone + octenol + cow urine and the octenol + cow urine odour combinations were found to be the most effective in trapping *G. morsitans submorsitans* and *G. tachinoides* as well as various mechanical vectors of trypanosomosis. For *G. tachinoides*, the *m*-cresol + octenol their 2:2 mixture appears to be more efficient than the 1:3 ratio. For *G. palpalis gambiensis*, however, no such effective odour could be identified. Of the various trap designed examined, the biconical (Challier-Laveissière) trap is recommended for use in Mali, especially as reinforcement barrier and to estimate apparent densities. The somewhat cheaper monoconical (Vavoua) trap can be considered for tsetse control.

4.3. Conclusions

4.3.1. Kenya

- The newly developed, modified sticky monopanel has proven to be effective for capturing *G. austeni*. For this trap, the octenol + acetone blend was the most promising bait identified for *G. austeni* (Table 4.1.12). The propylphenol + octenol + *p*-cresol + acetone (POCA) –based odour blends appear also to be attractive (Table 4.1.13).

- For *G. pallidipes*, the octenol + acetone and the POCA blends were identified as the most effective baits for the sticky monopanel trap (Tables 4.1.15 and 4.1.17). Furthermore, the combination of POCA either with decanal, octyl or decyl formate or pine essential oils also provides attractive blends for this tsetse fly species (Tables 4.1.16, 4.1.17 and 4.1.19).

- For *G. brevipalpis*, the combinations of POCA with decanal and/or *Pinus sylvestris* essential oil were identified as effective baits for the sticky monopanel trap (Tables 4.1.19 and 4.1.20). Complementing the POCA blend with isovaleric acid also gave increased catches of this tsetse species (Table 4.1.20).

4.3.2. Tanzania

- For *G. swynnertoni*, four unbaited trap types, namely the S3, the pyramidal, the biconical and the Nzi, were found to be promising (Table 4.1.22). Baiting the S3 trap with acetone significantly increased catches (Table 4.1.24). Inclusion of the POCA blend, or POCA with decanal or with octyl formate significantly increased catches of the S3 trap (Tables 4.1.23). Moving (e.g., swinging) traps should also be considered in future studies.

- For *G. morsitans centralis*, the S3 trap with the POCA or POCA + decanal (or octyl formate) blends performed best (Table 4.1.26). Of the pine essential oils, *P. sylvestris* oil also significantly increased the catches of the S3 trap (Table 4.1.26).

- For *G. brevipalpis*, the POCA + decanal or POCA + *Pinus sylvestris* oil blends increased catches of the H trap significantly (Table 4.1.27).

- For *G. pallidipes*, to POCA combined with synthetic odours such as octyl formate, decanal or with pine essential oils significantly increased trap catches of the H trap

(Table 4.1.27). Acetone alone, to some extent, was also effective for the H trap (Table 4.1.27).

4.3.3. Uganda

- For *G. fuscipes fuscipes* the most promising odour increasing the catches of the pyramidal trap was *Pinus pumilionis* essential oil (Table 4.1.32). Of the synthetic odours tested, the decanitrile + dodecanitrile blend significantly decreased catches of the pyramidal trap (Table 4.1.28).

- In experiments involving electrified grids and pyramidal traps, the addition of octenol and decyl formate significantly increased the numbers of attracted *G. f. fuscipes* females but not of males. However, the attracted *G. f. fuscipes* females did not enter the trap (Table 4.1.34).

- In comparison to the unbaited biconical trap, the unbaited H trap was ineffective in capturing this tsetse species (Table 4.1.35).

4.3.4. Burkina Faso

- Based on ELISA analysis of the blood meal of *G. palpalis gambiensis*, monitor lizard was established to be the most important host for this species (Fig. 4.7).

- Trials with monitor lizards in a stable revealed that *G. palpalis gambiensis* flies feed on mobile lizards but not on lizards that were completely restricted in their ability to move (Fig. 4.6).

- Biconical and monoconical traps are both suitable for trapping *G. palpalis gambiensis* (4.2.4).

- For *G. palpalis gambiensis*, the inclusion of the POCA blend in the biconical trap almost doubled catches although the difference was statistically not significant in the experiment conducted (Table 4.2.5).

- *For G. tachinoides*, the POCA, the POCA + *Pinus sylvestris* oil, and the POCA + *Pinus sylvestris* oil + decyl formate blends significantly increased catches of the biconical trap (Table 4.2.5). The monoconical trap was less effective with these odour blends (Table 4.2.6).

4.3.5. Mali

- Experiments using unbaited traps established the biconical as the best trap for *G. tachinoides*, *G. morsitans submorsitans,* and *G. palpalis gambiensis* (Table 4.2.7).

- For *G. tachinoides*, the *m*-cresol + octenol (2:2), the octenol + cow urine and the acetone + octenol + cow urine blends were found to be the best attractant odour combinations (Tables 4.2.10, 4.2.15 and 4.2.18). The *Pinus sylvestris* essential oil also significantly increased catches of the biconical trap but not of the monoconical one (Table 4.2.21).

- For *G. morsitans submorsitans*, the *m*-cresol + octenol (2:2) and the octenol + cow urine and the acetone + octenol + cow urine blends were found to be the best attractant odour combinations (Tables 4.2.8, and 4.2.14, 4.2.19 and 4.2.20).

- For *G. palpalis gambiensis*, octenol + cow urine (perhaps with added acetone) appear to be increasing catches of the biconical trap but only in the dry season (Table 4.2.18 and 4.2.19).

- For Tabanid flies, monoconical traps baited with the *m*-cresol + octenol (either in 1:3 or 2:2 ratio) and, especially, with the octenol + cow urine blends catch substantially more flies (Table 4.2.13). These odour blends also increased catches of biconical traps but the catches of Tabanid and Stomoxes flies were substantially lower (Table 4.2.13).

5. SEX PHEROMONES AND SURFACE HYDROCARBONS OF THE TSETSE FLIES

About the same time when the chemical nature of interspecific olfactory cues for *Glossina* was analysed in detail the chemical nature of signals involved in the intraspecific chemical communication was deciphered [142, 143]. These species-specific, non-volatile sex recognition pheromones are long chain, methyl-branched hydrocarbons and induce copulatory behaviour in males.

5.1. Summary of earlier work

Tsetse fly species that are under consideration for control schemes include the widespread, abundant and economically important members of the *morsitans*, *palpalis* and *fusca* groups. Until recently, the degree of genetic relatedness of the separate populations that comprise each of these species was unknown. For practical control purposes, even those involving modern attracticides, this lack of knowledge about the population structure of these species may not matter. However, for control efforts involving the sterile insect technique, relatedness among subpopulations of a taxon could be critical if sterile colonized males failed to respond to wild females for mating, or performed poorly. The similarity of populations of tsetse flies may be investigated using the base information of surface hydrocarbons for 24 of the 26 known species and sub-species [144, 145] that may include species-specific sex pheromones. In recent studies a number of very old pinned tsetse specimens were obtained undamaged from The Natural History Museum, London, and successfully extracted with the organic solvent hexane to obtain their surface hydrocarbons. The results were gratifying, as the old specimens provided apparently unchanged cuticular profiles that were little different from modern wild or laboratory specimens. The cuticular hydrocarbons including the high-molecular weight methylalkanes of the common members of the *morsitans* group and including *G. brevipalpis* had been previously characterized by GC-MS by Nelson and Carlson [146], and of the *palpalis* group by Nelson et al. [147]. More detailed study of the methylalkanes of each species was done in separate studies of the sexual behaviour as listed below. Several of these studies compared sex pheromones and hydrocarbons of laboratory and wild populations from different widely separated areas, showing distinct sexual dimorphism in all species but rather less in the *fusca* group flies.

All conspecific tsetse flies possess similar surface hydrocarbons that include species-specific contact sex pheromones. Knowledge of such a sex pheromone may help in modern biocontrol efforts against this disease vector, by ensuring that a compatible strain of fly is used in large-scale sterile male releases. If significant differences in the composition of surface hydrocarbons implicated in mediating sexual behaviour are found between the reared flies being considered for SIT and the individuals of the target population, then it is possible that the efficacy of the control effort will be compromised. Also, a synthetic pheromone might be used to increase the very slow rate of reproduction in laboratory-reared flies, and to test sexual behaviour in reared males intended for mass release. Furthermore, the presence of species-specific anti-pheromone hydrocarbons produced by males may play an important role in tsetse communications. This report compares recent research findings on several species under this CRP with earlier works where appropriate.

5.2. Recent progress in the isolation and characterisation of tsetse sex pheromones

5.2.1. G. austeni

Males of *G. austeni* from colonies in Bristol, UK, showed nearly obligatory sexual responses to conspecific females, but also responded well to several other species of females, and to

racemic synthetic 15,19-dimethyltritriacontane at 1 to 20 µg treatments [148], but this compound was essentially missing from laboratory-reared females obtained from this location. Also, the subsequent failure of separated natural fractions from females to release activity in colony males from Bristol was puzzling. The unseparated total hydrocarbon fraction was bioactive, and included alkenes and methylalkanes, but both the separated natural methylalkanes and alkenes were curiously inactive in repeated trials (P.A. Langley, unpublished data). This report presents the isolation and identification by GC-MS of dimethylalkenes found in females of *G. austeni* from colonies obtained from the FAO/IAEA Seibersdorf laboratory and in wild females from Natal, South Africa, and the first demonstration of biological activity in these unusual compounds. Also, the chemical analysis and comparison of the hydrocarbons from laboratory and wild female *G. austeni* from various locations is reported.

Evidence for a contact sex stimulant was found in the surface hydrocarbons extracted from female *G. austeni* in preliminary work done at the Seibersdorf Laboratory in 1994 (D. A. Carlson, unpublished data). Chromatography indicated that the bioactive hydrocarbon fraction contained alkanes and unsaturated hydrocarbons (alkenes) that were separated and analysed by GC/GC-MS. The structure and relative abundances of alkenes from laboratory and wild collected specimens appeared to be similar. However, the alkanes of wild and laboratory females of *G. austeni* showed only minor differences between northern and southern populations [149].

Recently, a contact sex stimulant was found in the cuticular hydrocarbons obtained from natural lipids extracted from female *G. austeni*. Chromatography on silica gel and argentation chromatography indicated that the bioactive hydrocarbon fraction contained alkanes and alkenes. The highest level of biological activity was associated with the alkene fraction but some activity was found in the equally prominent alkane fraction. Analysis by GC showed four major alkenes associated with female flies. The four alkenes were separated and collected by preparative GC and only the 33- and 35-carbon region showed biological activity against males, the 29- and 31-carbon region did not. There were four major alkene peaks in extracts of old colony females: Kovats Index (KI) 2965, KI 3165 and the isomers (KI 3355) 13,17-dimethyltritriacont-1-ene, and (KI 3555) 13,17-dimethylpentatriacont-1-ene, as verified by GC-MS after derivatization using deuterium and dimethyl disulfide [149]. Dose-response data showed ED_{50} at ca. 8 and 10 µg per decoy of the KI 3355 and KI 3555 natural materials respectively, using solvent-washed females as decoys. Up to 83% of the males responded at higher dose rates. This constitutes the first report of the release of sexual activity in the genus *Glossina* by natural alkenes in *G. austeni* males (D. A. Carlson and F. Mramba, unpublished data).

Synthetic samples including 8 stereoisomers of *G. austeni* [150] were tested in Tanga, Tanzania. Four enantiomers of the 33-carbon backbone eluting at KI 3355 (13,17-dimethyltritriacont-1-enes (*S*,*S*)-35, (*S*,*R*)-35, (*R*,*S*)-35, (*R*,*R*)-35) were sent at 1 mg each. The remainder is held frozen in the dark in Gainesville, Florida, USA. The other four were the 35-carbon backbone alkene enantiomers of 13,17-dimethylpentatriacont-1-ene (i.e. (*S*,*S*)-37, (*S*,*R*)-37, (*R*,*S*)-37, (*R*,*R*)-37) were also sent at 1 mg each. Two alkanes prepared from the corresponding alkenes from two stereoisomers were also shipped at 1 mg each. Bioassays showed highest activity with the (*S*,*R*)-35 (Fig. 5.1A) (ED_{50} = 12 µg), and less activity with the (*S*,*S*)-35 enantiomer at (ED_{50}= 20 µg). The 35 carbon backbone enantiomers showed little activity in these tests when tested independently (F. Mramba, MS Thesis, 2002, unpublished data). Mixtures or several candidate alkanes made from these alkenes are yet to be tested.

5.2.2. G. pallidipes

A sex stimulant pheromone was demonstrated in adult female *G. pallidipes* and the compound eliciting the maximal stimulatory response in males was identified as 13,23-dimethylpentatriacontane, with a smaller amount of the 11,21-isomer also being present. Both synthetic dimethylpentatriacontanes showed significant biological activity, whether racemic isomers [151], or diastereoisomers of 13,23-dimethylpentatriacontane [152]. The (13*R*,23*S*)-isomer (Fig. 5.1B) released biological activity with laboratory males at 4.5 µg (ED_{50}), whereas the diastereoisomeric mixture required twice as much [153]. The 13,23-isomer is the major hydrocarbon component in this species. A previous publication by McDowell [153] claiming the presence of major amounts of 13,17-dimethylpentatriacontane in females appears to be in error. The GC-MS of alkanes was recently obtained in samples of *G. pallidipes* females from Uganda and Ethiopia for comparison with specimens from other locations. The known sex pheromone components were very similar across populations. If minor differences were assigned, females appeared to cluster into two groups; 1) Zimbabwe wild; and 2) Amsterdam, ICIPE/Kenya, Kenya, Tanzania wild, Uganda/Bristol, Arba Minch/Ethiopia [149]. The absolute meaning of these minor differences are not known, since the activity of synthetic sex pheromones was shown conclusively for Wageningen and ICIPE males in 1984–1986 tests, although they were much less active against wild Zimbabwe males.

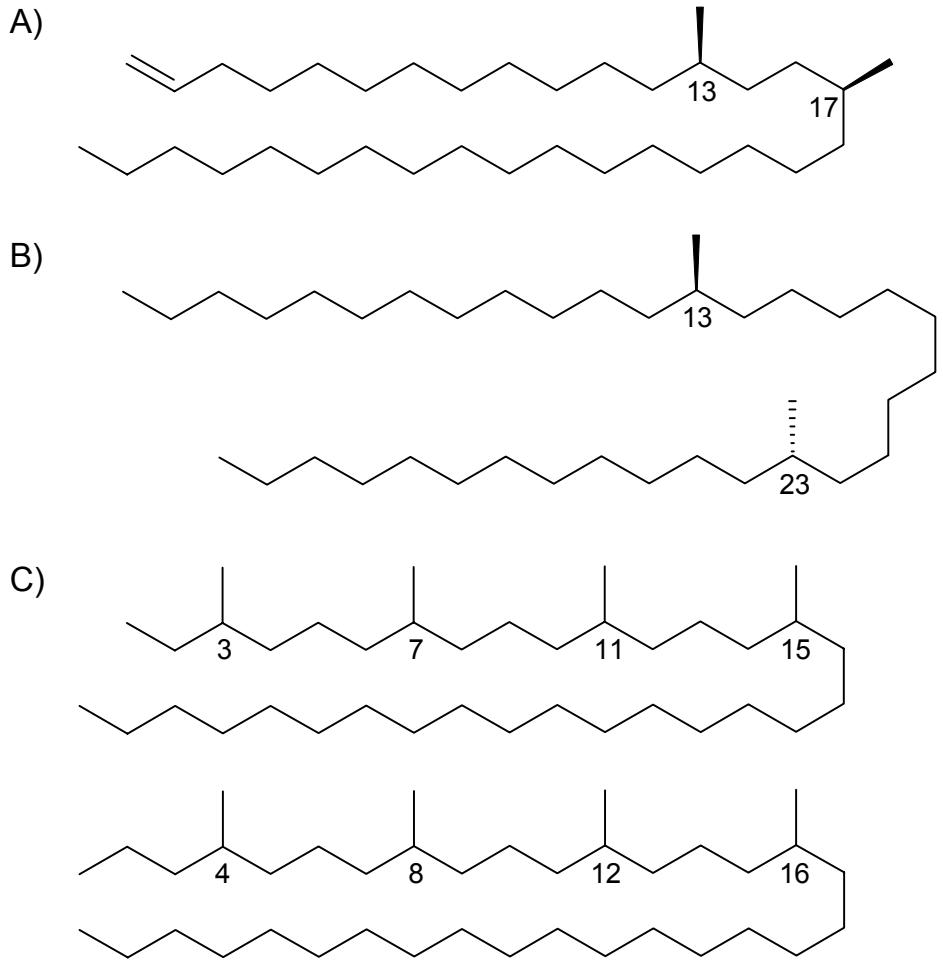


Figure 5.1. Structure of the most active sex stimulant cuticular hydrocarbons of female A) G. austeni, B) G. pallidipes, and C) G. brevipalpis.

5.2.3. G. brevipalpis

Evidence was found for the presence of a contact sex stimulant in the surface hydrocarbons extracted from female *G. brevipalpis* from Austria (D. A. Carlson, unpublished data, 1994). The structure and relative abundances of alkanes and the single alkene from several populations of laboratory and wild collected specimens appeared to be similar. The results were consistent with results from the earliest study by Nelson and Carlson [147]. The biological activity of total alkanes was compared using test males from Austria. The most bioactive fraction was the total hydrocarbon fraction that contained methyl branched compounds including two homologous alkanes with four methyl branches. A previous publication shows these to be 3,7,11,15-tetramethyltritriacontane and 4,8,12,16-tetramethyltetratriacontane [147] (Fig. 5.1C). These may be the first tetramethylalkanes to release sexual stimulant activity in an insect.

Table 5.1. Sex pheromones of tsetse flies (*Glossina* spp.)

Group	Demonstrated	Isolated/Identified	Synthesised	Strongly suspected
morsitans	*G. m. morsitans*[a]	*G. m. morsitans*[a]	*G. m. morsitans*[a]	*G. m. submors.*[a]
				G. m. centralis[a]
				G. swynnertoni[a]
	G. pallidipes[b]	*G. pallidipes*[b]	*G. pallidipes*[b]	
	G. austeni[c]	*G. austeni*[c]	*G. austeni*[c]	
palpalis	*G. tachinoides*[d]	*G. tachinoides*[d]	*G. tachinoides*[d]	
	G. p. palpalis[e]	*G. p. palpalis*[e]	*G. p. palpalis*[e]	
fusca	*G. brevipalpis*[f]	*G. brevipalpis*[f]	*G. brevipalpis*[f]	*G. longipalpis*[d]
Total				
6	6	6	6	7

[a] Major component in hydrocarbons from females identified as long chain trimethyl-alkanes similar or identical to females of *G. morsitans morsitans*: 15,19,21-trimethyl-C37 [143]. A summary of all species was given by Carlson et al. [144].

[b] Major component in hydrocarbons from females identified as long chain dimethylalkanes in *G. pallidipes*: 13,23-dimethyl-C37 [151]. Another species with these structures is *G. tachinoides*.

[c] Major components in hydrocarbons from females are C33 and C35 13,17-dimethylalkanes as proven by analysis (D. A. Carlson, unpublished results) and synthesis [150].

[d] Major component in hydrocarbons from females identified as long chain dimethyl alkanes in *G. tachinoides*: 13,25-, 11,21- and 11,23-dimethyl-C37 [154]. Other species with these structures are *G. longipalpis* from the *fusca* group.

[e] Major component in hydrocarbons from females identified as long chain trimethylalkanes similar or identical to females of *G. palpalis palpalis*: 13,17,21-trimethyl-C35 [155, 156].

[f] Major bioactive components in hydrocarbons from females identified are 3,7,11,15- and 4,8,12,16-tetramethyl-C33 [157; D. A. Carlson, unpublished results].

5.2.4. Other Glossina species

Strong sexual dimorphism is apparent in the cuticular hydrocarbon components of most of these species, and the dominant methylalkane component in each female has often been shown to comprise the sex pheromone for that species. Sexual stimulant compounds have been identified from females in several tsetse species including *G. morsitans morsitans* [143], *G. tachinoides* [154] and *G. austeni* (see above; also Carlson et al., submitted). Many of these compounds are chiral, and enantiomerically pure compounds for these species have been

synthesized and the chemistry and some bioassays published (K. Mori and co-workers). The identification of methylalkane sex stimulants for other species has been strongly suggested, but final evidence has been incomplete for *G. palpalis palpalis* [155]. Table 5.1 lists the currently known cuticular sex pheromones isolated from female tsetse flies.

There are numerous reasons for pursuing sex pheromones in tsetse flies. Knowledge of a sex pheromone may help in modern biocontrol efforts against this disease vector, by ensuring that a compatible strain of reared fly is used in large-scale sterile male releases. Significant differences in the composition of surface hydrocarbons implicated in mediating sexual behaviour could be found between the reared flies being considered for use in a sterile insect technique scheme and the target population. If there are significant differences, then it is very possible that the efficacy of the control effort will be compromised. Also, a synthetic pheromone might be used to increase the very slow rate of reproduction in laboratory-reared flies, and to test sexual behaviour in reared males intended for mass release.

Methods and materials

As an example for workers in the field, procedures used recently are included here for *G. brevipalpis*.

Biological samples and their chemical separation. Wild *G. brevipalpis* were obtained from Tanzania and Natal, South Africa, and from laboratory colonies in Seibersdorf, Austria, and shipped as dead dry specimens. Female *G. brevipalpis* were extracted in hexane as received. Crude extracts were reduced in volume and subjected to column chromatography on silica gel (60-200 mesh activated (Baker, Philadelphia, PA)) [154]. The first hexane fraction contained hydrocarbons and was used for further separation using argentation chromatography on silver nitrate impregnated silica gel to separate the alkanes from alkenes [143]. The other more polar lipids were also recovered from the column for bioassay: 10% ether/hexane (methyl ester fraction), 25% ether/hexane (triglyceride fraction), and the 50%ether/hexane plus chloroform / methanol (polar lipids). The alkene fraction from males was derivatized with dimethyl disulfide for GC-MS [158].

Gas chromatography - mass spectrometry. Cuticular hydrocarbons were quantified by GC utilizing a fused-silica capillary column (30 m, 0.32 mm i.d., 0.25 μm DB-1 stationary phase (J&W Scientific, Folsom, CA)) fitted to a Hewlett-Packard Model 6890 gas chromatograph fitted with a cool on-column injector, and a flame-ionisation detector. Hydrogen was used as the carrier gas at a linear flow velocity of 40 cm/sec. Each sample was reconstituted as necessary in 10 or 50 μl of hexane and 1 to 2 μl injected at 60^{o}C. Each GC run was temperature-programmed as follows: hold for two min (60^{o}C), ramp 20^{o}C/min (60 to 230^{o}C), ramp 2^{o}C/min to 320^{o}C, and hold at 320^{o}C to elute all components (10 min). A PC based data system, Turbochrom 3 (Perkin-Elmer Nelson, Cupertino, CA), was used for data recording and quantification. Manual adjustment of some small peaks was required for consistent integration, and peaks below a threshold of 0.02% were not considered.

Electron ionisation (EI) mass spectra were obtained using a Hewlett-Packard 5988A mass spectrometer interfaced to a HP 5890 GC fitted with an OCI-3 injector. Helium carrier gas at 9 psig gave a linear velocity of 40 cm/s with a DB-1 column as above, fitted with a retention gap (5 m × 0.53 mm i.d. deactivated FSOT column). The temperature program for GC-MS was: injection at 60^{o}C (hold 2 min), ramp at 10^{o}C/min to 220^{o}C, ramp at 3^{o}C from 220^{o}C to 310^{o} (hold 12–42 min). The mass spectrometer interface was maintained at 310^{o}C, electron voltage at 70 eV, and the system parameters manually optimised to enhance the EI spectra in

the critical region of *m/z* 200 to *m/z* 500. The mass spectral scan range extended from *m/z* 50 to *m/z* 700 with a scan rate (system limited) of 1.8 s per scan. Kovats retention indices (KI) were determined by injection of a normal hydrocarbon standard containing alkanes of 9 to 36 carbons in length plus 15,19,23-trimethylheptatriacontane (KI 3770). KI assignments were made in which KI 3100 represents a normal alkane of 31 carbons chain length, KI 3130 represents an internally-branched alkane of the same chain length. The assignment of KI narrows the range of possible methyl-branch configurations in cases of ambiguous or insufficient EI spectra [154]. The identification of methylalkanes followed the interpretations established previously for EI mass spectra [146, 147].

Bioassays. Bioassays were conducted at the Seibersdorf laboratory in 1994 in a manner similar to those for *G. tachinoides* [154]. Laboratory *G. brevipalpis* males were tested for sexual behaviour responses in dose-response studies of the hydrocarbon fraction obtained from conspecific females in doses from less than one to 100 female equivalents. Female fly decoys were killed by freezing and washed with hexane solvent which extinguished mating attempts on the wingless dried decoys. Individual decoys were treated with portions of the materials sent for bioassay. The alkene fraction was not separated or tested in initial bioassays. Later bioassays used field-caught flies in Natal, South Africa (Table 5.2).

Assays were scored as follows: active interest with mounting (Score 1), mounting with orientation to the copulatory position (Score 2), abdomen curving while gripping the female (Score 3), and extension of the male hypopygium (Score 4). Any score after 2 indicated very strong response, and the behaviour often proceeds immediately to Score 4. The highest score for each test was recorded, and then the scores averaged for each set of tests for statistical comparison.

Results of bioassays of G. brevipalpis *males*

Results of tests with the hydrocarbon fraction showed that males responded quite well to the highest dose used, that is 50 µg (Table 5.2). There were 7 full copulatory scores in 22 tests at this treatment, and many fewer full responses (2/22) at 20 µg, but 4 at 10 µg and 2 at 2 µg. There were no full responses to natural hydrocarbons at 1 and 5 µg (Table 5.2). The methyl esters and other lipid fractions showed no indication of bioactivity (data not shown).

Results of GC and GC-MS analyses of alkanes from G. brevipalpis *females*

Hydrocarbons of female flies were essentially identical to those shown to be present previously [147]. Major 2-methyl alkanes were found at KI 2865, 2965 and 3065. Dimethyl- and trimethylalkanes were present in very small quantities, but major tetramethylalkanes were present at KI 3065, 3265, and 3365 in females. The quantities found suggested that this group of homologous compounds were candidate sex pheromones. The major tetramethylalkanes were 3,7,11,15-tetramethylhentriacontane, and 4,8,12,16-tetramethyldotriacontane, consistent with the previous publication [147]. The hydrocarbon patterns in the gas chromatograms of *G. brevipalpis* females from different locations did not appear dramatically different qualitatively, although some quantitative differences in some larger methylalkanes were observed.

Table 5.2. Bioassays of *G. brevipalpis* males scored against treated decoys: Hydrocarbon fraction

Male No.	Dose (µg)					
	1	2	5	10	20	50
2	0	0	0	4	0	0
3	0	0	0	4	1	0
4	0	0	0	0	0	3
5	0	0	0	0	2	0
6	0	0	0	0	1	2
9	1	1	0	0	0	0
10	0	0	1	4	0	1
11	0	0	0	2	0	0
12	3	0	0	1	1	4
13	0	0	0	0	0	4
14	0	4	0	0	4	4
15	0	0	3	4	4	4
16	0	4	0	0	3	2
17	0	0	0	0	0	4
18	0	0	2	0	0	4
19	1	2	0	0	0	4
20	0	0	0	1	0	0
21		0			0	0
22					0	0
Total*	5 (0)	11 (2)	6 (0)	20 (4)	16 (2)	36 (7)

*Numbers in parenthesis after summarized scores represent the number of males responding of ca. 22 test specimens.

5.3. Anti-aphrodisiacs

Several indications of an anti-pheromone were seen in tests with extracts of male tsetse [159, 160]. The analytical chemistry of this phenomenon was followed and species-specific compounds were found and characterised in males of a number of species comprising a class of previously unreported compounds that were also species-specific [160, 161]. These compounds were shown to be exclusively male-produced and were essentially not found on virgin female flies, but appeared upon the cuticle of mated females. These findings were consistent with recent work showing consistently large amounts of a C-31 alkene (KI 3155) n the cuticle of mated female *G. austeni* colony flies produced by conspecific males (F. Mramba, unpublished data). This compound is a homologue of the recently described 33 and 35 carbon backbone dimethyl alkenes, KI 3355 and 3555, that comprise the female-produced sex pheromone in this species. There was much more of this male-produced material on the females than their own sex stimulant pheromone. An open question is posed, does the presence of this material on mature females inhibit mating by mature males in the tsetse colony. It is possible that the large amounts of such compounds produced and deposited on females by immature males could inhibit proper mating in large colonies, and thereby cause a decline in fecundity down to the 60% as noted in Tanga colonies (F. Mramba, unpublished data). A study of the presence of anti-aphrodisiacs called "abstinons" in laboratory-reared tsetse (*G. morsitans morsitans*) showed that males contributed a substance to females that inhibited further sexual contact [162]. This behaviour, reported first by Schlein et al. [159], was disputed by finding of no demonstrated effect in laboratory tests of females "marked" by males of the same species in Bristol [163]. However, such "abstinons" are claimed to be present in other insect orders. The compounds found in tsetse males are usually chemically related to the female-produced pheromone, usually by having 2 less carbons or by having one

double bond. These alkenes could be detected by gas chromatography in mated females collected in the lab after mating tests [160]. Similarly, mated wild females were so marked by wild males flies in the field, which distinguished them as mated by GC analysis.

It is curious that males would produce such a specific compound and transfer it to females when mating in every species investigated, yet no behaviour specifically assigned to these materials was observed. The structures are closely related to others identified as se pheromones active against conspecific males, and are therefore the most logical candidates as "Abstinons". The problem in proof of activity may be that if biologically active, such an anti-aphrodisiac would inhibit sexual behaviour, thus showing its action by a lack of activity. Nevertheless, such transfer of male-repellent or anti-aphrodisial pheromones occurs in many species of Diptera. Such an anti-pheromone could be used in the field as a contact material that would theoretically prevent males from mating with treated females. Moreover, it is logical that transfer of too much of such an anti-aphrodisiac substance could be deleterious to proper mating in laboratory tsetse colonies.

5.4. Larviposition pheromone

Saini et al. [164] reported a larviposition pheromone that induced pregnant females of *G. morsitans morsitans* and also *G. morsitans centralis* to deposit larvae on treated surfaces, and *n*-pentadecane and *n*-dodecane, respectively, were identified as the electrophysiologically and behaviourally active components for the two species. Carlson, however, was unable reproduce this effect in the Seibersdorf Laboratory in 1994 by washing larvae or surfaces upon which many larvae had crawled and testing the washings, or by treating surfaces with the synthetic alkanes. Furthermore, GC analysis of these two species of larvae in Seibersdorf colony materials did not show these or other unbranched alkanes under 20 carbons in crude larvae extracts. This was repeated later with larvae collected in Seibersdorf, sealed in glass, killed and sent to Gainesville, and no volatile alkanes were seen in the crude extract. Clearly, more research is needed to before the concept of tsetse larviposition pheromone can be exploited in practice.

5.5. Larval kairomone attracting parasitoids

Reports suggest the presence of tsetse-specific kairomones that could be produced by burrowing tsetse larvae or pupae in place in the soil. These materials could be present on the cuticle of last-instar larvae or pupae. Work in Seibersdorf recognized the presence of surface hydrocarbons on the cuticle of these life stages that resembled the compounds present in adult flies. The component profiles allowed differentiation by GC of older pupae by chemical analysis of hexane pupal washes without killing the pupae (D. A. Carlson, unpublished data). It is possible that these multiply branched alkanes could be kairomones as is known in other egg- or larvae-attacking parasitoids. However, supposedly tsetse-specific parasitoids were found in urban locations in Zimbabwe where there are no longer wild tsetse flies (Leticia Mattiacci, Harare, Zimbabwe, unpublished data).

5.6. Conclusions

Tetramethylalkanes are alkane structures rarely found in nature. The tetramethylalkanes described here are the first tetramethylalkanes shown to release sexual activity in tsetse flies, or among any insects known to date. The amounts of tetramethylalkanes shown in other species are extremely small or were undetectable.

It is interesting to note that the sex pheromones in tsetse flies examined to date are consistently long-chain (33- to 37-carbon) di-, and/or trimethylalkanes. They are consistently non-volatile sexual stimulants produced by the female and detected at close range by the male. The exception shown here is *G. brevipalpis*, the first member of the *fusca* group (Austenina) to be examined for the presence of a sex pheromone, and the only species that responds to tetramethylalkanes. This prompts the examination of females of other species for unique or major components as candidate sex pheromones. In only one species, *G. austeni*, do the males respond well to dimethyl-branched terminal alkenes produced by conspecific females, consistent with the notion that *G. austeni* are "aberrant" in many ways compared with others of the *morsitans* group. Thus it should be interesting to study other members of the poorly understood *fusca* group.

Another discovery is the presence of a long-chain methyl-branched alkene produced by the male and deposited onto the female during mating. Carlson and Langley [160] described this phenomenon in 10 species of tsetse, in which male flies produced a species-specific alkene, and deposited it upon conspecific females in the laboratory. The present work showed that the KI 2940 alkene produced by *G. brevipalpis* males has a chemical structure similar to the female-produced alkane. The significance of this phenomenon is not known, but it appears to be consistent in this genus of animals. Carlson and Schlein [162] showed reduced mating attempts in the presence of the male-produced alkene in *G. morsitans* using natural materials. Coates and Langley [163] suggested that such effects were due to physical masking of the sex stimulant but direct receptor-mediated behavioural effects cannot be discounted [162]. This anti-aphrodisial phenomenon was readily followed by GC even in wild-mated flies, since males of all tsetse examined to date possess species-specific alkenes that contaminate conspecific females upon mating [160]. It should be interesting to determine whether or not *G. brevipalpis* males reduce mating attempts by competitive conspecific males by dosing conspecific females with this unique alkene.

While there has been much progress in the structural identification and biological characterisation of tsetse sex pheromones and other hydrocarbons as indicators of population homogeneity, research on larviposition pheromones and larval kairomones that would attract parasitoids has not advanced, although these latter areas both have practical relevance.

6. FINDINGS REQUIRING / DESERVING FURTHER ATTENTION / EXPERIMENTS

The work conducted under this CRP resulted in several findings that require further attention in terms of additional research and methods development. The following points deserve particular attention:

- Laboratory screening of over tewenty newly designed compounds identified several promising substances (e.g., aldehydes and their analogous, stereoisomers of 3-propylcycloalkanols; Section 2.2) that should be further tested in the field.

- Based on the results of chemical analysis of the oxidative degradation products of linoleic acid ester, indicating that vegetable oils – rich in this fatty acid – could serve as locally available inexpensive sources of octenol (a known important tsetse kairomone; Section 2.4), appropriate field experiments should be carried out to corroborate and exploit this observation.

- Additional screening efforts should be invested towards the identification of optimal parameters (doses and blends of candidate kairomones) in order to achieve better results with species like *G. palpalis gambiensis* in wind tunnel experiments (Section 3.2).

- Certain plant essential oils, such as pine oils containing terpenes that appear to be characteristic to the habitat of tsetse flies and influence tsetse behaviour (Section 3.2) and enhance trap catches (Sections 4.1.2, 4.1.3 and 4.2.2), should be further investigated in field experiments.

- Further work on trap entering behaviour of attracted flies is needed for *G. fuscipes fuscipes* and *G. swynnertoni*.

- Further field studies, preferably complemented with the identification of semiochemicals involved in host recognition, should be conducted to identify practically useful attractrant odours for *G. fuscipes fuscipes* and *G. palpalis gambiensis*.

- Field studies with wind oriented or swinging traps should be conducted for *G. swynnertoni*.

- Inexpensive mobile attractant devices, with and without odour baits, are needed for subspecies of *G. morsitans*, possibly also for *G. swynnertoni*. As male tsetse flies of the above species appear to be more attracted to mobile attractant devices, such methods will be of particular importance before and during the phase of sterile male releases.

- The role of tetramethylalkanes in eliciting sexual responses among tsetse flies (*G. brevipalpis*) deserves further investigations.

- The function of long-chain methyl-branched alkenes (anti-aphrodisiacs?) produced and deposited by males onto females during mating deserves further investigation tsetse flies.

7. GENERAL DISCUSSION

The development and field use of odour baited traps and insecticide impregnated targets against several tsetse fly species in different parts of Africa [13, 62, 119] facilitated entomological monitoring and, to a certain extent, generated the basis for expanded community-based tsetse suppression efforts (the lack of sustainability [165, 166] that is generally observed for community-based tsetse control is an issue that will not be discussed here).

Table 7.1. Practically useful or promising odours and traps or targets for *Glossina* species studied in East Africa

Country	*Glossina* species	Trap type	Odour attractant*	Index of increase†
Kenya	*austeni*	Leg panel	Octenol + acetone	>2
			POCA	>2
	pallidipes	Leg panel	Octenol + acetone	>7
			POCA	>4
			POCA + decanal	2.8 – 9
			POCA + *P. sylvestris* oil	4 – 5
			POCA + *P. pumilionis* oil	4 – 5
	brevipalpis	Leg panel	POCA + decanal	>4
			POCA + *P. sylvestris*	>4
			POCA + isovaleric acid	>11
Tanzania	*swynnertoni*	S3	Acetone	~1.7
			POCA	~1.5
			POCA + decanal	~1.8
			POCA + octyl formate	~1.6
	pallidipes	S3	POCA + decanal	>4
			POCA + octyl formate	>5
			POCA + *P. sylvestris* oil	>4
			POCA + *P. pumilionis* oil	>4
		H	Acetone	>2
	morsitans centralis	S3	POCA	>9
			POCA + octyl formate	>12
			POCA + decanal	>10
			P. sylvestris oil	>2
	brevipalpis	S3	POCA + decanal	>6
			POCA + *P. sylvestris* oil	>7
Uganda	*fuscipes fuscipes*	Pyramidal	*P. pumilionis* oil	>2

*POCA is an odour blend of propylphenol, octenol, *p*-cresol and acetone.

†Numbers indicate catch increases of odour-baited traps relative to unbaited traps. Because trapping experiments with the different odour blends for a particular tsetse species were conducted at different times and under different circumstances, the figures provide only a rough guide.

The artificial bait technology also provides new perspectives for the (temporary) use of such devices in support of natural barriers in larger-scale tsetse intervention operations. At the time when this CRP was initiated, the available visually attractant devices and odours were not equally effective against all economically important tsetse fly species. For species like *G. austeni*, *G. brevipalpis*, *G. swynnertoni* and some species of the PALPALIS-group of tsetse flies no sufficiently effective combinations of visual or odour attractants were available for efficient suppression and standardized monitoring as part of an operational integrated intervention campaign against T&T.

Table 7.2. Practically useful or promising odours and traps or targets for *Glossina* species studied in West Africa

Country	*Glossina* species	Trap type	Odour attractant*	Index of increase†
Burkina Faso	*palpalis gambiensis*	Biconical	POCA	~1.9
	tachinoides	Biconical	POCA	>2
			POCA + *P. sylvestris* oil	~1.8
			POCA + *P. sylvestris* oil + decyl formate	>2
		Monoconical	POCA + *P. sylvestris* oil	>2
Mali	*morsitans submorsitans*	Biconical	*m*-Cresol + octenol (2:2)	~2
			Octenol + cow urine	2.5 – 10
			Octenol + cow urine + acetone	2 – 7
	palpalis gambiensis	Biconical	Octenol + cow urine	~2
			Octenol + cow urine + acetone	~2
		Monoconical	Octenol + dodecanal + acetone	~2
	tachinoides	Biconical	*m*-Cresol + octenol (2:2)	~2
			Octenol + cow urine	~2
			octenol + cow urine + acetone	~2
			m-Cresol + octenol + acetone	~7
			P. sylvestris oil	~4

*POCA is an odour blend of propylphenol, octenol, *p*-cresol and acetone.

†Numbers indicate catch increases of odour-baited traps relative to unbaited traps. Because trapping experiments with different odour blends for a particular tsetse species were conducted at different times and under different circumstances, the figures provide only a rough guide.

This CRP assessed several visual attractants (traps or sticky panels) for eventual use in tsetse monitoring or tsetse suppression operations or for placement in integrated barrier systems. Tables 7.1 and 7.2 list the traps that were found most efficient for use against the tsetse species under investigation in East and West Africa (see Section 1.5. Table 1.2). Furthermore, Tables 7.1 and 7.2 catalogue the most significant and promising odour

attractants obtained during the project in field experiments in East and West Africa, respectively. For a more detailed summary, see Section 4.3.

It appears worthwhile to consider the work done under this CRP (1995–2002) in the context of technical and techno-political developments over the past years that are highly relevant for ongoing and future research on and intervention operations against the T&T problem.

- In December 1997 the operational phase of T&T intervention on Unguja island of Zanzibar was successfully completed [10]. Early September 1996 the last wild tsetse fly had been captured on Unguja. The last case of nagana in cattle was detected in August 1997. In the five years after completion of AW-IPM campaign, no tsetse suppression nor any trypanocidal treatment were organised, but systematic entomological and veterinary surveys were conducted, involving a) the placement of 150 – 200 traps during more than 300 trapping days; and b) random sampling of more than 3,000 cattle. Not a single tsetse fly was captured between 1998 and 2002, and no cattle born on Unguja were found infected with trypanosomes; only one animal, which had been imported from the Tanzania mainland and was examined at Kisakasaka quarantine station, had trypanosomes. In spite of the presence of a dense population of other species of biting flies the epidemiological relevance of mechanical transmission appears to be negligible in the absence of the cyclical vector. An updated socio-economic assessment, following up on an earlier study on the agriculture and livestock situation on Unguja [167], is expected to quantify the benefits in terms of increased livestock and agricultural productivity that indirectly result from rendering Zanzibar free of tsetse and the trypanosomosis problem.

- In October 1999, OAU's International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), at the occasion of its golden jubilee (50th anniversary and 25th biannual meeting) in Mombassa, and in view of the escalating sleeping sickness and nagana problems and the continuous encroachment of tsetse into previously tsetse-free areas, passed a recommendation on the necessity for African governments to give highest priority in their development plans to T&T control.

- In July 2000 Ethiopia and Uganda took steps to ensure that the issue was presented to the 36th Summit of African Heads of State and Government, Lomé, Togo. In response the summit approved a resolution to eradicate tsetse flies from the African continent [168]. In this resolution the assembly

    - "RECOGNISES the seriousness of the problem as one of Africa's greatest constraints to socio-economic development severely affecting human and livestock health, limiting land use, causing poverty and perpetuating underdevelopment on the Continent;"

    - "ACKNOWLEDGES the trans-boundary nature of the problem" and

    - "URGES Member States to act collectively to rise to the challenge of eliminating the problem through concerted efforts in mobilising the necessary human, financial and material resources required to render Africa tsetse-free within the shortest time possible".

    - Furthermore, the summit "REQUESTS the Secretary-General to undertake all necessary consultations with a view to initiating the campaign from all

possible partners and seek their support and cooperation in the implementation of the Pan-African Tsetse Eradication Campaign".

- In September 2000, the FAO, IAEA, WHO and other collaborating partners welcomed the "summit decision" as a historic declaration since it gives recognition to the problem of tsetse and trypanosomosis at the highest political level in Africa. There was a consensus [169] on following issues:

    - To generate tsetse-free zones, various steps are needed and these will involve disease management and tsetse suppression prior to rendering an area tsetse free. Integrated area wide campaigns will likely involve a SIT component for final vector eradication in almost all ecosystems;

    - Human trypanosomosis control will, for the foreseeable future, continue to rely on disease surveillance and treatment as the principal priority, with tsetse suppression as a complementary tool;

    - Strategies for the creation of tsetse fly free zones need to be developed not only for immediate problem alleviation in identified priority and opportunity areas but also as a component of longer-term human trypanosomosis prevention.

- In December 2000 a meeting of a task force of African tsetse/trypanosomosis specialists was held in Nairobi, Kenya, and the participants developed an outline action plan [170] for a Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC).

- In February 2001 IAEA organised in Vienna a thematic planning meeting on tsetse and trypanosomosis [171]. Representatives of the specialised UN Agencies interacting in the PAAT forum recommended that the PATTEC plan of action be accepted and implemented by the OAU. The meeting emphasised that, to ensure that the SIT becomes just another routine technique in the tsetse intervention armoury, highest priority be given to the development of tsetse fly factories for the supply of high-quality, low-cost sterilised tsetse flies in strategic locations in Africa.

- In October 2001 PATTEC was officially launched at the occasion of the 26th ISCTRC held at Ouagadougou, Burkina Faso.

- In September and November 2001 the IAEA General Conference and the FAO Conference, respectively, adopted resolutions in support of the PATTEC initiative [172, 173].

- In May 2002 participants from PAAT, PATTEC and the mandated organisations (AU, FAO, IAEA and WHO) agreed at a workshop on PAAT–PATTEC harmonisation [174] on criteria for identifying priority areas for intervention against African animal trypanosomosis (AAT) and specified the approach for intervention, namely the area wide integrated pest management (AW-IPM) concept in the wider context of sustainable agriculture and rural development (SARD). This approach capitalises on factors such as agricultural practices, climatic and other trends, and brings together all tsetse control technologies including the use of sterile flies. Two proposals in areas, where T&T interventions are planned, namely within the "cotton belt" or moist savannah zone (MSZ) of West Africa and within the Ethiopian valley systems in East

Africa, were screened according to the criteria. The participants concluded that both programmes deserve full international support for implementation. Following the workshop, the four mandated international organizations (AU, FAO, IAEA and WHO) that collaborate in PAAT, issued a joint press release highlighting the consensus reached in the battle against the T&T problem [175].

- In September 2002, participants at a meeting organised by the DFID Animal Health Programme in Edinburgh, UK, welcomed the ultimate PATTEC objectives as a long-term vision and commitment at the political level. There was also consensus that creating tsetse fly free zones in identified priority areas will probably result in the highest possible benefits and should therefore be supported.

- September 2002: 2nd IAEA GC resolution in support of PATTEC was adopted [176].

- Also in September 2002, the PAAT Advisory Group Co-ordinators and the larger "PAAT community", which includes members from the mandated international organizations (AU/IBAR, FAO, IAEA, WHO), tsetse-affected countries, NARS, ARIs and relevant international institutes (ILRI, ICIPE, CIRAD and IFAD) met in Nairobi. The meeting supported the outcome and the associated joint press release resulting from the PAAT-PATTEC harmonization workshop, Rome, May 2002 [174, 175], and broadened consensus [177] regarding the ultimate objectives and the need for starting intervention in identified priority areas.

- In November 2002, representatives of the private sector who participated in PAAT Programme Committee meeting at WHO, Geneva, also confirmed their willingness to enter into public/private partnerships and join the international alliance against the T&T problem.

The above reflects a growing international consensus to specifically address the T&T problem in the context of an internationally concerted effort that is targeted at poverty reduction and generating food security for the drastically growing population in sub-Saharan Africa. It is obvious that this can only be achieved by enabling the introduction of higher productive [178] agriculture and livestock systems. In the T&T affected areas of sub-Saharan Africa there is evidence that this necessitates the creation of tsetse free zones, starting in identified prime opportunity areas for sustainable agricultural and rural development. First examples are the Ethiopian valley system, including adjacent areas in Sudan, in East Africa and tsetse infested areas in the moist savannah zone in West Africa. The criteria and guidelines drafted at the May 2002 PAAT–PATTEC harmonisation meeting [174] meanwhile facilitate for T&T affected Member States the process of mapping out and fund raising for additional opportunity / priority areas for AW-IPM measures against T&T in the context of SARD. One example is the area infested with *G. fuscipes fuscipes* and *G. pallidipes* in the lake Victoria basin, and a few more areas that – following the outlined criteria – deserve joint international efforts for T&T intervention will undoubtedly be identified. However, in order to generate the necessary funding for larger-scale AW-IPM campaigns against T&T, efforts in the forthcoming years need to focus on the creation of success stories, i.e. the establishment of some sustainable tsetse free zones, starting in the already identified priority areas for T&T intervention and agricultural development.

Community based operations will continue to be a major component of tsetse suppression particularly in small-scale tsetse control activities. It appears though that recent success stories (the application of the AW-IPM concept on Zanzibar [10], involving the SIT for

rendering the island free of tsetse flies and the trypanosomosis problem; the successful re-introduction of the SAT[2] for tsetse control in Botswana [179, 180]) appear to have revitalised confidence among decision makers that there are now concepts and sets of intervention tools available that can sustainably solve this "root" problem for agricultural and overall socio-economic development in several areas of sub-Saharan Africa.

The success with SAT in the Okavango Delta of Botswana in 2001–2002 will likely have implications for the selection of tsetse suppression tools in other T&T affected countries. Particularly in larger areas with dense or inaccessible tsetse habitat, SAT may play an increased role, provided there is evidence that it will be technically feasible and environmentally acceptable. The findings under this CRP will, nevertheless, be vital for the implementation of more efficient standardized entomological monitoring operations. Outputs of this CRP will also contribute to the establishment of more effective (temporary) barrier systems that aim at preventing a reinfestation of tsetse into freed areas, until the AW-IPM operations can be expanded into such adjacent areas and, eventually, to the natural distribution limits of the target populations, thus eliminating sources of potential reinfestation.

The above summarised past years' developments underline the interdependence of technical concepts, technologies and (techno-) political developments. The problem holders, T&T research and control personnel and decision makers appear to face a unique "window" of opportunities that obliges the international T&T community to get together and jointly initiate action against the T&T problem. Besides the implementation of developed concepts, particularly the AW-IPM against T&T, and the application of available, environmentally accepted intervention techniques, it will be important to a) continue paying close attention to the applicability of findings from various blue-sky / upstream research and b) generate increased funding for focussed R&D in direct support of efforts against the T&T problem in the context of SARD. Additional investigations on improved attractants for tsetse flies, as outlined in this CRP report, are among the topics that deserve continued support for focussed research and methods development.

[2] Sequential Aerosol Technique (repeated aerial spraying of ultra low volume formulations of non-persistent insecticides)

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