SEMIOCHEMICAL BASIS OF FEEDING PREFERENCES ON WATERBUCK *KOBUS DEFASSA*, BUFFALO *SYNCERUS CAFFER* AND OX, BY SOME *GLOSSINA MORSITANS* GROUP OF TSETSE

BY

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DECLARATION

I hereby declare that the material contained in this thesis is my original work and has not been presented for a degree in any other university.

signed ..... date 3/12/99

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Dedicated

To

Lewis Gathua
Denis Kamau
Mark Gikonyo
Agnes Waithera

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ABSTRACT

This study was carried out based on the speculation that gradation of hosts as sources of bloodmeal by tsetse flies is due to the absence and/or presence of specific semiochemicals. The study was carried out to establish whether kairomones and/or allomones from waterbuck *Kobus defassa* (nonhost of tsetse), buffalo *Syncerus caffer* and ox (hosts) emanate from the skin surface of the animals and whether these semiochemicals are responsible for the relative refractoriness of the waterbuck to tsetse flies.

The behavioural responses of caged individual teneral *Glossina morsitans morsitans* on waterbuck and ox and on feeding membranes with and without smears of different doses of waterbuck sebum, were compared. No significant difference was found in the initial landing behaviour on the two animals (waterbuck and ox), nor on treated and control parts of the membrane. However, the subsequent behaviours of the flies were significantly different. Whereas none of the flies that landed on the ox showed any escape behaviour, more than a third of those that initially landed on waterbuck escaped. Similar results were obtained on feeding membranes treated in part with the waterbuck sebum. Moreover, flies that landed on waterbuck or its sebum changed probing sites more often, probed significantly longer and showed a general delay in initiation of feeding. Comparison of the behaviour of flies that landed on control zones of the membrane and those on untreated (double control) membrane also showed significant differences, indicating that the insect's behaviour was affected by a more volatile chemical signal in addition to a less volatile contact signal associated with the sebum. Analysis of the waterbuck sebum by gas chromatography-linked electroantennographic detector (GC-EAD) revealed the presence of two electrophysiologically (EAG) active constituents of relatively high molecular weights which may have a role in the close-range/contact effects. The compounds were identified from their mass spectral data as the diacylglycerol, glyceryl 1-butanoate-3-myristate and the triacylglycerol, glyceryl 1-butanoate-3-hexanoate-2-myristate.

An effective technique for trapping odour from a specific area on the body surface of a live animal was developed. The odour from the waterbuck body surface was trapped on various adsorbents including activated charcoal, glass wool and

reverse-phase silica (octadecyl bonded silica) while odours from buffalo and ox were trapped on reverse-phase silica. EAG-active compounds from the trapped volatiles and non-volatile skin secretions of the animals on tsetse antennae (*G. m. morsitans* and *G. pallidipes*) were detected using GC-EAD and identified using gas chromatography-linked mass spectrometry (GC-MS), gas chromatography (GC) co-injection, synthesis and confirmed using GC-EAD. Fourteen EAG-active peaks on antennae of *G. m. morsitans* were detected in the reverse phase- and activated charcoal-trapped volatiles of the waterbuck. These compounds were identified as: (E)-2-heptenal, 2-octanone, 4-methylphenol, 2-nonanone, nonanal, 2-decanone, decanal, 3-*n*-propylphenol, δ-octalactone, 2-undecanone, 3-isopropyl-6-methylphenol, undecanal, 2-dodecanone and (E)-6,10-dimethyl-5,9-undecadien-2-one. Laboratory-reared *G. pallidipes* showed no EAG responses while field-trapped *G. pallidipes* showed EAG responses to thirteen peaks in the waterbuck body volatiles, eleven of which were identified as: (E)-2-heptenal, 2-octanone, 4-methylphenol, 2-methoxyphenol, 2-nonanone, nonanal, (E)-2-nonenal, decanal, 3-*n*-propylphenol, δ-octalactone and (E)-6,10-dimethyl-5,9-undecadien-2-one. Ten EAG-active peaks on female *G. m. morsitans* were detected in body volatiles of the buffalo. Eight of these were identified as: (E)-2-heptenal, octanal, 4-methylphenol, nonanal, decanal, undecanal, (E)-2-undecenal and dodecanal. Nine EAG-active peaks on female *G. m. morsitans* were detected in the ox body volatiles and six of them identified as: 4-methylphenol, nonanal, decanal, undecanal, (E)-2-undecenal and dodecanal. Candidate compounds from the waterbuck body volatiles suspected to be EAG-active on *G. m. morsitans* but were not available commercially were synthesized. These compounds included 2-methyl-3-octanone, 2,6-dimethyloctane, *δ*-octalactone, 2-isopropyl-4-methylphenol and 3-isopropyl-4-methylphenol. *δ*-Octalactone and 2,6-dimethyloctane were EAG-active on *G. m. morsitans*, but only *δ*-octalactone co-eluted with the target peak in the volatiles of waterbuck. The structure of *δ*-octalactone was confirmed using ^{1}H and ^{13}C NMR in addition to the other methods described above.

The EAG-active compounds identified from volatiles of buffalo and ox were similar in structure and elicited attraction responses in *G. m. morsitans* when bioassayed in a choice wind-tunnel. On the other hand, EAG-active compounds

identified from waterbuck volatiles comprised of most of the compounds identified in volatiles of both ox and buffalo in addition to a series of other compounds that elicited aversive behaviour in *G. m. morsitans* in the wind-tunnel bioassays. The blend of all EAG-active compounds from the waterbuck was slightly repellent to the flies in the wind-tunnel. Apart from 4-methylphenol and 3-*n*-propylphenol, the other compounds that elicited attraction behaviour to tsetse flies (in the wind-tunnel) are being reported as new candidate attractants (kairomones) from the body surface of the animals in this study. Whereas the waterbuck was shown to emit similar attractants, it also emits a series of repellents (allomones) for defense against the biting flies from its skin surface. The use of allomones by higher mammals for defense against tsetse flies is being reported for the first time in this study.

These results provide a clear evidence that the relative refractoriness of waterbuck to tsetse flies is mediated by allomones. The allomones (both volatile and non-volatile), account for the low numbers of the flies that are attracted to and feed on the waterbuck in the wild. The allomones identified from the waterbuck, when appropriately formulated, may be used to protect cattle and humans against tsetse attack.

CHAPTER 1.

1.0 INTRODUCTION

Tsetse flies (Diptera: Glossinidae: *Glossina*) are haematophagus insects which infest about 40% (11 million km^2) of tropical Africa, affecting 38 countries between 15^0N and 30^0S. The flies transmit protozoa, *Trypanosoma* spp., which cause trypanosomosis in human (referred to as human sleeping sickness) and animal trypanosomosis in domestic livestock (where it is referred to as nagana). About 150 million cattle and 50 million people are at risk of the disease (Murray and Gray, 1984; UNDP/WORLD BANK/WHO, 1990).

As a vector of trypanosomosis, the tsetse fly imposes a constraint on orderly rural development in over 7 million square kilometres of good agricultural land in the tsetse infested region of Africa (Murray and Gray, 1984; Turner, 1986). The persistence of the tsetse flies, therefore, leads to under-exploitation of infested land and over-exploitation and degradation of fly-free areas. The disease may be fatal to domestic animals if untreated; otherwise, it causes severe losses in production due to poor growth, weight loss, low milk yield, reduced capacity for traction power in mixed agriculture, infertility and abortion. Losses in beef production alone due to trypanosomosis in cattle was estimated at $5 billion annually in 1963 with a similar figure ten years later (Murray and Gray, 1984; Lehane, 1991). Animal trypanosomosis is a disease primarily of the wild animals, but whereas domestic animals succumb to the infection, wild animals show a high degree of resistance. These wild animals act as efficient reservoir hosts from which the tsetse fly obtains an infection during the process of feeding (Ashcroft, 1959; Jordan, 1986).

Surveys on feeding patterns of different species of tsetse (*Glossina* spp.) based on blood-meal analyses have shown varying degree of specialisation on different groups of vertebrate hosts (Weitz, 1963; Moloo, 1993; Clausen *et al.*, 1998). Although overlap or conjunction of habitats preferred by tsetse and their hosts may be a factor in promoting some specialisation (Mihok *et al.*, 1996; Clausen *et al.*, 1998), no relationship exists between the relative abundance of different vertebrates that are available in different habitats and the frequency with which they are fed on (Vale, 1974b; Turner, 1987; Snow *et al.*, 1988; Clausen *et al.*, 1998). For example, in a study carried out in Lambwe Valley in Kenya where *Glossina pallidipes* is the dominant species, over 80% of feeds derived from bushbuck, buffalo and bushpig, but none or hardly any from other numerically common game species such as oribi, impala, waterbuck, reedbuck and hartebeest (Turner, 1987). Similarly, Lamprey *et al.* (1962) reported that impala constituted 70% of fauna in some parts of Tanzania while 3, 0.2 and 0.02% comprised of warthog, rhinoceros and buffalo respectively. Whereas the impala provided only 1% of tsetse bloodmeals, the latter three hosts provided 77, 2 and 14% of the bloodmeals, respectively.

In summarizing the observed host preferences by the *Glossina morsitans* group, Weitz and Jackson, (1955); Weitz and Glasgow, (1956), grouped the animals in terms of the frequency with which they were bitten by the flies: those that were i) always bitten included warthog and rhinoceros; ii) commonly bitten included roan antelope, reedbuck, buffalo, kudu, bushpig, bushbuck, elephant and giraffe; iii) rarely bitten included eland, duiker, waterbuck, impala, baboon, monkey, dog and cats; and iv) never bitten included hartebeest, topi, zebra and wildebeest. In their reviews on tsetse and trypanosomosis, Jordan (1986) and Leak (1998) reported that the impala, gazelles,

zebra, waterbuck, wildebeest and hartebeest were very common in many tsetse infested areas of Africa but were rarely fed on. Examples are, the Luangwa Valley of Zambia, the Galana ranch of Kenya, the Comoe National Park of Cote de'Ivoire (Clausen *et al.*, 1998) and the Mkwaja ranch in NE Tanzania (Tarimo *et al.*, 1983), where the waterbuck, hartebeest and impala were reported among the available hosts but were not fed on.

The natural hosts of the 31 species and subspecies of *Glossina* in Africa based on bloodmeal analysis has been documented by Moloo (1993). The 9394 bloodmeals analysed from *G. m. morsitans* obtained from Tanzania, Zambia and Zimbabwe revealed that the ten natural hosts for this fly in order and proportion of importance as food source were: warthog, (37.4%), kudu (14.1%), elephant (5.2%), buffalo (3.4%), bushpig (2.8%), bushbuck (2.5%), cattle (1.9%), man (1.5%), rhinoceros (1.5%) and eland (1.1%). The waterbuck was quoted as providing only 0.1% of the bloodmeals in this species. 7342 bloodmeals from *G. pallidipes* were obtained from Tanzania, Kenya, Uganda, Zimbabwe and Ethiopia. The natural hosts of *G. pallidipes* in order and proportion of importance were bushbuck (28.2%), bushpig (14.8%), warthog (11.8%), buffalo (10.6%), cattle (3.8%), kudu (2.5%), man (2.3%), elephant (2.1%), rhinoceros (1.0%) and roan antelope (1.0%). The waterbuck contributed only 0.8% of the bloodmeals. The nonpreference in some of these animals has been confirmed by direct observations made on the responses of tsetse flies to various hosts in the natural tsetse infested areas. Vale (1974c) observed very few landings and no engorgement on the impala by *G. m. morsitans* compared to the ox, bushpig, dog and warthog on which many flies alighted and engorged. Field experiments in which ox, buffalo, eland, oryx and waterbuck were observed in enclosures in a *G. pallidipes* habitat provided clear

evidence that the fly has a gradation of host preferences (Grootenhuis, 1986; Grootenhuis and Olubayo, 1993). In this study, buffalo and cattle were equally attractive to tsetse (600-800 flies during observation period), 20-25% of which engorged. On the other hand, very few flies (<10) appeared to be attracted to the waterbuck, none of which were seen to engorge. Eland and oryx were intermediate attracting a third and a sixth, respectively, of the number associated with cattle (or buffalo), of which even smaller proportions (10% and 3%, respectively) engorged.

No systematic studies have been carried out to elucidate the bases of the apparent feeding nonpreferrence in these animals. Although host body size and mass are known to affect close-range attraction and landing behaviour of tsetse (Vale, 1974b,c; Hargrove, 1976), they cannot account for differences between animals of roughly the same sizes nor the extent of differences observed. A key question is whether preferences by tsetse are related to suitability of host blood, which may have influenced the adaptive behaviour of the fly, or whether they reflect the existence of defense mechanisms of varying effectiveness in different wild animals. Moloo *et al.* (1988) studied the survival and reproductive performance of *G. m. morsitans* when maintained in vitro on the blood of eight wild mammals comprising of both unpreferred (e.g. waterbuck, oryx) and preferred (e.g. buffalo, cattle, warthog) hosts. No significant differences were found, showing that tsetse host preferences are not based on host blood characteristics. A well-documented defensive reaction of hosts to tsetse involves their grooming behaviour such as tail flicking, skin twitching, kicking and stamping (Vale, 1977; Torr, 1994; Baylis, 1996; Torr and Hargrove, 1998). However, detailed observations to date have not revealed significant negative correlation between host grooming and feeding success by tsetse. Such defensive behaviour were observed to

increase not only the number of tsetse attracted but also their feeding success (Baylis, 1996; Torr and Hargrove, 1998).

Dethier (1976), pointed out that if an animal is to select foods, it must be able to discriminate between foods. Therefore, for an obligate blood feeder like the tsetse fly, selection of a host for bloodmeal obviously should involve the ability to distinguish all hosts and nonhosts.

Tsetse flies locate stationary hosts beyond their visual range upwind (60-120m) through odour-mediated anemotaxis (Vale, 1977). Closer to the host (~10m), visual cues supplement host odours (Vale, 1974c). Thermal, tactile and contact chemical stimuli on the host induce landing, probing and feeding, and contribute to host acceptability (Dethier, 1954; Reinouts van Haga and Mitchell, 1975; Sutcliffe, 1987; Packer and Warnes, 1991; Van der Goes van Naters and Rinkes, 1993; Saini *et al.*, 1993; Van der Goes van Naters and Den Otter, 1998; Van der Goes van Naters *et al.*, 1998). Some of the kairomones associated with host odours used by some tsetse species to locate their hosts have been identified and include breath constituents, such as carbon dioxide, acetone and 1-octen-3-ol (Hall *et al.*, 1984; Vale and Hall, 1985a) and phenolic microbial breakdown products of host skin secretions and urine, particularly the blend of 4-cresol and 3-n-propylphenol (Hassanali *et al.*, 1986; Owaga *et al.*, 1988; Bursell *et al.*, 1988; Vale *et al.*, 1988; Warnes, 1990; Saini *et al.*, 1993). The identified attractants are not host specific and have been found to vary among the hosts. The urine of waterbuck (nonhost) was found to contain the phenolic attractants, 4-cresol and 3-n-propylphenol in about the same ratio as in the urines of buffalo and cattle, while the urines of bushpig and warthog (hosts) lacked 3-n- propylphenol (Madubunyi *et al.*, 1996). Although combinations of these kairomones have been

successfully used as baits to enhance the efficacy of traps or insecticide-treated targets for the control of *G. pallidipes* and *G. m. morsitans* in the field (Vale *et al.*, 1988; Brightwell *et al.*, 1991; Willemse, 1991), they are still less than 50% as attractive as the natural cattle odour. This implies that there are other components of the host odour that are yet to be identified (Vale, 1981; Willemse and Takken, 1994; Torr *et al.*, 1995; Hargrove *et al.*, 1995; Holmes, 1997), and these may include the host-specific and/or host-discriminating semiochemicals.

1.1 Conjectural basis of the study

This study was carried out based on the speculation that the refractoriness of wild animals like waterbuck to tsetse could be due to the absence and/or presence of specific semiochemicals. That is, key kairomone components are absent, or present in sub-threshold amounts, rendering the flies relatively indifferent to the animals; or allomones may be present that repel the flies from distance and, when close to the animal, deter them from feeding.

1.2 Objective of the study

1.2.1 Overall Objective

Establish whether kairomones and/or allomones from waterbuck (nonhost of tsetse), buffalo and ox (hosts) emanate from the skin surface of the animals; and whether these semiochemicals are responsible for the relative refractoriness of the waterbuck to tsetse flies.

1.2.2 Specific Objectives

1) Study the feeding behaviour of *G. m. morsitans* on live animals (waterbuck and ox) and on membranes treated with various doses of waterbuck sebum.

2) Trap volatile compounds from the body surface of animals (waterbuck, ox and buffalo) and carry out GC-EAD studies on *G. m. morsitans* and *G. pallidipes* and identify any EAG-active compounds using instrumental methods (GC and GC-MS). Similarly, carry out GC-EAD studies on *G. m. morsitans* on waterbuck sebum and identify any active compounds.

3) Synthesize unavailable candidate EAG-active compounds for chemical identification and assays.

4) Carry out behavioural bioassays (repellency/ attractancy) against *G. m. morsitans* on the blends of the identified EAG-active compounds from the animals.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 The Genus *Glossina*

The genus *Glossina* contains 31 species and subspecies placed in three subgenera, *Glossina s. tr.*, *Nemorhina*, and *Austenina*, constituting, respectively, the species groups *morsitans*, *palpalis* and *fusca*. The classification of tsetse into the three subgenera is based on morphological differences in the structure of the genitalia (Potts, 1970). The groups have several important differences in terms of their ecology and their role as disease vectors.

The *fusca* group contains 15 species and subspecies, 11 of which inhabit rain forests. This group is generally not considered important in the transmission of trypanosomosis (Nash *et al.*, 1950; Jordan, 1986), partly because most species do not feed on man and partly because their habitat (forest) does not allow extensive fly-livestock contact, except for livestock grazed at the edge of such forests.
The most preferred host of this group, based on bloodmeal analysis (Moloo, 1993) is the bushpig *Potamochoerus porcus* (L.). Others include the hippopotamus *Hippopotamus amphibius* (L.), bushbuck *Tragelaphus scriptus* (Pallas), buffalo *Syncerus caffer* (Sparrman) and cattle *Bos* spp.(L.).

The *palpalis* group consists of 9 species and subspecies occurring primarily in West Africa and inhabiting lowland rain forest as well as the linear riverine-forest habitat found in savanna areas (Challier *et al.*, 1977; Jordan, 1986). The riverine species are vectors of *Trypanosoma brucei gambiense*, which causes gambian sleeping sickness in humans. It is essentially a human-fly-human transmitted disease which

develops into a chronic disease with increasing lethargic syndromes, ending in death in two to three years (Lambrecht, 1980). The most preferred hosts of the *palpalis* group are man and bushbuck followed by the domestic pig *Sus scrofa* (L.) and monitor lizard *Varanus niloticus* (Linnaeus) (Moloo, 1993).

The *morsitans* group with 7 species and subspecies is the most important economically and includes the species, *G. morsitans morsitans* Westwood 1850, *G. m. submorsitans* Newstead 1910, *G. m. centralis* Machado 1970, *Glossina swynnertoni* Austen 1923, *G. longipalpis* Wiedemann 1830, *G. pallidipes* Austen 1903, and *G. austeni* Newstead 1912. These species occupy the woodland - savanna habitat and are the main vectors of *T. brucei rhodesiense*. This parasite causes the rhodesian sleeping sickness in humans and nagana in cattle. The parasite causes an acute disease with symptoms similar to gambian sleeping sickness but ending in death in a matter of a few weeks (Lambrecht, 1980). The disease is a zoonosis with wild animals acting as reservoir for parasites. The most preferred hosts of the *morsitans* group are warthog *Phacochoerus aethiopicus* (Pallas), cattle and buffalo, followed by bushbuck, bushpig and man (Moloo, 1993).

Whereas tsetse flies are known to derive their bloodmeals from other wild animals like the giant forest hog *Hylochoerus meinertzhageni* (Thomas), elephant *Loxodonta africana* (Brummenbach), rhinoceros *Diceros bicornis* (L.), aardvark *Orycteropus afer* (Pallas), porcupine *Hystrix* spp., giraffe *Giraffa camelopardalis* (L.), kudu *Tragelaphus* spp. and duiker *Cephalophus* spp., all species of *Glossina* hardly feed on the waterbuck *Kobus ellipsiprymnus ellipsiprymnus* (Ogilby), *K. e. defassa* (Rupell).

2.2 Waterbuck *Kobus ellipsiprymnus defassa*

The waterbuck is an antelope which is coarse-haired with a neck mane and ruff. The males weigh on average 236 kg while females weigh 186 kg. Their colour varies from grizzled grey to red brown, darkening with age.

The waterbuck is distributed in most parts of tropical Africa, (Fig.1), an expanse within the tsetse region, (Fig.2). There are two subspecies of the waterbuck, *Kobus ellipsiprymnus ellipsiprymnus* found mainly in west and central Africa and *K. e. defassa* found in eastern and southern Africa. The two subspecies overlap and hybridise in several places around the great Rift Valley (Estes, 1991; Dorst and Dandelot, 1993).

The waterbuck is generally a grazer that drinks water frequently but also browses when green grass is in short supply. Its habitat is therefore near drainage lines, valleys or near other water systems that are neighbouring grasslands.

Waterbucks have numerous diffuse sebaceous follicles that make the coat oily and emit a cloying, musky smell (Estes, 1991; Mihok *et al.*, 1991; Dorst and Dandelot, 1993; Haltenorth and Diller, 1996; Amin *et al.*, 1997).

Apart from being an unpreferred host of tsetse, the waterbuck was not affected by the great rinderpest epidemic of 1889-1890 that killed hundred of thousands of cattle and other wild mammals like the buffalo, eland, bushbuck, giraffe, warthog, bushpig and forest hog (Ford, 1971; Leak, 1998).

2.3 Buffalo *Syncerus caffer caffer*

The buffalo is one of the most successful African mammals, in terms of geographical range, (Fig.3). The buffaloes have a wide range of habitat extending from the sea level to forests of the highest mountains including the arid savanna as long as water is available in the form of rivers, swamps and lakes.

There are two subspecies of the African buffalo: *Syncerus caffer nanus,* which occurs in West African forests and is small in size, weighing at most 300 kg; *Syncerus caffer caffer* is the common bulky black buffalo that inhabits most of the African savanna. The males weigh an average of 686 kg while females weigh 576 kg.
S. c. caffer adults have a black or seal brown coat colour while the young calves have a black or dark brown colour, changing to dirty yellow-brown and then chocolate brown after several months.
Buffaloes whose scent or that of their excrement is just like that of cattle (Estes, 1991), are bulk grazers but may browse when grass is scarce or of poor quality.

2.4 Cattle (*Bos* spp.)

The cattle found in Africa are grouped into two broad categories based on whether or not they have a hump (Leak, 1998). The humpless cattle *Bos taurus,* include the Shorthorns of West Africa, and the Longhorns such as the Kuri and N'Dama. Humped cattle *Bos indicus,* include the West African zebu such as the Fulani, East African zebu which is mainly the East African Shorthorn and the Sanga comprising of the Ankole, Nilotic Sanga and Afrikander.

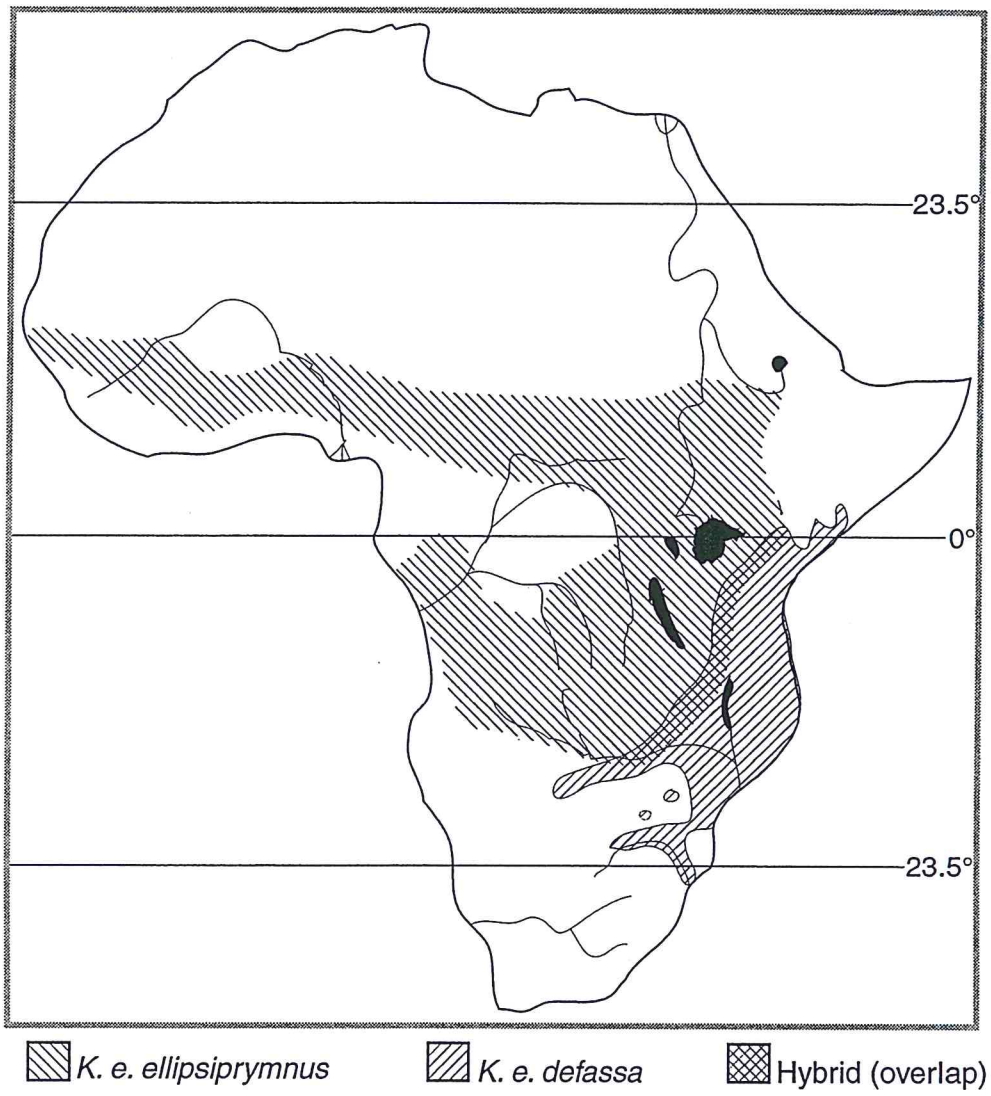


Fig. 1. Distribution of the Waterbuck in Africa (from Estes, 1991)

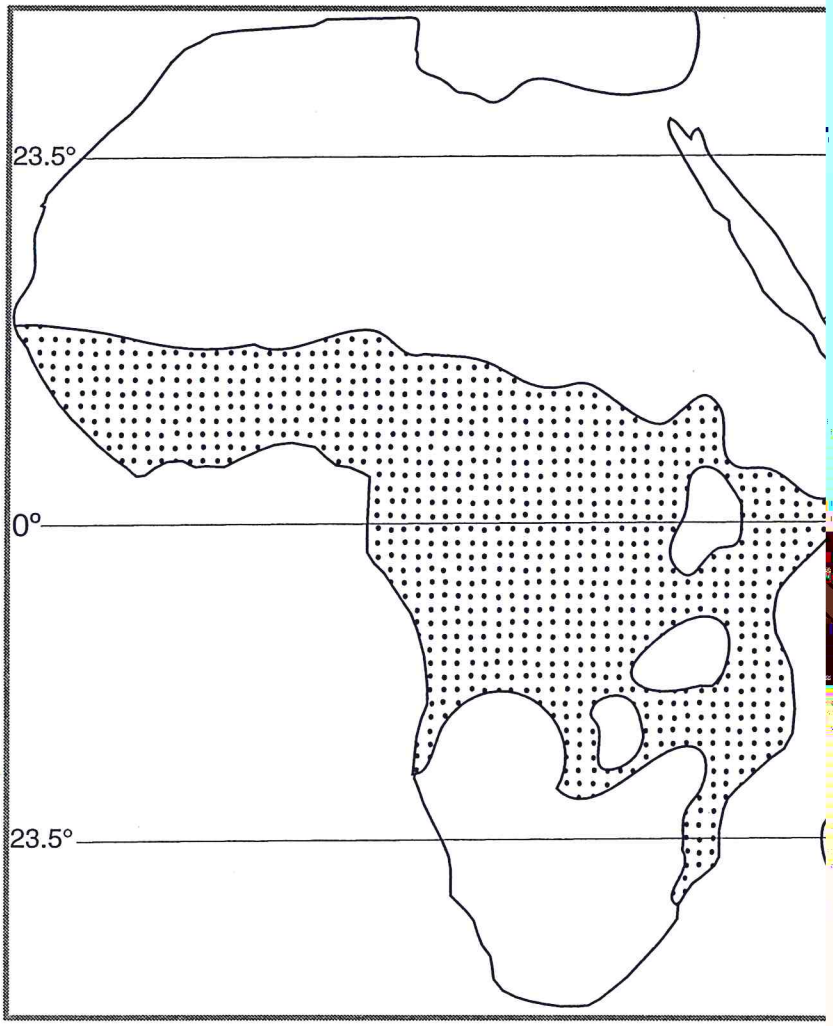


Fig. 2. Distribution of tsetse flies in Africa (from

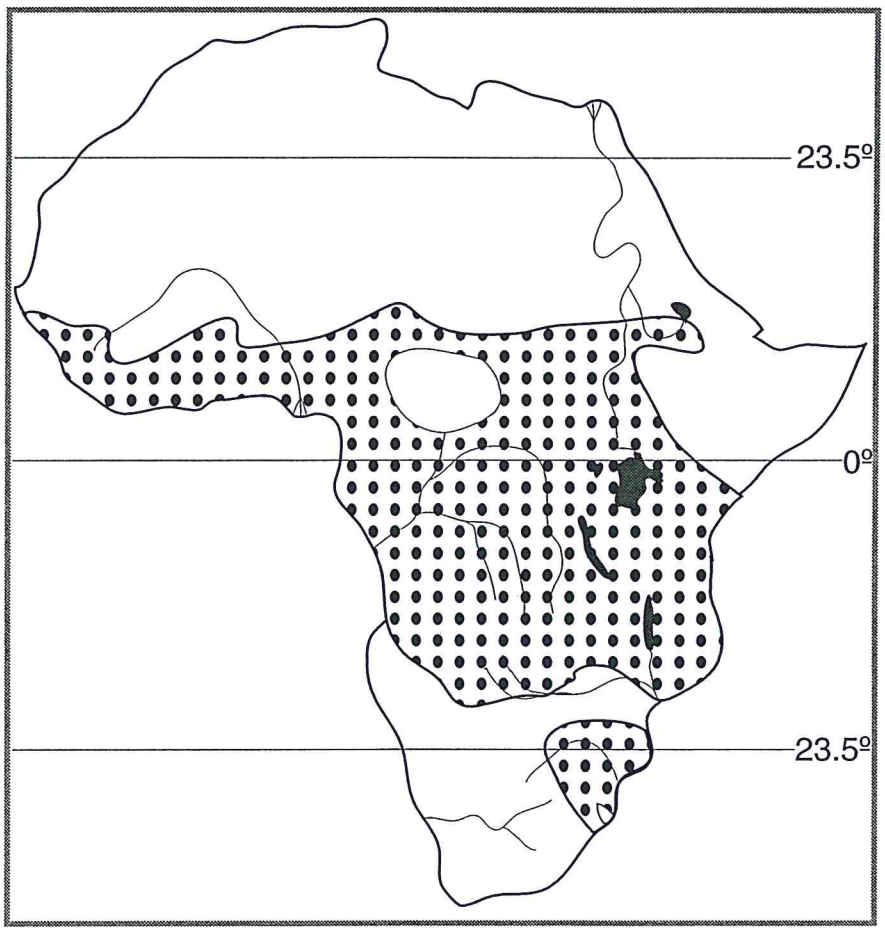


Fig. 3. Distribution of the buffalo in Africa (from Estes, 1991)

2.5 Control of tsetse-host contact

Tsetse flies transmit and acquire trypanosomes in the process of taking a bloodmeal from a host. Absolute prevention of feeding on a host would therefore be necessary to avoid the transmission of the trypanosomes. Since this has not been achieved on livestock, two methods have been used to control the feeding success of flies: the use of repellents and the reduction of tsetse challenge which is achieved by keeping the populations of flies at low levels.

2.5.1 Use of repellents

Repellents are meant to prevent either host-fly contact or the initiation of feeding process on the host. The search for repellents started in the 1940s with several compounds and plant extracts being tested (Hornby and French, 1943; Holden and Findlay, 1944; Findlay *et al.*, 1946) but all suffered from lack of persistence of repellent activity. More recently, several other anti-insect compounds such as N,N-diethyl-m-toluamide (DEET), indalone, citronyl and those associated with hosts such as, 2-methoxyphenol (from cattle urine), acetophenone, pentanoic and hexanoic acids (from cattle sebum) have shown variable repellence to *Glossina* (Schmidt, 1977; Wirtz *et al.*, 1985; Torr *et al.*, 1996); but none is being used commercially. This is probably due to lack of appropriate dispensing techniques for these repellents on livestock.

2.5.2 Reduction of tsetse populations

The control of tsetse populations is achieved by using chemical or non-chemical methods to kill the tsetse flies.

2.5.2.1 Chemical methods

Insecticides are sprayed on vegetation or applied on livestock.

a) Application of insecticides to tsetse habitats.

Two methods for application of insecticides to tsetse habitats have been used.

i) Ground spraying.

This method aims at applying a deposit of residual insecticide to tree trunks, lower branches and other tsetse fly resting sites where it remains lethal to the flies for many weeks. The most commonly used insecticides were DDT and dieldrin. Although this technique was used with some success in Nigeria, rapid reinvasion was reported to occur in Central African Republic, Senegal and Zimbabwe (Allsopp, 1984). The use of residual insecticides is an environmental pollution problem that affects the entire ecosystem and is, therefore, no longer being used (FAO/WHO, 1969; Kaaya, 1994; Holmes, 1997).

ii) Aerial spraying.

This is a technique that aims to kill adult flies over the period of application. The insecticide is applied at low doses in form of aerosols either from low flying fixed-wing aircrafts or from helicopters. Although there are no residual effects in this technique, it remains an environmental hazard since at least five applications of the insecticides (endosulfan, deltamethrin, DDT, BHC and dieldrin) have to be carried out in a single eradication operation.

The use of sophisticated application equipment makes this method unsustainable in many African countries unless substantial funding from international sources is made

available. Moreover, the problem of reinvasion or re-establishment is widespread unless this technique is combined with ground spraying of residual insecticides around the periphery of the earmarked eradication zone (Allsopp, 1984; Hursey and Allsopp, 1984; Turner, 1984; Turner and Brightwell, 1986; Holmes, 1997).

b) Application of insecticides on livestock.

Pyrethroids such as deltamethrin, cypermethrin, alpha-cypermethrin, flumethrin and cyfluthrin are applied on cattle as pour-ons. Animals treated this way act as insecticide-impregnated targets on which tsetse that land to feed are killed by contacting lethal amounts of the insecticide. This method has been widely used with various degrees of success in the reduction of trypanosome prevalence (Bauer *et al.*, 1988; Lohr *et al.*, 1991; Thomson and Wilson, 1992a,b; Leak *et al.*, 1995). However, like the application of insecticides to tsetse habitats, the use of insecticides on livestock may pose a health hazard to humans resulting from contamination of animal products such as milk and meat.

2.5.2.2 Non-Chemical methods

i) Destruction of vegetation.

The earliest methods used in combating trypanosomosis was the destruction of the vector habitats, where large tracts of land were cleared of vegetation. This exercise proved not only too costly in terms of labour but also promoted soil erosion and is therefore no longer being used.

ii) Killing of wild animals.

Game animals considered as tsetse preferred hosts were eliminated with the aim of removing the food supply of the flies (FAO/WHO, 1969; Jordan, 1986). This method proved unsuitable since tsetse flies can shift their feeding preferences once their regular food source is removed (Vale and Cumming, 1976).

iii) Biological control.

Natural enemies of *Glossina* such as predators, parasites and pathogens are deliberately used for purposes of controlling the flies. No significant progress has been made in the use of this technique because most trials of parasites of tsetse flies have used insects that have naturally coexisted (possibly mutually adapted) with the tsetse flies (Jordan, 1986; Leak, 1998). The use of entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* which effected a 100% mortality against *G. m. morsitans* in the laboratory (Kaaya, 1989), may provide a better alternative for the control of the fly.

iv) Sterile insect technique (SIT).

Laboratory reared male flies are sterilised and released in large numbers into the field. The success of this technique depends on the sterile males being competitive with wild fertile males for the insemination of females. This method may succeed in an ecological island where a population estimate of the target tsetse species is known, and there is no possibility of reinvasion. For example, successful eradication of *G. austeni* from Unguja Island (Zanzibar) was achieved by using this technique (Saleh *et al.*, 1997; Saleh *et al.*, 1999).

v) Baited traps and targets.

In this technique, flies are attracted to traps or targets (screens) and are either caught and restrained thereby dying of starvation and exposure to the sun or are killed by insecticides on the devices. The method attempts to exert a daily mortality of 2-3% of the tsetse population leading to a 95% reduction in the population annually in the absence of reinvasion (Holmes, 1997). The efficiency of traps and targets in tsetse control has been greatly improved by modifying their appearance, shape, colour and contrast between colours, and by dispensing tsetse attractants near them (Laveissiere *et al.*, 1990). The use of traps and targets has been acknowledged as the most effective and sustainable method of tsetse control since it is safe for the environment, easy to use, very cheap and can be used by the rural communities (Laveissiere *et al.*, 1990; Holmes, 1997).

2.6 Behavioural specificity to hosts

Tsetse flies are host-specific and are known to exhibit different behaviours once in the vicinity of the hosts in terms of final attractivity, landing sites, and the feeding success. The number of flies approaching a mobile or a stationary host has been studied using either a ring of electric nets surrounding the host or by direct observation (Vale, 1974c). Close-range responses of flies to a host have been studied by observations made by man in a glass-shielded pit (Hargrove, 1976).

Using electric nets, Vale (1977) demonstrated that wild and domestic animals have varying degrees of attractiveness to tsetse flies. The warthog was the most attractive to *G. pallidipes* and *G. m. morsitans* followed by buffalo, ox, goat, bushpig and kudu. The least attractive animals were donkey, bushbuck, sheep and impala

although the catches were about half those caught near the ox. Different proportions of flies eventually engorged on these animals but not on the impala.

By direct observation, Vale (1974c) reported no engorgement by the few flies attracted to the impala or bushbuck compared to ox, dog, bushpig and adult warthog. Similarly, Grootenhuis (1986), Grootenhuis and Olubayo (1993), observed few *G. pallidipes* approaching the waterbuck and none engorged, while among the few attracted to the oryx, only 3% engorged. On the other hand, the buffalo and cattle attracted large numbers of flies, similar to those reported by Vale (1977), and a quarter of them engorged. The eland was of intermediate attractancy.

Tsetse flies are known to form swarms behind mobile objects, especially after man for whom they rarely land on to feed unless when they are extremely hungry (Ford, 1970). For example, when a party of men walked alongside a bull, *G. m. morsitans* landed on the bull (but not on man) and fed in smaller numbers than when the bull was unaccompanied. Hargrove (1976) and Vale (1977) demonstrated that the presence of men not only reduced attractiveness of an ox to tsetse flies but also reduced the proportion of engorging flies. It was concluded that men reduce the odour attraction of the ox. Reduction of odour attractiveness to cattle implies that man emits an odour that is repellent to the flies. This appears to be the case since Vale (1969, 1974a,b) demonstrated that a model animal alone was a much more effective bait for *G. m. morsitans* than man, and that man accompanying a model animal reduced the catches from the model.

The differences in the various responses by flies in the vicinity of other hosts was attributed to host size or complacency. Whereas the host's size may play some role in numbers attracted, it cannot account for differences among hosts of the same

size or between small sized hosts like the warthog or bushpig and large animals like the eland or waterbuck.

Defensive behaviour against nuisance biting flies is common among all animals and is characterised by skin ripples, tail switches, leg kicks, head movements, ear flicks and rubbing the snout against objects or on the ground for warthog and bushpig (Torr, 1994; Baylis, 1996; Torr and Hargrove, 1998). The defensive movements are expressed with various frequencies that may discourage biting insects from feeding (Lehane, 1991) or reduce the mean size of bloodmeal taken by the flies. However, these defensive behaviours increased the number of flies attracted to an ox and did not affect the feeding success of *G. pallidipes* on the ox (Baylis, 1996; Torr and Hargrove, 1998) or on an adult warthog by both *G. pallidipes* and *G. m. morsitans* (Torr, 1994).

The defensive behaviours of any host are density dependent and can be lethal to the flies, but a change of the feeding strategy rather than escape to the bush has recently been reported. Torr and Hargrove (1998) observed that as the number *of G. pallidipes* per ox increased from 10 to 40, the proportion of flies on the legs increased from 60% to 70%. Similarly, the proportion of *G. pallidipes* and *G. m. morsitans* alighting on the head of a warthog increased from 25% to 50% as the number of flies increased from less than 100 to more than 800 (Torr, 1994). The shift to the legs of an ox or the head of a warthog presumably allowed the flies to feed unperturbed.

When complacency was induced on ox and goat by sedation using xylazine (Vale, 1977), the number of flies attracted to the goat was slightly reduced while attractivity to the ox was reduced to a quarter. In both cases there was an increase in the proportion of engorging flies compared to unsedated animals. In a related experiment, Baylis and Nambiro (1993) compared the attractiveness of cattle to *G. pallidipes* and

their feeding success on cattle infected with *T. congolense* and healthy ones. There was no difference in the attractiveness of infected and uninfected cattle to *G. pallidipes* neither was there any difference in the mean fat content or blood meal size of male flies which fed successfully on the two groups of animals. However there was a highly significant difference between the feeding success of *G. pallidipes* on uninfected (36%) and infected (63%) cattle. In both cases, it appears that complacency of a host acts at close range and only affects the feeding success but not the attractiveness of the host. Sedation of animals makes them sleep and remain still most of the time, hence reducing their conspicuousness to resting and ranging tsetse. Vale (1977) suggested that the xylazine may also have reduced the quantity and nature of odours emanating from the sedated animals, suggesting the importance of host odour in host location.

2.7 Host location

Feeding on blood, which is not only important for the acquisition and transmission of trypanosomes but also for the survival and reproduction of all adult tsetse flies, occurs every 2-5 days (Madubunyi, 1989; Leak,1998). This implies that within this interval a tsetse fly must locate a suitable host from which to take the bloodmeal.

The host-seeking behaviour of tsetse flies is influenced by both endogenous factors such as circadian rhythm of activity, level of starvation, age, sex , pregnancy status of female flies and exogenous factors which include temperature, vapour pressure deficit, visual, mechanical and olfactory host stimuli (Colvin and Gibson, 1992). Exogenous factors such as visual cues and odours, associated with the host are

the only ones considered important since they can be manipulated in the field for the control of tsetse.

Tsetse locate and recognize potential hosts by utilizing visual and olfactory cues. In general, the fly is activated by the host's odour even when beyond its visual range and after orientation takes off upwind until it comes in the vicinity of the host where visual cues such as shape, size, colour contrast and movement or close-range olfactory stimuli, which may be different from those detected at long range, assist the fly in alighting on the host (Sutcliffe, 1987; Lehane, 1991; Willemse and Takken, 1994; Leak, 1998). Once on the host, the fly utilizes other stimuli sensed through touch, taste, and thermoreception to determine the suitability of the host and also to locate a feeding site (Dethier, 1954; Reinouts van Haga and Mitchell, 1975; Sutcliffe, 1987; Van der Goes van Naters and Rinkes, 1993; Van der Goes van Naters and Den Otter, 1998; Van der Goes van Naters *et al.*, 1998). After piercing the skin of the host, engorging is stimulated by blood constituents, ATP, ADP, and AMP (Galun and Margalit, 1969; Galun, 1987).

2.7.1 Odour-induced host location

The importance of odour in long-distance host location by tsetse flies was demonstrated using hidden animals, separating visual from olfactory stimuli. Llyod, (1935) was among the first researchers to demonstrate this by showing that *G. swynnertoni* could locate a calf hidden 23m away.

Experiments carried out in Zimbabwe where live animals such as cattle, goats, sheep, donkey, bushpig, buffalo and bushbuck were incarcerated in roofed subterranean pits from which air was blown through electric nets at ground level revealed that even

without visual stimuli, large numbers of tsetse approached the odour source from downwind. These experiments also revealed that about 90% of both *G. m. morsitans* and *G. pallidipes* that approached the stationary host were attracted by its odour (Vale, 1974b; Vale and Hall, 1985a). The number of flies attracted to the odour source was found to be positively correlated with body weight, where catches increased as a power of bait mass (Hargrove and Vale, 1978; Hargrove and Vale, 1980; Vale and Hargrove, 1975; Hargrove *et al.*, 1995). In the Central African Republic, odour emanating from a monitor lizard hidden near a trap increased trap-catches of *G. fuscipes fuscipes* by 1.7 times that of control traps (Gouteux *et al.*, 1995).

2.7.2 Sources of the attractive odour

The attractive odour to tsetse has been shown to emanate from excretory products of hosts such as urine, dung, breath or glandular secretions and exudates. In Uganda, Chorley (1948) found large numbers of *G. m. morsitans* in areas previously used by elephants for resting than elsewhere. Similarly, *G. pallidipes* or *G. palpalis* and *G. pallidipes* were found in great numbers in areas used for resting by buffaloes and hippopotami, respectively. After concluding that the animals' dung and urine were responsible for the larger numbers of flies, he demonstrated that cattle-dung and urine could increase catches of *G. pallidipes* near shelters made of grass and branches.

More recently using traps and targets, the urine of several mammals, both hosts and nonhosts of tsetse, have been shown to be attractive to various species of tsetse. Cattle urine increased biconical trap catches of *G. pallidipes* by 1.8 times while catches using buffalo urine were increased by 9.6 times compared to control traps (Owaga, 1985). Aged urine samples were found to be more effective than freshly collected

urine (Owaga, 1984, 1985). In Zimbabwe, jars containing 200 ml of ox or buffalo urine roughly doubled trap catches of *G. pallidipes* and *G. m. morsitans* (Vale *et al.*, 1986), whilst Zebu urine increased trap catches of *G. f. fuscipes* by 1.4 times that of control traps in Central African Republic (Gouteux *et al.*, 1995). Rhinoceros-dung and urine were shown to be as attractive to *G. pallidipes* and *G. longipennis* as the cattle urine in Kenya (Mihok *et al.*, 1996) while urines of warthog, domestic pig and bushbuck increased trap catches of *G. longipalpis* by 1.58, 1.91 and 2.5 times respectively in Cote d'Ivoire (Spath, 1997). The mean catch of either *G. pallidipes* or *G. longipennis* in traps baited with aged urine of buffalo and cattle (tsetse hosts) and waterbuck (a nonhost of tsetse) did not differ significantly (Madubunyi *et al.*, 1996). After demonstrating that patches of soil on which fresh urine of cattle was deposited and in which it was aged failed to effect significant increase in the trap catch of either *G. austeni*, *G. brevipalpis* or *G. pallidipes*, Madubunyi *et al.* (1996) concluded that under natural conditions tsetse flies are unlikely to utilise urine scent to discriminate hosts from nonhosts or locate favoured hosts.

Skin secretions obtained as washes or scrapings from various animals contain olfactory attractants for tsetse flies. Fresh pig skin washings were shown to significantly increase catches of *G. pallidipes* in Uganda (Persoons, 1966) while trap catches of *G. pallidipes* and *G. m. morsitans* were doubled by the presence of two bushpig sacks in Zimbabwe (Vale *et al.*, 1986). Skin washings from oxen when impregnated on screens and placed adjacent to traps increased catches of *G. m. morsitans* and *G. pallidipes* by 80% and 29% respectively in Zimbabwe (Warnes, 1990b). In Cote d'Ivoire, hexane skin washings of monitor lizard or warthog dispensed in small quantities improved the trap catches of *G. tachinoides* significantly by 1.34

and 1.46, respectively, while the skin washings of bushbuck reduced the catch (Spath, 1997).

These results suggest that secretions from skins of animals may have constituents, recognised by the flies, that vary from host to host.

2.8 Identification of kairomones

The identification of components of odour that stimulate tsetse flies (semiochemicals) has been made possible through the improved techniques of analysis including electroantennogram recordings of column chromatographic effluents of odour passed over the tsetse antenna (Hall *et al.*, 1984). Combined with wind tunnel experiments in the laboratory (Bursell, 1984), video recordings (Gibson and Brady, 1985) and electric nets in the field (Vale, 1974a; Hargrove, 1980b; Parker and Brady, 1990) to investigate the responses of tsetse without human interference, specific kairomones have been identified in host odours.

2.8.1 Kairomones from host breath

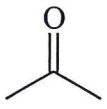
Components of ox breath that are attractants to tsetse are carbon dioxide (CO_2), acetone *(1)* and 1-octen-3-ol *(2)*. CO_2, which is released by an ox at approximately 2.5 l/min (Gaddum, 1961) was first shown to increase trap catches of *G. pallidipes* in Uganda by Rennison and Roberts (1959).

In Zimbabwe, CO_2 alone dispensed at 2.5 to 15 l/min or acetone alone dispensed at 0.3 to 300 g/hr, increased trap catches of *G. m. morsitans* and *G. pallidipes* by up to six times. Trap catches were increased further when the two compounds were dispensed together, being as effective as natural ox odour at high concentrations (Vale, 1980). CO_2 induces alighting responses of tsetse (Vale, 1983; Vale and Hall, 1985b)

and in the presence of acetone triggers upwind anemotaxis while acetone activates and elicits upwind anemotaxis only at very high concentrations of between 80 and 400 g/l (Bursell, 1984, 1987).

Hall *et al,* (1984) trapped odour from cattle on Porapak resin by drawing air over the animals enclosed in a tent. The trapped volatiles were recovered in dichloromethane, fractionated by liquid chromatography and components identified using gas chromatography linked to mass spectrometry. By performing electroantennographic recordings from *G. pallidipes* and *G. m. morsitans*, Hall *et al.* (1984) showed that 1-octen-3-ol (octenol) was the most potent olfactory stimulant in the cattle volatiles.

Octenol significantly increased upwind flight of *G. m. morsitans* in wind tunnel experiments (Bursell, 1984). In the field, trap catches of *G. m. morsitans* and *G. pallidipes* were increased by up to 3 times when octenol was dispensed 1m upwind of the traps, otherwise when dispensed just in front of the traps, catches were reduced. In addition, octenol increased the attractiveness of ox odour to *G. m. morsitans* by about 2.5 times and *G. pallidipes* by 1.5 times and also increased trap catches when mixed with CO_2 and acetone. However when dispensed in large doses, octenol reduced the attractiveness of ox odour (Hall *et al.*, 1984; Vale and Hall, 1985a,b).



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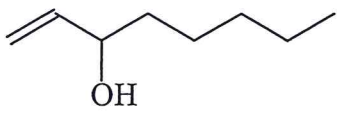

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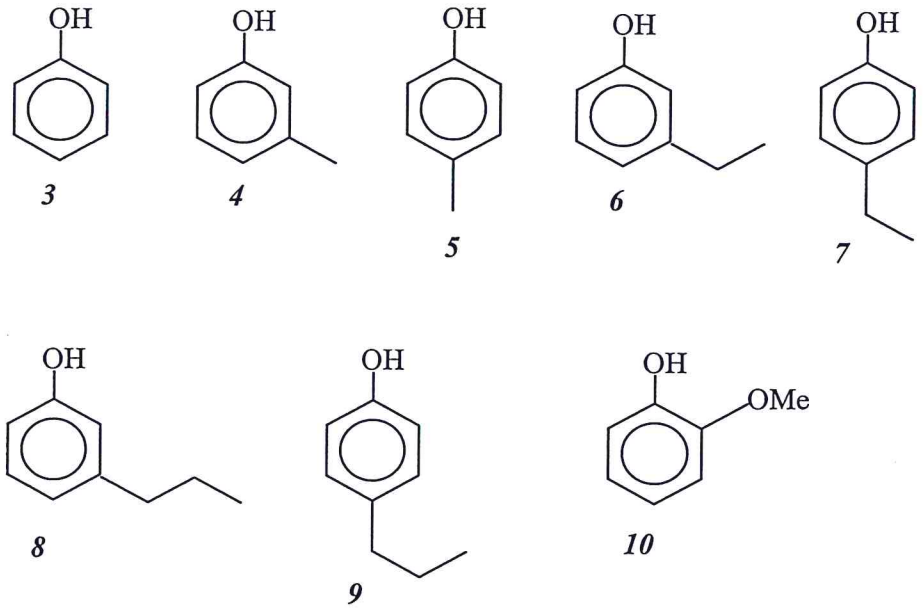
2.8.2 Kairomones from host urine

The attractiveness of buffalo or ox urine to tsetse flies was shown to be due to phenols in the urine. Hassanali *et al*, (1986) extracted constituents of the buffalo urine using dichloromethane. A chromatographic fraction of the urine extract containing seven simple phenols [phenol (***3***), 3-methylphenol (***4***), 4-methylphenol (***5***), 3-ethylphenol (***6***), 4-ethylphenol (***7***), 3-n-propylphenol (***8***), and 4-n-propylphenol (***9***)] increased field trap catches of *G. pallidipes* by up to seven folds when dispensed in biconical traps. Out of these phenols, 4-methylphenol (***5***), and 3-n-propylphenol (***8***), acting synergistically were shown to be the most important for the attractancy of the urine (Owaga *et al.*, 1988). These two compounds when dispensed in biconical traps increased catches of *G. pallidipes* by 5 - 7 fold relative to unbaited traps.

Using chemical methods of fractionating cattle urine, Bursell *et al.* (1988) found similar phenols in the phenolic fraction. However, in addition to 4-methylphenol (***5***) and 3-n-propylphenol (***8***), two other phenols, 3-methylphenol (***4***) and 3-ethylphenol (***6***) elicited upwind flight in wind tunnel experiments and increased trap catches of *G. m. morsitans* and *G. pallidipes* in the field. A minor component of the cattle urine, 2-methoxyphenol (***10***) was found to be repellent to both *G. m. morsitans* and *G. pallidipes* in the field (Bursell *et al.*, 1988; Vale *et al.*, 1988).

The host's urine or phenols derived from the urine do not appear to have any role in host discrimination since these phenols are gradually formed from pro-attractants broken down by microbial activity (Okech and Hassanali, 1990). Besides, these phenolic attractants were found in urine of waterbuck (nonhost), while 3-n-propylphenol (***8***) was missing in urines of highly preferred suids, warthog and bushpig (Madubunyi *et al.*, 1996).



2.8.3 Kairomones from ox sebum

Kairomones from body wash (sebum) of cattle were identified by chemically fractionating the sebum followed by instrumental analysis (Warnes, 1990; Saini *et al.*, 1993). Simple phenols similar to those identified in cattle urine were found in the phenolic fractions. However both groups of researchers demonstrated attractive properties to *G. pallidipes* and/or *G. m. morsitans* of other fractions of the sebum, namely, the non-acidic fraction (Warnes, 1990) and all the other fractions, acidic, neutral, basic and ethyl acetate-insoluble fractions (Saini *et al.*, 1993).
Although fractionation of the sebum could have been incomplete, it is possible that other unidentified kairomones may have been present in the other fractions.

2.9 Use of attractants in tsetse control

Combinations of the identified attractants have been used to improve trap catches (Politzar and Merot, 1984; Vale and Hall, 1985b; Vale *et al.*, 1988; Cheke and Garms, 1988; Jaenson *et al.*, 1991; Randolph *et al.*, 1991; Baylis and Nambiro, 1993; Mhidurwa, 1994; Torr *et al.*, 1995; Hargrove *et al.*, 1995), sampling (Dransfield *et al.*, 1986) and control (Vale *et al.*, 1986; Willemse, 1991; Knols *et al.*, 1993; Vale *et al.*, 1994) of various species of tsetse flies. Attempts are currently being made to use the attractants in the development of targets as barriers against reinvasion of cleared areas (Muzari and Hargrove, 1996; Muzari, 1999).

CHAPTER 3.

3.0 MATERIALS AND METHODS

3.1 Insects

G. m. morsitans flies were obtained from the ICIPE insectary where they were maintained under 12:12 normal photoperiod, 25 ± 2 °C, 70 ± 5% r.h. and fed off rabbits' ears. These flies were used for feeding bioassays and the electrophysiological recordings. For olfactory responses in the wind tunnel, *G. m. morsitans* pupae were obtained from the International Atomic Energy Agency (IAEA), Vienna, Austria or from the International Livestock Research Institute (ILRI), Nairobi, Kenya. Colonies from the two sources were maintained in the insectary and fed through membranes. *G. pallidipes* flies for electrophysiological recordings were either obtained from ICIPE insectary where they were maintained under conditions described above or caught from the wild (Nguruman, Kenya).

3.2 Animals

The animals used for experiments were four female waterbucks *K. e. defassa* Ruppel, two (male and female) buffaloes *S. c. caffer* Sparrman and two oxen. Waterbucks, and buffaloes were born and reared in captivity at the Wildlife Disease Research Center, Kenya Agricultural Research Institute (KARI), in an area free of tsetse flies. They were maintained on hay and concentrates and grazed freely within the pens. The oxen were kept within the ICIPE animal confinement pens and maintained on hay and concentrates. The animals were maintained free of acaricides for more than one year before experiments were conducted. At the time of experiments, the animals were herded into an outdoor metallic crush where they were restrained.

3.3 Trapping odour from animal body

3.3.1 Adsorbents

The adsorbents used for trapping odour from the animals were activated charcoal (80/100 mesh, Chrompack), glass wool and octadecyl bonded silica having a particle size of 40 μm (J.T. Baker, Phillipsburg, N.J. USA).

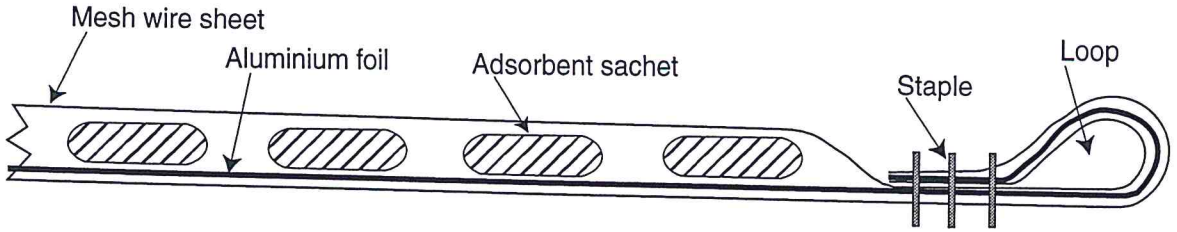
The adsorbents were first soxhlet cleaned using dichloromethane (99%, Aldrich) for 24 hours and dried in an oven at 40 ^{0}C overnight.

3.3.2 Sachets for holding adsorbents

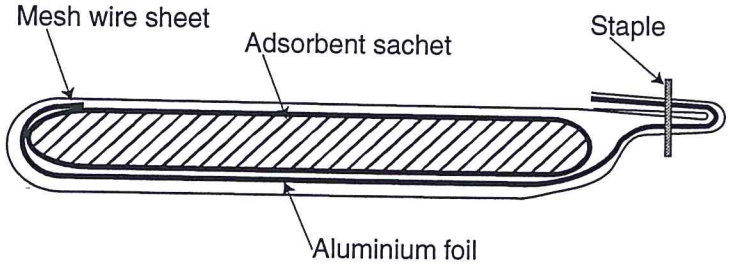
Sachets of dimensions 4 cm x 4 cm for holding the adsorbents were made from filter paper (Whatman No. 1) and from cream mesh sheets (Estal mono, 120T mesh; Switz Silk Company, Switzerland). Approximately 450 mg of the octadecyl bonded silica was placed in each filter paper sachet while about 200 mg of the glass wool or 350 mg of the activated charcoal was placed in each cream mesh sachet.

3.3.3 Adsorbent belt

Belts of dimensions 8 cm x 41 cm or 8 cm x 126 cm, were made from strips of the cream mesh sheet folded lengthwise in the middle. A strip of aluminium foil was placed inside on one side to act as a lining. Sachets containing adsorbents were arranged inside the belt above the aluminium foil lining. The belt was then hemmed and stapled tightly so that the sachets were exposed, through the mesh, to the air on one side and sealed by the aluminium foil on the other side, Fig.4.



a. Longitudinal section of adsorbent belt



b. Cross-section of the adsorbent belt

Fig. 4. The adsorbent belt

The ends of the belt (about 5 cm) were folded and stapled to provide a loop through which a cord would pass for tying the belt round an animal (Plate-1).

The belts were soxhlet cleaned using dichloromethane for 24 hours and then dried overnight at 40 ^{0}C in an oven. Each belt was wrapped lengthwise in an aluminium foil and used immediately on the animal.

3.3.4 Adsorption of body odours on to adsorbents

Soxhlet cleaned (dichloromethane) sisal twine was passed through the loops at the ends of the belt. When the animal (waterbuck, buffalo or ox) was restrained in the metallic crush, the aluminium foil wrapper was removed and the belt tied with the sachet exposed side touching the body, round the neck, the belly next to the front legs and next to the hind legs of the animal (Plate-1). The animal was then released, with belts on, into the paddocks to feed freely for the rest of the day. Buffaloes, however, could remove belts tied round their bellies, therefore trapping from this animal was carried out from the neck region. Trapping of odour was for about seven hours starting from 10 am. All trappings were carried out on sunny days when the average temperature was 26 ± 2 ^{0}C and relative humidity of 40 ± 5 %.

For the control, the aluminium foil wrapper was removed from the belt, thus exposing the adsorbent sachets to the air around the crush for the same length of time taken to tie a single belt round an animal.

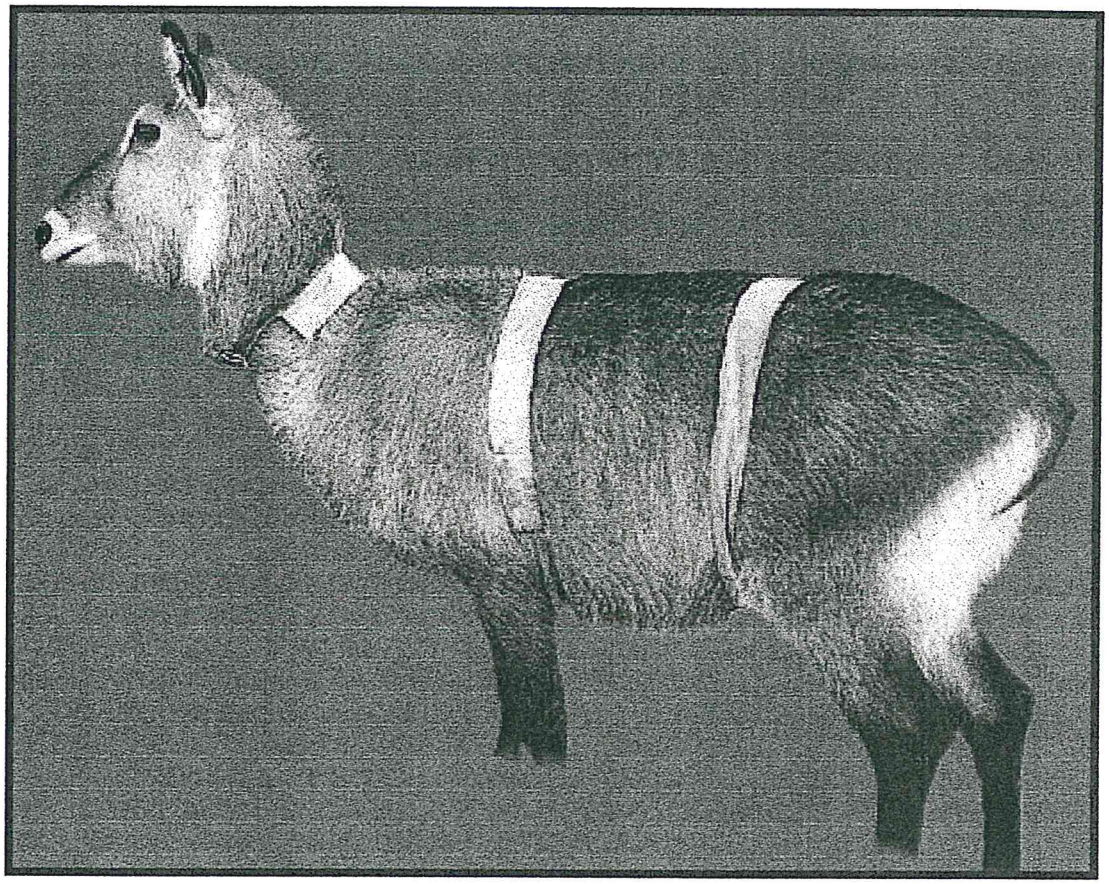


Plate I: The waterbuck, *K. e. defassa* Ruppel, wearing adsorbent belts for collecting body odours

At the end of trapping, the animals were herded into the crush and belts removed. These belts were immediately wrapped in clean aluminium foil and placed in a cool box. They were then transferred into a freezer (-20 ^{0}C) at ICIPE, and desorption of the volatiles carried out.

3.3.5 Extraction of volatiles from adsorbents

The adsorbent material in sachets was carefully transferred into elution tubes (either Pasteur pipettes or funnel shaped tubes made from the female part of a ground joint tube). Redistilled dichloromethane (over 99.9%; GC) was passed through the adsorbent and collected in a clean 4ml vial. 1ml of dichloromethane was used to elute adsorbent from 2 sachets. The sample was concentrated under ice to about 20 µl using a gentle stream of high purity nitrogen and stored in a freezer at -20 ^{0}C until used for instrumental analyses.

3.4 Extraction of skin secretions (sebum)

Dichloromethane washed (soxhlet, 48 hours) pieces of cotton wool held in clean forceps and moistened in distilled dichloromethane were used to swab parts of the animal's body except the anal region, lower belly and lower legs to avoid contamination from faecal and urine residues. The swabs were then shaken in 5% methanol in dichloromethane overnight. The extract was filtered and concentrated under reduced pressure, weighed and then stored at –20 ^{0}C until used.

The quantity of sebum produced by the waterbuck per unit area of the skin was determined by taking successive swabs from a marked out area on the lateral side of a waterbuck.

3.5 Fractionation of waterbuck sebum

The waterbuck sebum (1g) dissolved in 50 ml of dichloromethane was fractionated into acidic, phenolic, basic and neutral fractions.

The sample was shaken successively with four aliquots (30 ml each) of 10% Na_2CO_3 solution and the aqueous layers combined leaving an organic fraction (A). The aqueous fraction was neutralised with 4M HCl and then extracted with four 30 ml portions of dichloromethane to give the acidic fraction.

Organic fraction A, was extracted with 25 ml (four times) of 10% NaOH solution. The aqueous layers were combined leaving an organic fraction (B) and then neutralised with 4M HCl. The phenolic fraction was obtained by extracting the aqueous solution with three lots (50 ml each) of dichloromethane.

The basic fraction was separated from the neutral fraction by extracting fraction B successively with 4 aliquots (25 ml each) of 2M HCl. The combined aqueous fraction was neutralised with 10% NaOH and extracted with three 50 ml aliquots of dichloromethane.

All the final organic fractions were dried over anhydrous magnesium sulphate ($MgSO_4$) overnight followed by filtration. The filtrates were then concentrated in vacuo at room temperature to about 20 ml, transferred into vials and solvent removed using a gentle stream of filtered nitrogen.

3.6 Instrumental analysis

Analysis of samples from the animals was carried out using gas chromatography, gas chromatography-linked mass spectrometry and gas chromatography-linked electroantennography.

3.6.1 Gas chromatography (GC)

GC analyses were conducted on a Hewlett Packard (HP) model 5890 Series II gas chromatograph equipped with a splitless capillary injector system, a flame ionization detector (FID) and an HP 3396 Series II integrator.

For the analysis of body volatiles, a fused silica capillary column, 50 m x 0.2 mm internal diameter (i.d.) coated with cross linked methyl silicone gum (0.33 μm film thickness) HP was used with nitrogen as carrier gas at a flow rate of 0.7 ml/min. The oven temperature was isothermal at 40 ^{0}C for 15 minutes following injection of a sample and then programmed at 8 ^{0}C/minute to 280 ^{0}C where it was held for 15 minutes.

The sebum from animals was analysed in a fused silica capillary column (HP); 15 m x 0.32 mm i.d. coated with cross linked methyl silicone gum having a film thickness of 0.17 μm. The flow rate of the carrier gas (nitrogen) was 1.4 ml/minute. The initial oven temperature was held at 150 ^{0}C for 2 minutes and then programmed at 10 ^{0}C/minute to 220 ^{0}C where it remained for 5 minutes and then at 5 ^{0}C/minute to 280 ^{0}C where it was held for 30 minutes.

In all cases, 1-2 μl of the sample was injected in the splitless mode with a 45 seconds delay before injection purging. The injector and detector temperatures were held at 280 ^{0}C.

3.6.2 Gas chromatography-linked mass spectrometry (GC-MS)

GC-MS analyses were carried out on a VG Masslab 12-250 mass spectrometer coupled to an HP 5790 Series A gas chromatograph. The mass spectrometer was operated in the electron ionization (EI) mode having an electron energy of 70 eV and

an emission current of 200 mA. The source temperature was held at 180 ^{0}C and the multiplier voltage at 1400 V. The spectrometer had a scan cycle of 1.5 seconds and a mass range of m/z 1 - 1400. The scan range for the samples was however from m/z 38 - 700. The instrument was calibrated using heptacosafluorotributylamine $[CF_3(CF_2)_3]_3N$, (Apollo Scientific Ltd. UK).

The GC columns and oven temperature programming were the same as those described for the GC analysis.

3.6.3 Gas chromatography-linked electroantennographic detection (GC-EAD)

3.6.3.1 Antennal preparation for GC-EAD recording

The antennae for EAD recordings were prepared from 3 day old female non-teneral *G. m. morsitans* and *G. pallidipes*. A tsetse fly was held with a pair of fine jeweller's forceps (no. 5) under the objective of a dissecting microscope and using a similar pair of forceps, one of the antennae was held by the second segment and gently pulled off the head capsule. The antenna was then cut with a scapel blade at the base of the third broad segment into which a glass micropipette containing tsetse saline (Gee, 1976) was inserted. The micropipette with the antennal preparation was sheathed over a silver wire (0.5 mm in diameter) recording electrode on one arm of a micromanipulator (Syntech). The indifferent electrode was a similar glass micropipette which was sheathed over a similar silver wire held on the other arm of the micromanipulator and grounded through a wire. To complete the circuitry for recording electroantennographic (EAG) activity, the tip of the micropipette on the indifferent electrode was gently but firmly positioned on the intact end of the antennal

preparation. The recording electrode was connected via a probe to a Universal AC/DC UN 05 amplifier (Syntech) and to the computer (PC) with the EAD card. The micromanipulator was positioned such that the antennal preparation was directly in the path of the effluent from the opening of the stimulus delivery tube connected to the GC.

3.6.3.2 GC-EAD recording

GC-EAD was performed on an HP model 5890 Series II gas chromatograph equipped with an FID and an HP 3396 Series II integrator.

The same capillary column and GC conditions were employed as in the GC analysis described above. However, the column effluent was split into two at the end of the column and passed through two 50 cm long deactivated fused silica columns, one connected to the FID and the other to the antennal preparation. A make-up gas having a flow rate of 40 ml/minute was added just before the split point to accelerate the effluent through the deactivated columns (Fig. 5).

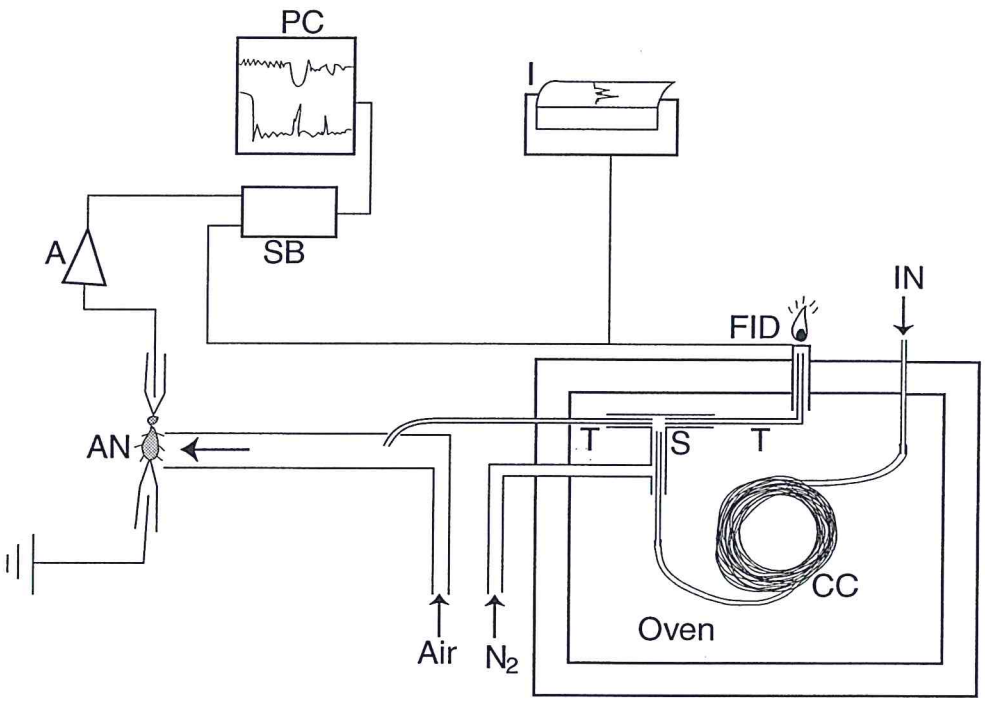


Fig.5. Coupled GC-EAD system

A—Amplifier
AN—Antenna
FID—Flame ionisation detector
CC—Capillary column
I—Integrator for FID signal
IN—Injector of the GC
PC—Computer with EAD card
SB—Signal connection box
S—Glass-lined T-Splitter
T—Deactivated fused silica transfer lines

The effluents were driven from the chromatograph through a transfer line maintained at 150 ^{0}C by a THC-3 temperature control unit (Syntech) and into a moistened airstream maintained at 25 ^{0}C, 90% r.h. and flowing at 4.0 ml/second. This effluent was delivered over the anntennal preparation via a stainless steel tube of 5 mm i.d. FID and EAD signals were monitored synchronously with a programme on a GC-EAD interface (Syntech) card installed in the PC.

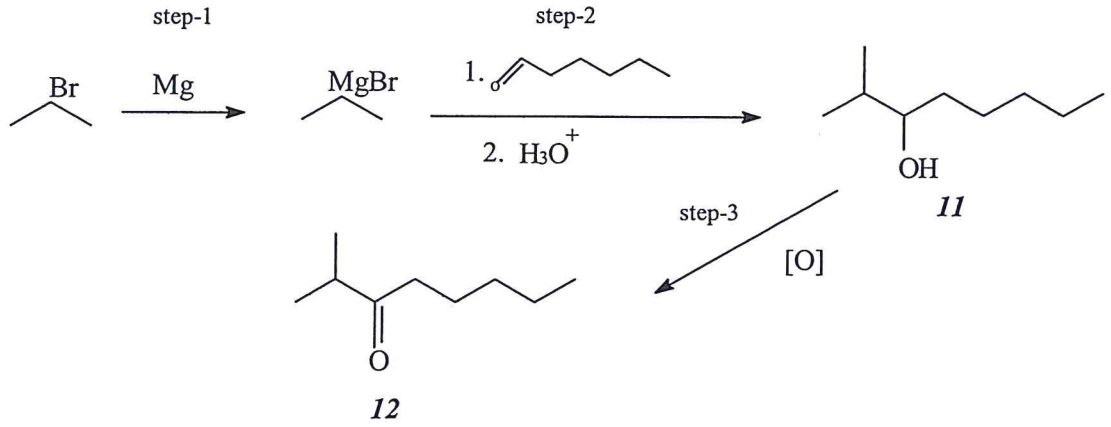
3.6.4 Nuclear Magnetic Resonance (NMR) Spectrometry

The ^{1}H and ^{13}C NMR analyses were carried out on a Varian Mercury-200 NMR Spectrometer. The sample was dissolved in deuterated chloroform and ^{1}H NMR run at 200MHz while the ^{13}C NMR run at 50 MHz.

3.7 Synthesis

Candidate compounds identified by GC-MS in the waterbuck body volatiles and suspected to be EAG-active on *G. m. morsitans,* but were not available commercially were synthesized. After GC analysis, if the synthesized compound co-eluted with the target peak in the volatiles of the waterbuck, the compound was analysed by GC-EAD. These compounds included, 2-methyl-3-octanone (***12***) [Scheme-1], δ-octalactone (*17*) [Scheme-2], 2-isopropyl-4-methylphenol (***26***), 3-isopropyl-4-methylphenol (*27*) [Scheme-3] and 2,6-dimethyloctane (*31*)[Scheme-4].

3.7.1 2-Methyl-3-octanone



Scheme-1. Synthetic route for 2-methyl-3-octanone (*12*).

Step-1

The reaction was carried out in a 500 ml 3-necked flask fitted with a dropping funnel, condenser and a thermometer. A calcium chloride guard tube was fitted on top of the funnel and dry nitrogen passed through the top of the condenser to keep the flask dry before and during the reaction.

3.75 g (0.154 moles) of ether-washed dry magnesium turnings and 100 ml of dry ether were placed in the flask and a small crystal of iodine added. 18.5 g (14.2 ml, 0.150 moles) of dry 2-bromopropane (99%, Aldrich) in 100 ml of dry ether was placed in the dropping funnel and 25 ml of this solution added into the flask. When the reaction started (indicated by the disappearance of the iodine colour), the stirrer was put on and the rest of 2-bromopropane added dropwise. Refluxing of the reaction mixture continued for another one hour.

Step-2

The flask was then cooled in ice, and a solution of 18.37 ml (0.153 moles) of hexanal (99%, Aldrich) in 25 ml of dry ether added dropwise from the dropping funnel.

When all the aldehyde had been added, the reaction product was poured into 200 g of crushed ice. The basic MgBr was dissolved by adding 50 ml of 30% HCl.

The alcohol was extracted from the aqueous solution using four 50 ml portions of ether and then dried over anhydrous Na_2CO_3. The solvent was removed in vacuo and sample transferred into a 25 ml vial where it was concentrated by blowing nitrogen over it, yielding 15.4729 g. Analysis of the product by GC and GC-MS showed that the reaction yielded over 90% 2-methyloctan-3-ol (***11***), with traces of hexanal (8%).

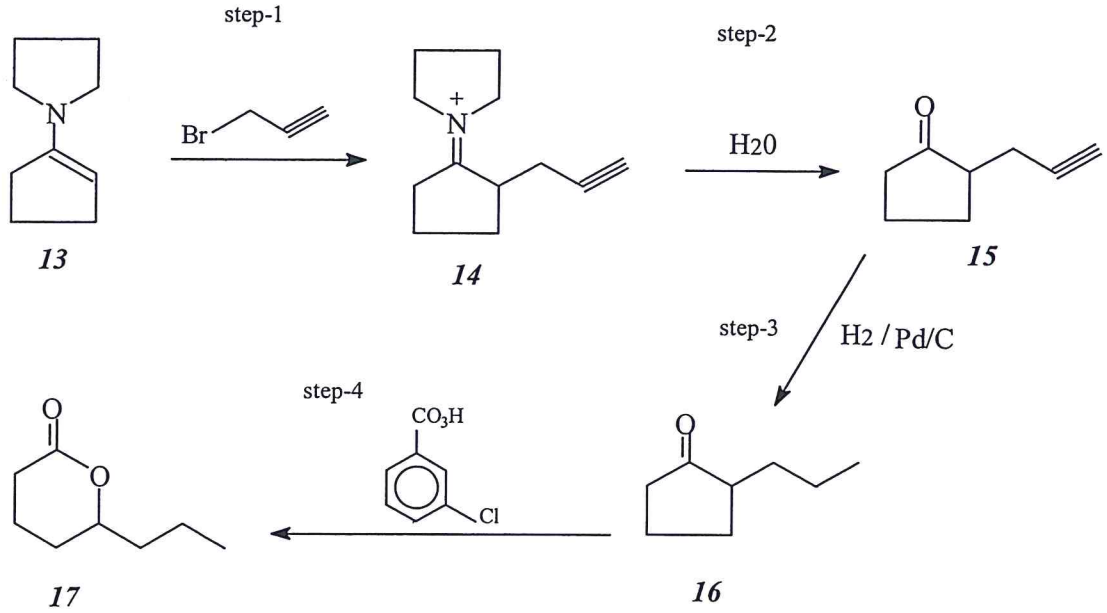
Step-3

The oxidation of compound ***11*** to 2-methyl-3-octanone (***12***), was by chromic acid. Chromic acid was prepared by dissolving 20 g (0.067 moles) of sodium dichromate dihydrate in 50 ml of distilled water and slowly adding 14.6 ml (0.268 moles) of concentrated sulphuric acid. After cooling, the solution was diluted to 100 ml with distilled water. The oxidation was carried out in a 3-necked 100 ml flask fitted with a dropping funnel, condenser and a thermometer.

6.09 g (about 0.042 moles) of compound ***11*** was placed in 50 ml of ether in the flask and 21.08 ml (0.014 moles) of the chromic acid added dropwise with stirring for a period of 15 minutes. The reaction mixture was stirred at room temperature for a further $2^1/_3$ hours. The ether layer was separated and the dark green aqueous layer extracted using four, 25 ml portions of ether. The combined ether extracts were washed with 20 ml of saturated sodium hydrogen carbonate solution and then with 20 ml of brine. The ether extract was dried over anhydrous sodium sulphate for 24 hours, filtered and ether removed in vacuo. The sample was transferred into an 8 ml vial and concentrated by blowing nitrogen over it, yielding 5.3374 g.

GC and GC-MS analysis showed the sample to be about 90% compound *12*, 2% hexanal and 8% compound *11*. However, 2-methyl-3-octanone (*12*) failed to co-elute with the target peak in the waterbuck body volatiles and therefore, further work on the compound was discontinued.

3.7.2 δ-Octalactone



Scheme-2. Synthetic route for δ-octalactone (*17*).

Step-1

The reaction was carried out in a 100 ml 3-necked flask maintained under nitrogen and fitted with a condenser, thermometer and a dropping funnel.

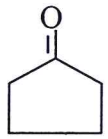
20 ml of 1-pyrrolidino-1-cyclopentene (*13*), [(98%, Aldrich); (71%, GC); unstable in air] in 15 ml of dry acetonitrile (99.9%, Aldrich) was placed in the flask. 12 ml of propargylbromide (80% wt in toluene, Aldrich) in 35 ml of the acetonitrile was placed in the dropping funnel. This solution was added into the reaction flask dropwise with stirring for about 15 minutes while cooling the flask in ice. After 40

minutes, ice was removed and stirring continued at room temperature for the next 2 hours.

Step-2

Ice was added in small amounts to the reaction mixture while stirring. The reaction mixture was transferred into a separatory funnel and the ethereal layer (dark brown in colour) separated out. The ethereal fraction was washed once with 50 ml of distilled water and then dried over anhydrous $MgSO_4$. Although the expected weight of the product was 12.2 g, the weight of the organic fraction was 16.64 g probably due to the presence of impurities including bromine salt of pyrrolidine.

GC and GC-MS analysis of this sample showed that in addition to the expected product (36%), double alkylation giving compounds ***19*** and ***20*** (6 and 15%) occurred. Impurities included, cyclopentanone (***18***) (6%), toluene(32.9%) (in which propargyl bromide is preserved) and traces of compound ***13***.



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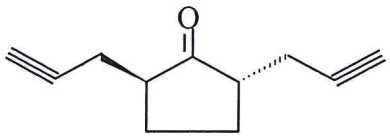

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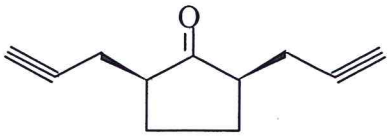


20

13.1 g of the sample was partially purified by passing it through a column packed with silica gel (230-400 mesh) and eluting with hexane, dichloromethane and then dichloromethane/methanol (9:1). Fractions that contained compounds ***15, 18, 19*** and ***20*** eluted in n-hexane and dichloromethane (GC analysis). These were combined and solvent removed in vacuo to give about 6.9 g. The sample was loaded onto a column packed with activated (grade III) silica gel (230-400 mesh) and eluted using hexane with polarity increasing gradually using dichloromethane.

GC analysis of fractions showed that compounds ***15, 19*** and ***20*** were present in the same fractions. Fractions containing from 70% and above of compound ***15*** were combined and solvent removed in vacuo to give 3.8 g of sample, resulting to about 78% (GC) of this compound.

Attempts to use preparative TLC (DC-Fertigplatten SIL G-25 UV_{254}; 0.25 mm silica gel) to separate the two groups of compounds by developing with various combinations of pentane, hexane, heptane and dichloromethane were not successful.

Step-3

The sample was dissolved in 80 ml of ethyl acetate (99.5%, Aldrich) and placed in a 100 ml round bottomed flask. A little palladium on activated charcoal was added and hydrogen bubbled through the mixture with stirring overnight. After filtering and removing solvent in vacuo, 3.1 g of a clear product was obtained consisting of 70% (GC) of 2-propylcyclopentanone (***16***).

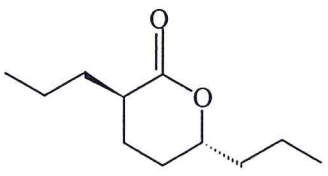
Step-4

This reaction was carried out in a 3- necked 100 ml flask, fitted with a dropping funnel, thermometer and condenser. About 7.5 g of m-chloroperbenzoic acid (80-90%, Aldrich) was dissolved in 35 ml of anhydrous chloroform (99%, Aldrich) while raising the temperature to allow solubility. The 3.1 g of sample containing 2-propylcyclopentanone was dissolved in 20 ml of anhydrous chloroform and introduced dropwise through the dropping funnel into the reaction flask while stirring. The reaction mixture was refluxed at about 57 ^{0}C for 48 hours.

The reaction mixture was cooled in ice water and the white precipitate formed (m-chlorobenzoic acid) filtered off. Solvent was removed from filtrate in vacuo and residue taken up in 50 ml of dry ether. This was washed with four, 20 ml portions of

10% Na_2CO_3 solution. The organic fraction was then washed once with 50 ml of brine and then dried over anhydrous $MgSO_4$. The $MgSO_4$ was filtered off and solvent removed in vacuo to give 2.5 g of sample comprising 81% (GC, GC-MS) of δ-octalactone (*17*).

To purify δ-octalactone, 2.4 g of the sample was dissolved in 5 ml dichloromethane and 50 ml of 10% NaOH added. The mixture was stirred overnight. The alkali solution was extracted with four, 20 ml portions of dichloromethane. The organic layer was placed aside and the aqueous fraction acidified with 4M HCl until acidic (Litmus paper). The acidified solution was extracted with four aliquots (20 ml each) of dichloromethane. The combined organic fractions were dried over anhydrous $MgSO_4$, filtered and solvent removed to give 1.7 g of product. GC and GC-MS analysis showed that δ-octalactone formed close to 88% and compounds *21* and *22* comprised about 6% each. The δ-octalactone was purified to 99.5 % (GC) by passing the sample through a column packed with activated silica gel (grade III). Compound *17* eluted at the same retention time as the target peak in the waterbuck body volatiles and was EAG-active on antenna of female *G. m. morsitans*. The structure of compound *17* was further confirmed using ^{1}H and ^{13}C NMR Spectrometric analysis. No attempt was however made to resolve the two possible optical isomers of δ-octalactone since the configuration of the one emitted by the waterbuck is unknown.



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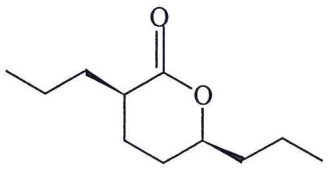

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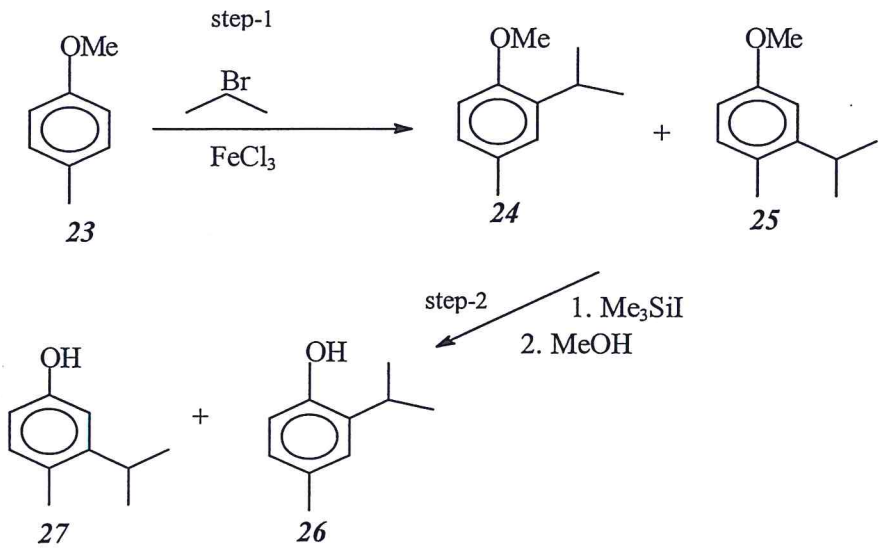
3.7.3 2-Isopropyl-4-methylphenol and 3-isopropyl-4-methylphenol



Scheme-3. Synthetic route for 2-isopropyl-4-methylphenol (*26*) and 3-isopropyl-4-methylphenol (*27*).

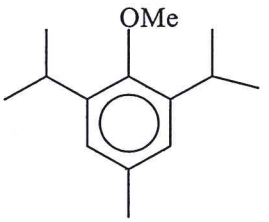
Step-1

This reaction was carried out in a 3-necked 500 ml flask fitted with a thermometer, condenser and a dropping funnel onto which a $CaCl_2$ guard tube was fixed. The top of the condenser was fixed to a water drainage pipe from the same condenser for the purpose of dissolving uncondensed HCl fumes.

25 g (0.15 moles) of $FeCl_3$ was placed in the flask and 40 ml of nitrobenzene added with stirring until all the $FeCl_3$ dissolved. 41.3 ml (0.33 moles) of 4-methoxytoluene (*23*) (99%, Aldrich), was added into the flask. 23.8 ml (0.25 moles) of 2-bromopropane was placed in the dropping funnel and introduced into the reaction flask over 10 minutes. Temperature was raised to about 40 ^{0}C and stirring continued for 5 hours. 150 ml of 2.4M HCl was added with vigorous stirring followed by addition of 100 ml of distilled water. The reaction mixture was distilled off and the distillate transferred into a separatory funnel. The organic layer was separated out and the aqueous layer washed with three 50 ml portions of ether. The organic fractions

were combined and dried over $MgSO_4$. After removing solvent, the residue was placed in a 250 ml flask and fractionally distilled. Four fractions were obtained, distilling at 176-177 ^{0}C (distillate-1; 20.7 g), 188-192 ^{0}C (distillate-2; 15 g), 196-198 ^{0}C (distillate-3; 32.9 g) and 204-208 ^{0}C (distillate-4; 10.1 g).

From the GC and GC-MS analyses, distillate-1 consisted mainly of compound ***23*** (87%) while distillate-2 had compound ***23*** and nitrobenzene as the major components. Distillate-3 and -4 consisted of the four possible products, ***24, 25, 28*** and ***29*** together with nitrobenzene.



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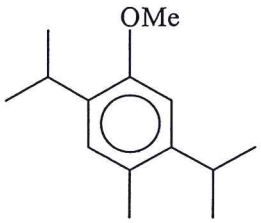

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Distillate-3 consisted of 44% compound ***24***, 8% compound ***25***, and 6% each of compounds ***28*** and ***29***.

Step-2

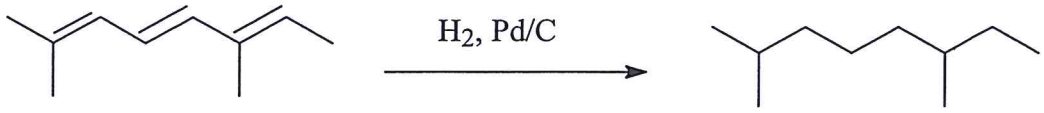
This reaction was carried out in a 3-necked 250 ml round bottomed flask fitted with a thermometer, a condenser and a rubber stopper. The system was maintained under nitrogen introduced through the top of the condenser. The sample (distillate-3; 32.9 g) was placed in the flask and 95 ml of dry chloroform added. About 22 ml of iodotrimethylsilane (97%, Aldrich) was added using a dry syringe through the rubber stopper. The temperature was maintained at 45-50 ^{0}C and reaction carried out with stirring for 140 hours. 30 ml of methanol was added into the reaction mixture and stirring continued at 38 ^{0}C for seven hours. The reaction mixture was filtered and

residue washed severally with dichloromethane. Solvent was removed in vacuo from the combined filtrates giving 40.9 g of product. This was taken up in 50 ml ether and washed with (i) six, 75 ml aliquots of 0.5M $Na_2S_2O_4$, (ii) four, 50 ml portions of 10% $NaHCO_3$ and (iii) one lot of 50 ml brine. The sample was dried and solvent removed to give 24.9 g of product. The sample was loaded onto a column packed with activated silica gel (grade III) and elution carried out from n-hexane and polarity increased using dichloromethane. Fractions were combined based on TLC analysis. Solvent was removed in vacuo and residue dissolved in 100 ml ether.

The phenols were extracted into an aqueous layer by partitioning in 10% NaOH (7 portions of 100 ml each). The aqueous fraction was acidified using 4M HCl until acidic (Litmus paper), and divided into two fractions which were extracted with three portions of 100 ml each of dichloromethane. The organic fractions were combined and washed twice with 5% $NaHCO_3$ (100 ml each) and once with 100 ml brine. The sample was dried over anhydrous $MgSO_4$. After filtering, solvent was removed in vacuo to give about 4 g of an oily product.

GC and GC-MS analysis showed that 60.0% of the product was compound ***26***, 38.8% was compound ***27*** while the double alkylated phenols formed 0.4 and 0.5%. Compounds ***26*** and ***27*** eluted at different retention times from the target compound in the waterbuck body volatiles and these compounds were not EAG-active on *G. m. morsitans*. No further work on these compounds was carried out.

3.7.4 2,6-Dimethyloctane



30 *31*

Scheme-4. Synthetic route for 2,6-dimethyloctane (***31***)

2,6-Dimethyloctane (***31***) was prepared by a one step reduction of 2,6-dimethyl-2,4,6-octatriene (***30***). 10 ml of compound ***30*** was dissolved in 30 ml of ethyl acetate and placed in a 100 ml round bottomed flask. A few grams of palladium on activated carbon was added and hydrogen bubbled gently while stirring overnight. A quantitative product was obtained with over 95% (GC and GC-MS) compound ***31***. This compound failed to co-elute with the target peak in the volatiles of waterbuck. Although the compound elicited an EAG response from *G. m. morsitans*, no further work was carried out on it.

3.8 Behavioural experiments

3.8.1 Feeding responses

Teneral 2- or 3-day old *G. m. morsitans* females were used for the experiments. On the day of experiments, the flies were individually placed in cages (18 x 7 x 5 cm^3) made of PVC frames covered with nylon mesh (Kaaya and Alemu, 1984).

3.8.1.1 Feeding responses on live animals (waterbuck and ox)

A cage containing one fly was firmly held on a randomly selected part of the body of the animal, generally when the fly was sitting quiescent at the top of the cage away from the animal's body. The fly was then observed for a maximum of 10 minutes, the observer standing in a downwind position. The following observations and measurements were made:

a) if the fly landed on the animal and time taken to do so;

b) if landing was accompanied by probing and feeding;

c) if no feeding took place after initial probing, number of probing sites changed by hoping or walking before the fly settled down to feed;

d) if the fly showed an escape behaviour after landing or initial probing by hoping or flying away from the animal without feeding;

e) if the fly alighted again on the animal, and its subsequent behaviour;

f) time spent in probing before initiation of feeding;

g) duration between initial exposure to the animal and onset of feeding.

Probing was scored when the protruded haustellum was brought into contact with the animal skin. An observation cycle was terminated once a fly started to feed as

indicated by the changing colour of the abdominal wall and swelling abdomen. For each treatment 35 flies were observed.

3.8.1.2 Feeding responses on membranes

The silicone feeding membranes (Bauer and Wetzel, 1976) and the aluminium base were cleaned thoroughly in a mixture of organic solvents (hexane/dichloromethane/methanol), water, rinsed using ethanol and then dried in an oven at 100 ^{0}C for 2 hours. The top part of a membrane was marked out using a waterproof leukoplast adhesive (a l'oxyde de zinc), BDH Beiersdorf AG Hamburg, such that two equal zones of dimensions 6 cm x 6 cm were separated by a strip of 2 cm width (except in double control experiments involving untreated feeding membrane). Waterbuck sebum of known weight was dissolved in 1ml of ethanol and applied evenly on one zone of the membrane using a micropipette, to give one of the following concentrations of the sebum: 0.1, 0.7, 1.0 and 1.4 mg cm^{-2} (the concentration of sebum on the waterbuck was estimated to be close to 1.0 mg cm^{-2}). For the control (blank) zone, the membrane was similarly treated with an equal volume of the solvent. The solvent was allowed to evaporate, and the membrane then placed on the aluminium plate containing heparinised ox blood such that the experimental arena was directly above the blood. The preparation was placed on a heating mart and the temperature on the membrane maintained at 34.5 ± 0.5 ^{0}C. The temperature 5 cm above the mart was 27.5 ± 0.5 ^{0}C. A cage containing a single fly resting on its upper part was cautiously placed on the membrane such that the lower meshed side sat squarely on top of the marked out experimental arena. The observations made were similar to those on the animals except that a direct comparison of the behaviours of the flies landing on treated

and untreated zones could be made. About ten flies were individually observed in any one treatment, after which a fresh membrane was used. Five to seven replicates were carried out in each treatment.

3.8.2 Olfactory responses in the wind tunnel

3.8.2.1 Wind tunnel

Tests were conducted in a cylindrical plexiglass tunnel (180 cm long, 24 cm internal diameter). A duct (20 cm diameter) in the middle of the tunnel was connected via a PVC pipe in which an air extracting fan was mounted. When the fan was switched on, air flowed into the tunnel from either arms thereby making the middle of the tunnel downwind. The duct in the middle thus divided the tunnel into two equal arms with a 20 cm wide middle zone where air from either arms mixed.

The upwind ends of the tunnel were closed with white PVC gauze, while the downwind end was closed with a metallic wire mesh cover. The two upwind ends of the tunnel were connected to activated charcoal (4 - 14 mesh, Sigma) air filters made of PVC (Fig.6). The wind tunnel had three windows, one on either arm (15 cm x 10 cm) for introducing sample dispensers and one in the middle (4.7 cm in diameter) for introducing cylindrical (3cm in diameter, 4cm long) PVC cages containing flies.

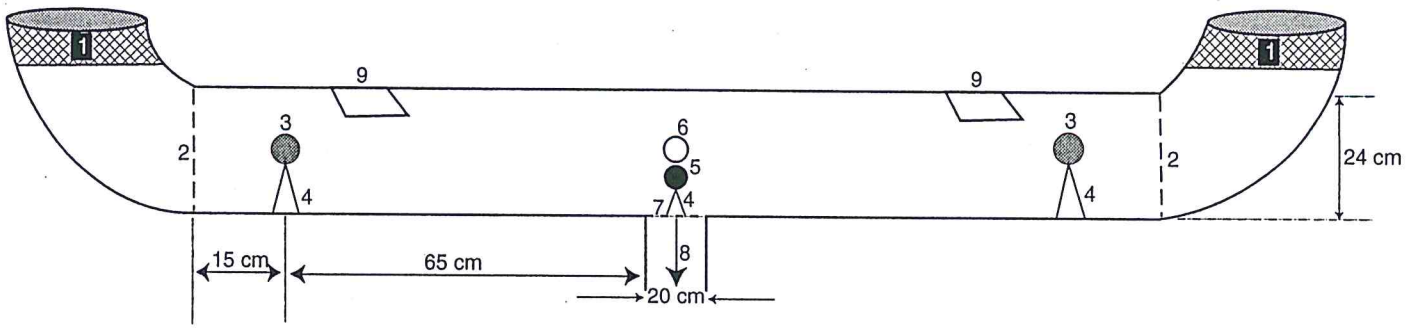


Fig. 6. The wind tunnel

1—activated charcoal
2—PVC gauze
3—odour dispenser
4—metallic rack
5—insect release cage
6—window for introducing insect release cage
7—metallic wire mesh
8—air flow
9—window for introducing odour dispenser

Light from fluorescent tubes and bulbs (connected to a light intensity regulator) was diffused through fiber glass filter sheeting placed 35 cm above the tunnel and offering about 1000 lux light intensity. A white sheet of paper with black stripes marked about 2 cm apart was placed beneath the tunnel floor to offer contrast to the fly during anemotaxis. The wind speed in the tunnel was adjusted to 10 cm/sec while the bioassay room was maintained at 26 ± 1 ^{0}C and 65 ± 5 % r.h.

3.8.2.2 Olfactory stimuli

The test odour consisted of the identified compounds in body volatiles of waterbuck (unpreferred host of tsetse), buffalo and ox (preferred hosts) that had elicited EAG responses in antennae of *G. m. morsitans.* A total of fifteen compounds were tested, 14 of which were obtained commercially while one was synthesized as described in **3.7.2** above.

The odour was prepared and tested in blends based on structural comparisons of compounds from the three animals as follows, (with abbreviations in parentheses):

i) whole waterbuck volatiles, comprising of all the EAG-active compounds from waterbuck (WBV)

ii) compounds present in waterbuck but absent in ox and buffalo volatiles (REP)

iii) compounds common in waterbuck, ox and buffalo volatiles (WOB)

iv) compounds present in buffalo and ox volatiles minus known attractants (BUX).

The compounds were mixed in ratios derived from GC profiles of the body volatiles of the animals. In each experiment, three to four doses were tested; 0.01 mg and / or 0.1 mg, 1.0 mg and 10.0 mg (in 200 µl of paraffin oil) against a control comprising of 200 µl of paraffin oil.

In the push-pull experiment where flies were exposed to two different blends of odour simultaneously in the tunnel, WOB was tested against REP and WBV at doses of 1 or 2 mg in 200 µl of paraffin oil.

For the blank test, dispensers (see section 3.8.2.3 below) in either arms of the tunnel were treated with equal volumes (200 µl) of paraffin oil.

In testing the experimental set up, a blend of the known tsetse attractants (acetone, 1-octen-3-ol, p-cresol and 3-n-propylphenol) was dispensed at a dose of 1.0 mg (in 200 µl of paraffin oil) in the wind tunnel against a control. In this experiment, the ratio of acetone to 1-octen-3-ol was 100:1 (Paynter and Brady, 1993) while that of p-cresol to 3-n-propylphenol was 4:1 (Owaga *et al.*, 1988; Saini, 1990; Saini and Hassanali, 1992).

3.8.2.3 Odour dispenser

The odour dispensers consisted of a black cloth tied using dichloromethane soxhlet-cleaned rubber bands on one end of an open ended plexiglass tube (5 cm long and 4.5 cm in diameter). The dispensers were each placed, through the window on the upper side of either arm of the tunnel onto a metallic rack positioned 15 cm from upwind end such that they were mid-height of the wind tunnel, with the cloth facing downwind. The required dose of the odour, dissolved in paraffin oil was pipetted onto the centre of the cloth on one of the dispensers, while air was allowed to flow at 10 cm/sec. For the control, the same volume of paraffin oil was pipetted onto a similar dispenser placed in the other arm of the tunnel. With all the windows closed, air was allowed to flow inside the tunnel for 5 minutes before the first fly was introduced.

3.8.2.4 Bioassay procedure

Teneral female *G. m. morsitans* after emergence were placed in a 20 cm x 15 cm x 15 cm cage and kept under 26 ± 1 ^{0}C and 70 ± 5% r.h. until they were three days old.

30 minutes before experiments, the flies were individually transferred into cylindrical PVC release cages (4 cm long and 3 cm in diameter) closed on one side with PVC gauze. The open end was closed loosely with a small piece of cotton wool plug.

The release cage containing a quiescent female was held with a long pair forceps and placed cautiously through the window in the middle of the tunnel onto the metallic rack placed on the middle of the mesh wire cover at the downwind end (middle zone), so that the cotton plugged end of the cage faced the observer. The cotton wool plug was removed slowly using forceps and the window closed tightly so that only air passing through the charcoal filters passed over the release cage carrying odour from the dispensers.

The behaviour of each fly was observed and recorded for three minutes after which it was aspirated out and destroyed using a vacuum pump. The following observations were recorded in a time scale score chart:

i) activation (walking or hoping within or out of the release cage)

ii) direction of flight

iii) distance covered in flight and position landed relative to the dispenser

iv) hovering around the dispenser

v) grooming while resting

vi) turning at 180^0 in flight prior to landing, and

vii) attempting to escape out of the tunnel (gliding against wall of tunnel while in flight or trying to fly through the wall of the tunnel).

In each experiment, about 10 flies were tested after which the tunnel, the metallic racks and the release cages were cleaned using 70% ethanol. With the humidifier switched off, the temperature of the room was raised to above 30 ^{0}C and air exhausted out of the room for about six hours using a powerful extraction fan fixed on the room wall. At the same time the tunnel was exhausted at maximum wind speed overnight.

Between five and eight replicates were carried out in each treatment.

3.9 Statistical analysis of data

The differences in proportions of flies that were engaged in specific behaviours (extended probing, escaping from a feeding surface, flight preferences and escape likelihood in the choice wind-tunnel) was analysed by Chi-square (SAS 1996). The average number of probing sites changed was calculated and means compared by Student's *t* test (SAS 1996). The percentage of flies that fed on waterbuck and ox, and on treated and control zones of the membrane, was plotted against observation time (T) and regression curves computed using either the Gauss-Newton method for nonlinear regression analysis or probits (SAS 1996) with $P > \chi^2 = 0.3 - 0.99$, and $P = 0.0001$ for estimates of intercepts and ln(T). Similar plots were used to calculate the probing times for 50% of flies that had initiated feeding under different conditions.

CHAPTER 4.

4.0 RESULTS

4.1 Feeding behaviour of *G. m. morsitans* on hosts and on membranes treated and untreated with waterbuck sebum.

4.1.1 Landing

No significant difference between the initial response of *G. m. morsitans* females placed on the waterbuck and ox was found; in both situations the flies responded within 1.20 ± 0.09 minutes (± s.e.) by landing on the respective animals (Table-1A). On the membrane, there was some delay in the reaction of the flies. Landing occurred after 2.61 ± 0.09 minutes (± s.e.) with no clear pattern in relation to the presence or absence of waterbuck sebum or its concentration (Table-1B). However, in both environments (animals and membranes) there were significant differences in the subsequent behavioural responses of the flies.

4.1.1.1 Behaviour after landing on hosts

Whereas none of the flies that landed on the ox exhibited escape behaviour, more than a third of those that initially landed on waterbuck escaped (Table-2A), although they all subsequently alighted again. Out of these flies, 25% escaped more than once but eventually 41.7% engorged. This aversion to proximity to and feeding on the waterbuck was also reflected in significant differences ($\chi^2_{(1)}$; 40.7, $P < 0.001$) in the proportion that changed probing sites after first or subsequent landings (Table-3A).

Table 1. Average time (minutes) taken by 2- and 3-day teneral *G. m. morsitans* females (from the commencement of experiment) to land on: **A.** live animals (waterbuck and ox); **B.** membranes treated/untreated with waterbuck sebum.

		N		Average time (mins ± s.e) taken to land	
		2-day	3-day	2-day	3-day
A: Live animals					
Waterbuck		-	34	-	1.2 ± 0.09
Ox		-	35	-	1.2 ± 0.08
B: Waterbuck sebum (dose, mg/cm^2)					
0.0	Untreated	46	35	2.9 ± 0.3	2.8 ± 0.4
0.7		44	42	2.4 ± 0.2	2.2 ± 0.2
1.0		60	67	2.9 ± 0.2	3.0 ± 0.2
1.4		-	56	-	2.3 ± 0.2

Table 2. Proportion of 2- and 3-day teneral *G. m. morsitans* females that escaped after initial landing prior to feeding on: **A.** live animals (waterbuck and ox); **B.** membranes treated/untreated with waterbuck sebum.

		N		% that escaped on initial landing and probing	
		2-day	3-day	2-day	3-day
A: Live animals					
	Waterbuck	-	34	-	35.3 ***
	Ox	-	35	-	0.0
B: Waterbuck sebum (dose, mg/cm^2)					
0.0	Untreated (double control)	46	35	0.0	2.9
0.7	Treated	21	21	9.5	4.8
	Control	24	21	4.0	0.0
1.0	Treated	38	44	34.2	34.1 **
	Control	34	32	26.5 ###	9.4
1.4	Treated	-	30	-	26.7 **
	Control	-	28	-	3.6

, * - indicate significant differences at $P < 0.01$ or $P < 0.001$, respectively, between live animals or treated and control zones of the membrane

- indicate a significant difference at $P < 0.001$, between control zone of the membrane and untreated (double control) membrane.

Table 3. Proportion of 2- and 3-day teneral *G. m. morsitans* females that changed probing sites prior to feeding on: **A.** live animals (waterbuck and ox); **B.** membranes treated/untreated with waterbuck sebum.

		N		% that changed probing sites after initial and subsequent landing	
		2-day	3-day	2-day	3-day
A: Live animals					
	Waterbuck	-	34	-	88.2 ***
	Ox	-	35	-	11.4
B: Waterbuck sebum (dose, mg/cm^2)					
0.0	Untreated (double control)	46	35	10.9	20.0
0.7	Treated	21	21	19.0	38.1
	Control	24	21	25.0	28.6
1.0	Treated	38	44	68.4 ***	50.0 ***
	Control	34	32	26.5 ǂ	15.6
1.4	Treated	-	30	-	46.7 **
	Control	-	28	-	17.9

, * - indicate significant differences at $P < 0.01$ or $P < 0.001$, respectively, between live animals or treated and control zones of the membrane

ǂ - indicate a significant difference at $P < 0.05$, between control zone of the membrane and untreated (double control) membrane.

Table 4. Average number of probing sites (± s.e.) changed by 2- and 3-day teneral *G. m. morsitans* females between initial landing and eventual feeding on: **A.** live animals (waterbuck and ox); **B.** membranes treated/untreated with waterbuck sebum.

		N		Average number of probing sites changed (± s.e)	
		2-day	3-day	2-day	3-day
A: Live animals					
Waterbuck		-	34	-	3.94 ± 0.79***
Ox		-	35	-	0.11 ± 0.05
B: Waterbuck sebum (dose, mg/cm^2)					
0.0	Untreated (double control)	46	35	0.17 ± 0.07	0.31 ± 0.14
0.7	Treated	21	21	0.29 ± 0.10	0.38 ± 0.11
	Control	24	21	0.25 ± 0.09	0.29 ± 0.10
1.0	Treated	38	44	1.74 ± 0.24***	1.20 ± 0.19***
	Control	34	32	0.59 ± 0.14‡‡	0.25 ± 0.09
1.4	Treated	-	30	-	0.90 ± 0.24*
	Control	-	28	-	0.39 ± 0.18

*, *** - indicate significant differences at $P < 0.05$ or $P < 0.001$, respectively, between live animals or treated and control zones of the membrane

‡‡ - indicate a significant difference at $P < 0.01$, between control zone of the membrane and untreated (double control) membrane.

About 88.2% of flies that contacted the waterbuck's skin changed probing sites by either hoping, walking or short flight, while only 11.4% of flies that contacted the ox skin exhibited this behaviour. The flies probed on significantly more sites on the waterbuck (3.94 ± 0.79) compared to the ox (0.11 ± 0.05, Table-4A). Significantly more flies (30%, $P < 0.001$) spent time grooming themselves after probing on the waterbuck compared to only 3% that groomed while on the ox.

4.1.1.2 Behaviour after landing on membranes

In the absence of the waterbuck sebum on the membrane (untreated), there were no significant differences in responses of 2- and 3-day teneral flies. Whereas none of the 2-day teneral flies escaped, only one 3-day teneral fly escaped from the membrane. Although the proportion of 3-day teneral flies that changed probing sites was higher (20%) and probed on more sites (0.31 ± 0.14) than the 2-day tenerals (10.9% and 0.17 ± 0.07 respectively), these figures were not statistically different (Tables-3B, 4B). All flies in the two groups fed to repletion by the sixth minute after landing.

2-Day teneral flies presented to a blank choice bioassay, landed and fed equally on either zones. There were no significant differences in any response observed between the two zones and all flies fed within six minutes of landing.

The waterbuck sebum was estimated at about 0.7 mg/cm^2 on the hairs and skin of the live animal. However when the same marked out portion on the lateral side of waterbuck was washed out at intervals, similar GC profiles were obtained from all the washings. When 2- and 3-day teneral *G.m.morsitans* females were presented to a membrane partly treated with 0.7 mg/cm^2 of the waterbuck sebum in a choice bioassay, there was no significant difference in all responses recorded between the treated and

the control arenas (Table-2B, 3B, 4B). Similarly, studies carried out at a waterbuck sebum concentration of 0.1 mg/cm^2 resulted in no differences at all between the treated and the control zones.

Flies presented to membranes treated with higher doses of waterbuck sebum showed significant differences in responses on treated compared to control zone. The 2- and 3-day teneral flies presented to membranes partly treated with either 1.0 mg/cm^2 or 1.4 mg/cm^2 of waterbuck sebum exhibited significantly higher searching behaviours on the treated than on the control zone.

The proportion of 2- and 3-day teneral flies that changed probing sites on the part of membrane treated with 1.0 mg/cm^2 of waterbuck sebum (68.4% and 50.0% respectively) was significantly higher than on the control part (26.5% and 15.6% respectively). Similarly, a significantly higher proportion (46.7%) of 3-day teneral flies that landed on the part of membrane treated with 1.4 mg/cm^2 of the sebum changed probing sites compared to 17.9% on the control part (Table-3B). In the search for probing sites on the part of membrane treated with 1.0 mg/cm^2 , the 2- and 3-day teneral flies made significantly more movements (1.74 ± 0.24 and 1.20 ± 0.19 respectively) than on the control zone (0.59 ± 0.14 and 0.25 ± 0.09 respectively). Similarly, on the membrane treated with 1.4 mg/cm^2, the number of probing sites changed on the treated zone (0.90 ± 0.24) was significantly higher than on the control (0.39 ± 0.18, Table-4B).

At the higher doses (1.0 and 1.4 mg/cm^2) of waterbuck sebum on the membrane, 3-day teneral flies that landed on the treated zones showed significantly higher escape behaviour (Table-2B). Although the difference in proportion of 2-day teneral flies that escaped from the zone treated with 1.0 mg/cm^2 of the sebum was not

significant from that escaping from control; the proportion escaping from the treated zone was similar to that of 3-day tenerals escaping from a similar zone (Table-2B). Most of the flies that escaped from the membranes landed again and continued with searching for feeding sites. In all cases, similar proportions (37%) of flies that landed again escaped more than once from the treated zone.

Comparison of the behaviour of the flies that landed on control zones of the membrane and those on untreated (double control) membrane also showed some differences particularly with less hungry flies (Table-2B, 3B, 4B), indicating that the insect's behaviour may be affected by a volatile chemical signal in addition to a less volatile contact signal associated with the sebum.

4.1.2 Engorgement

The proportions of flies that fed on the two animals, and on treated and untreated zones of the membrane were plotted against observation time and regression curves derived using probit analysis (Figs. 7-12). When the proportions of flies that initiated feeding on the animals and on the membranes were plotted against time taken probing, regression curves were derived using two methods: probit analysis and the Gauss-Newton method for nonlinear regression analysis (SAS, 1996).

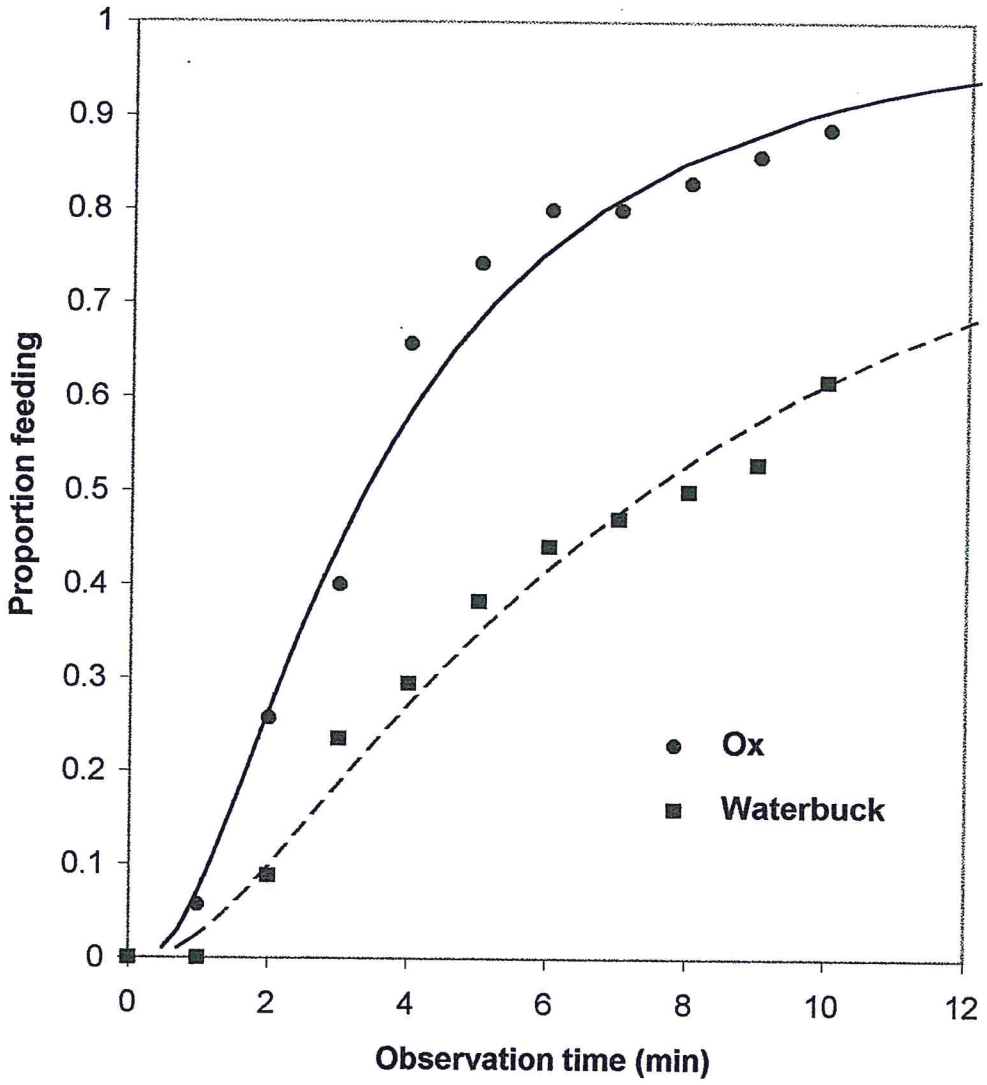


Fig. 7 Proportion of 3-day teneral *G. m. morsitans* females feeding on waterbuck and ox. Regression curves were derived using probit analysis.

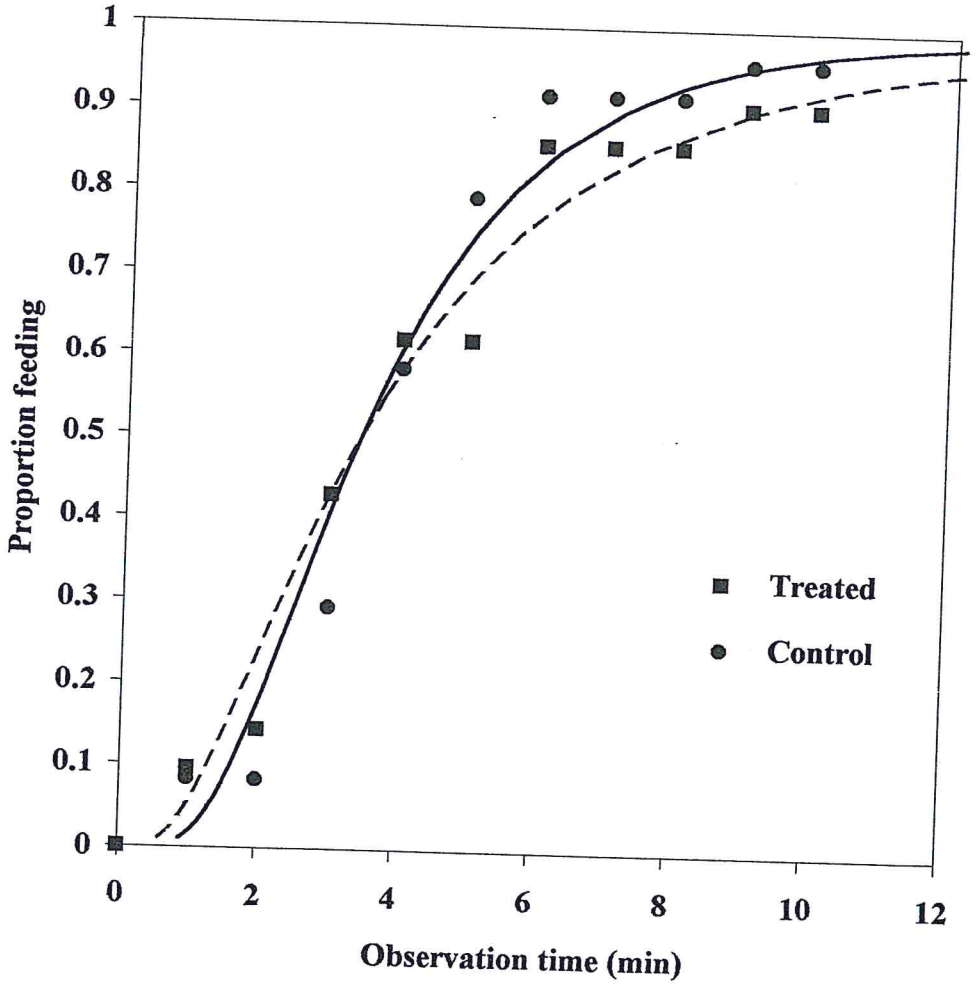


Fig. 8 Proportion of 2-day teneral *G.m. morsitans* females feeding through a membrane partly treated with 0.7 mg/cm^2 of waterbuck sebum. Regression curves were derived using probit analysis.

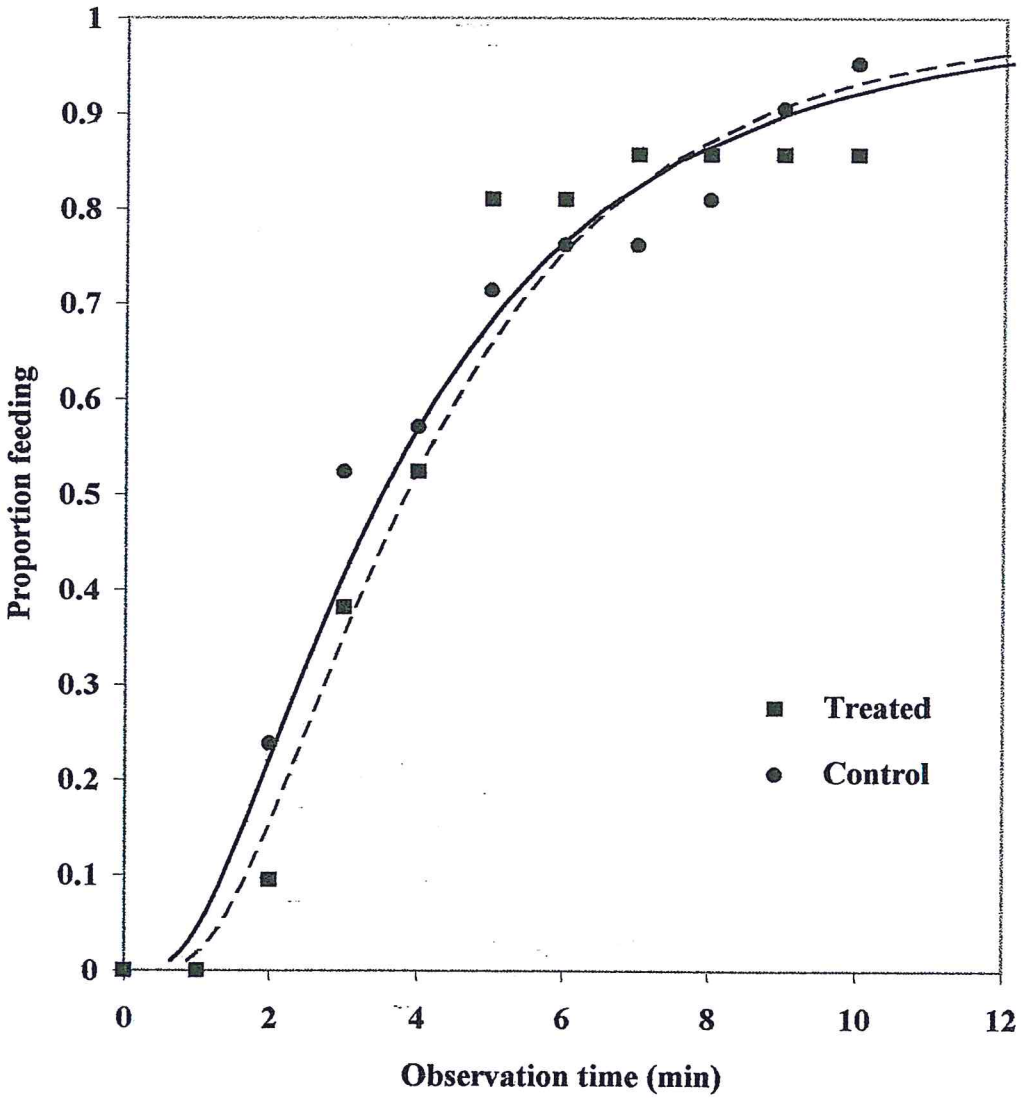


Fig. 9 Proportion of 3-day teneral *G. m. morsitans* females feeding through a membrane partly treated with 0.7 mg/cm^2 of waterbuck sebum. Regression curves were derived using probit analysis.

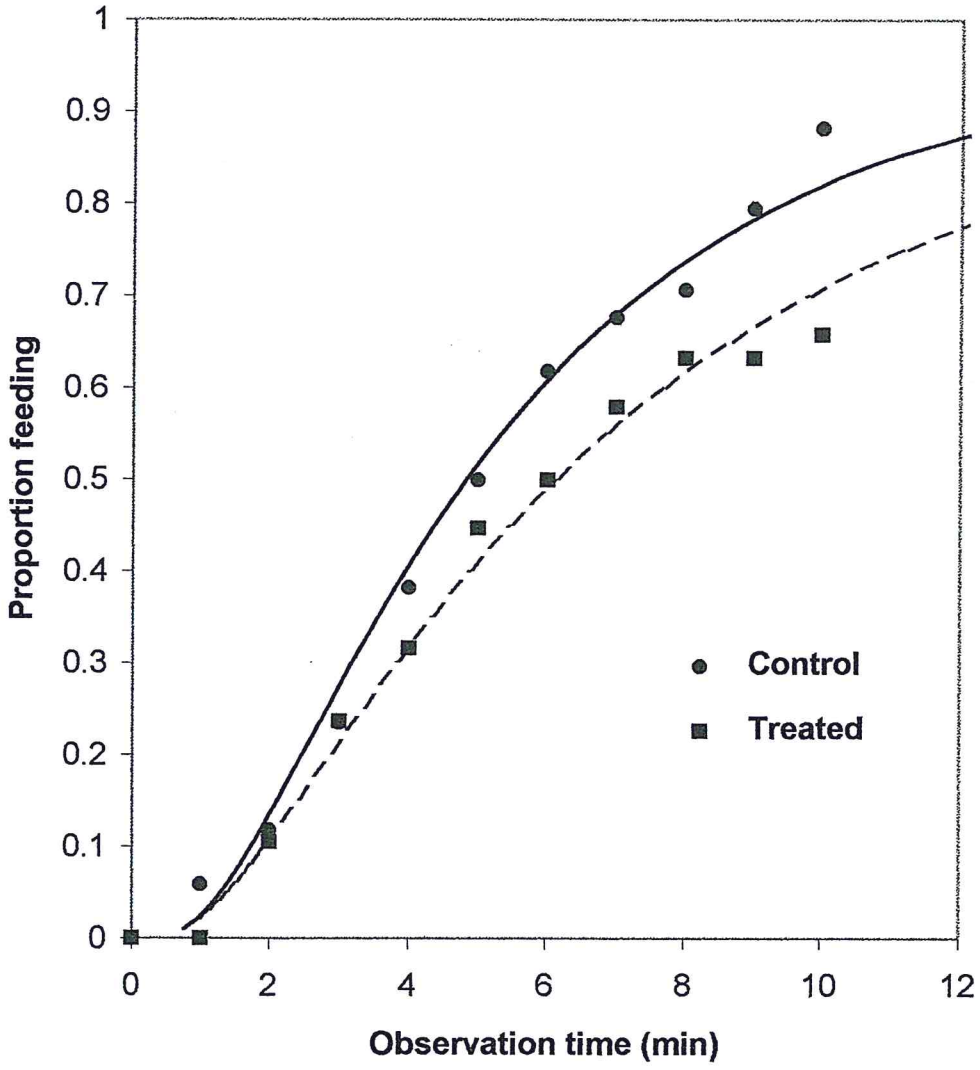


Fig.10. Proportion of 2-day teneral *G.m. morsitans* females feeding through a membrane partly treated with 1.0 mg/cm^2 of waterbuck sebum. Regression curves were derived using probit analysis.

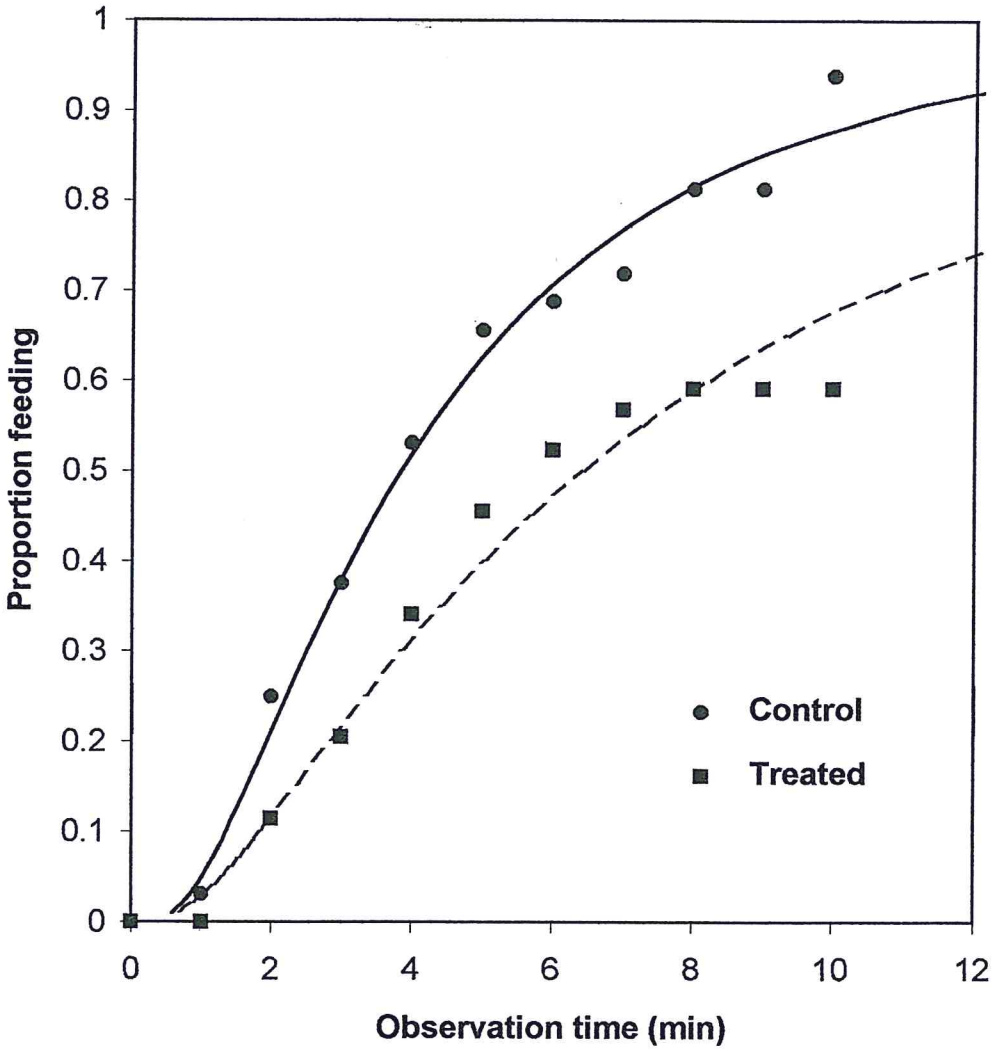


Fig.11. Proportion of 3-day teneral *G.m. morsitans* females feeding through a membrane partly treated with 1.0 mg/cm^2 of waterbuck sebum. Regression curves were derived using probit analysis.

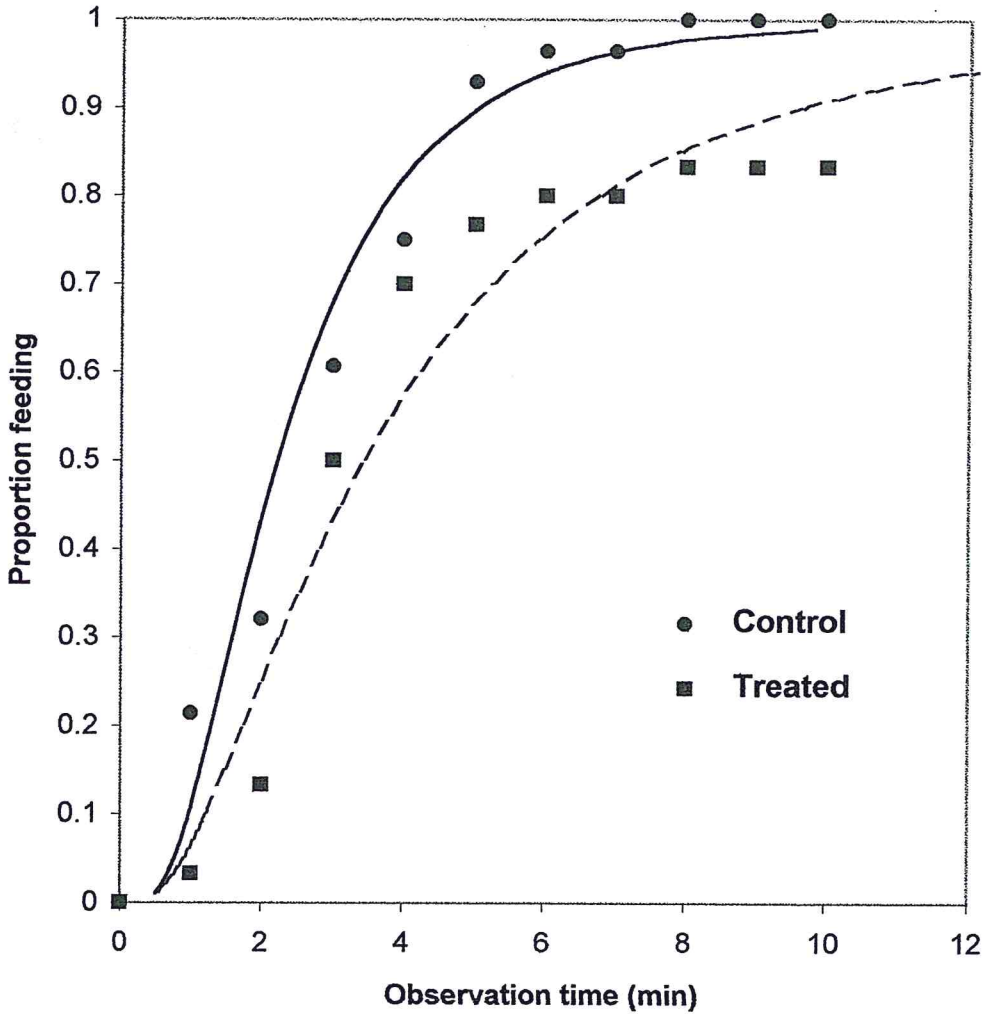


Fig.12. Proportion of 3-day teneral *G.m. morsitans* females feeding through a membrane partly treated with 1.4 mg/cm^2 of waterbuck sebum. Regression curves were derived using probit analysis.

Using the probit analysis, the time taken in probing by the first 50% of flies before onset of feeding (F_{50}) was computed (Table-5). The Gauss-Newton method for nonlinear regression analysis gave exponential curves described by the equation:

$$Pr = a(1-e^{-bt})$$

where,

Pr = proportion of flies that fed

t = probing time (minutes)

a and ***b*** = constants whose values are indicated in Table-6.

The nonlinear regression equation (NRE) was used to estimate the proportion of flies that would engorge after probing for any length of time.

4.1.2.1 Feeding on hosts

The flies showed a highly significant reluctance to feed on waterbuck compared to the ox (Fig. 7). Proportions of flies feeding on waterbuck and ox differed significantly from the 2nd to 10th minute ($P < 0.05$ - $P < 0.001$) of observation period. At the end of observation period (10 minutes), significantly fewer flies (61.8%) presented to waterbuck had initiated feeding compared to 88.6% on ox. Although the flies that never fed were still probing or had escaped from the skin surface, those that fed on waterbuck spent significantly more time (5.14 ± 0.60 minutes) probing compared to those on ox (3.65 ± 0.38 minutes, Table-7A). Similarly, the F_{50} on waterbuck was significantly higher (7.3 minutes) compared to that on ox (3.1 minutes, Table-5A). The NRE indicated that only 65% of flies presented to the waterbuck would engorge after infinite time compared to 99% that would engorge on the ox (Table-6A).

Table 5. Probing time (minutes) for the first 50% of 2- and 3-day teneral *G. m. morsitans* females to start feeding on: **A.** live animals (waterbuck and ox); **B.** membranes treated/untreated with waterbuck sebum.

		N		Probing time (mins) for first 50% of flies to start feeding[a]	
		2-day	3-day	2-day	3-day
A: Live animals					
Waterbuck		-	34	-	7.3 *
Ox		-	35	-	3.1
B: Waterbuck sebum (dose, mg/cm^2)					
0.0	Untreated (double control)	46	35	1.4	1.4
0.7	Treated	21	21	1.9	2.0
	Control	24	21	2.2 ǂ	2.3 ǂ
1.0	Treated	38	44	4.5 *	5.1 *
	Control	34	32	2.1 ǂ	1.6
1.4	Treated	-	30	-	2.6 *
	Control	-	28	-	1.4

* - indicate a significant difference at $P < 0.05$, between live animals or treated and control zones of the membrane.

ǂ - indicate a significant difference at $P < 0.05$, between control zone of the membrane and untreated (double control) membrane.

[a] - calculated from regression curves, derived using probit analysis, in plots of proportions of flies that started feeding against probing time.

Table 6. Values of a and b (± s.e.), calculated at $P < 0.05$, for the exponential equation $Pr = a(1 - e^{-bt})$ derived from the nonlinear regression (Gauss-Newton method, SAS 1996) curves in plots of proportion (Pr) of *G. m. morsitans* females that started feeding against observation time (t): **A.** on live animals; **B.** through membranes treated/untreated with waterbuck sebum.

	2-Day teneral flies		3-Day teneral flies	
	a	***b***	***a***	***b***
A: Live animals				
Waterbuck	-	-	65.00 ± 13.94	0.17 ± 0.07
Ox	-	-	99.00 ± 11.72	0.22 ± 0.06
B: Waterbuck sebum (dose, mg/cm^2)				
0.0 Untreated (double control)	100.0 ± 13.64	0.24 ± 0.08	100.0 ± 10.61	0.27 ± 0.07
0.7 Treated	96.00 ± 13.78	0.24 ± 0.08	100.0 ± 21.09	0.21 ± 0.09
Control	100.0 ± 20.83	0.23 ± 0.11	100.0 ± 12.88	0.22 ± 0.06
1.0 Treated	75.00 ± 16.02	0.17 ± 0.07	67.00 ± 12.89	0.20 ± 0.08
Control	96.00 ± 23.26	0.15 ± 0.07	99.00 ± 12.43	0.19 ± 0.05
1.4 Treated	-	-	93.50 ± 13.61	0.25 ± 0.09
Control	-	-	100.0 ± 6.92	0.34 ± 0.07

Table 7. Proportions of by 2- and 3-day teneral *G. m. morsitans* females that initiated feeding by the 10th minute and the time taken between initial landing and feeding on: **A.** live animals (waterbuck and ox); **B.** membranes treated/untreated with waterbuck sebum.

	% that initiated feeding by the 10th minute		Average time (mins ± s.e.) taken between initial landing and feeding[b].	
	2-day	3-day	2-day	3-day
A: Live animals				
Waterbuck	-	61.8 ***	-	5.14 ± 0.60 *
Ox	-	88.6	-	3.65 ± 0.38
B: Waterbuck sebum (dose, mg/cm^2)				
0.0 Untreated (double control)	100	100	2.10 ± 0.15	2.10 ± 0.17
0.7 Treated	90.5	85.7	2.47 ± 0.26	2.50 ± 0.30
Control	95.8	95.2	2.87 ± 0.28 ‡‡	2.65 ± 0.28 ‡
1.0 Treated	65.8 **	59.1 ***	3.20 ± 0.31	2.92 ± 0.25 *
Control	88.2	93.8	2.70 ± 0.25 ‡	2.30 ± 0.23
1.4 Treated	-	83.3 **	-	2.76 ± 0.25 *
Control	-	100	-	2.07 ± 0.20

*, **, *** - indicate significant differences at $P < 0.05$, $P < 0.01$ or $P < 0.001$, respectively, between live animals or treated and control zones of the membrane

‡, ‡‡ - indicate significant differences at $P < 0.05$, and $P < 0.01$, respectively, between control zone of the membrane and untreated (double control) membrane.

[b] - only flies that initiated feeding within the observation time were considered

4.1.2.2 Feeding through membranes treated and untreated with waterbuck sebum.

Teneral (2- and 3-day) flies presented to untreated (double control) membranes had similar feeding responses. All flies engorged after having spent 2.1 minutes after initial landing (Table-7B) and had the same F_{50} value of 1.4 minutes (Table-5B). When flies were presented to membranes partly treated with various doses of waterbuck sebum, they were reluctant to feed through the treated zone. Fewer flies, though not statistically significant, fed through the part of membrane treated with 0.7 mg/cm^2 compared to the control (Figs. 8, 9). Moreover, the 2- and 3-day teneral flies that fed through the control parts spent significantly more time probing (Table-7B) and had significantly higher F_{50} values (Table-5B) compared to the untreated membranes, implying that flies that landed on the control zone were affected by a volatile chemical signal from the waterbuck sebum on the treated zone. Similarly, a significantly higher effect of sebum on flies that landed on the control zone, compared to the untreated membrane, was observed for the 2-day tenerals presented to the membrane treated with 1.0 mg/cm^2 of waterbuck sebum (Tables-2B,3B,4B,5B,7B).

The 2- and 3-day teneral *G. m. morsitans* females presented to membranes partly treated with higher doses (1.0 and 1.4 mg/cm^2) of waterbuck sebum showed significant reluctance to feed through the treated zone compared to the control zone (Figs. 10 - 12). For the 2-day teneral flies presented to the membrane treated with 1.0 mg/cm^2, the proportion feeding on treated and control differed significantly in the 10th minute ($P < 0.01$). The proportion of 3-day teneral flies feeding on the treated and control zones differed significantly from the 3rd to 10th minute ($P < 0.05$ - $P < 0.001$) for the same dose of waterbuck sebum. When the dose of waterbuck sebum was increased

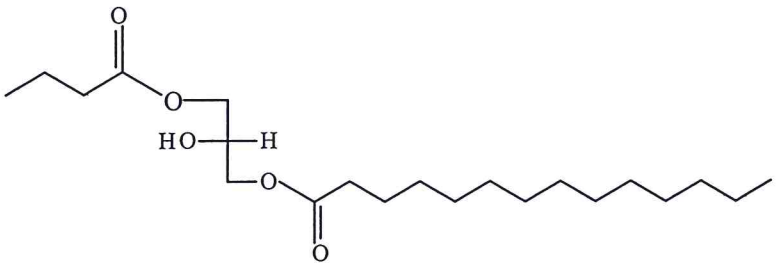
to 1.4 mg/cm^2, the proportion of 3-day teneral flies feeding on the treated and control zones differed significantly in the first two minutes ($P < 0.05$) and from the 5th to 10th minute ($P < 0.05$ - $P < 0.01$). Moreover, the proportion of 3-day teneral flies feeding through the membrane treated with 1.4 mg/cm^2 of the waterbuck sebum were higher compared to flies at the same state of hunger feeding through membranes treated with lower doses (Table-7B). Similarly, at the higher dose (1.4 mg/cm^2), flies spent less probing time before feeding, had a lower F_{50} value (Table-5B) and had higher values of ***a*** and ***b*** in the NRE (Table-6B), than flies presented to a membrane treated with 1.0 mg/cm^2 of the sebum. However, for the 1.0 mg/cm^2 dose, proportions of flies feeding through the treated zone were comparable to those feeding on a live waterbuck (Table-7) and had similarly higher F_{50} values and lower values of ***a*** and ***b*** in NRE compared to membranes treated with higher or lower doses of the sebum (Tables-5B, 6B).

4.2 Identification of electrophysiologically active components of skin secretions from animals (waterbuck, buffalo and ox).

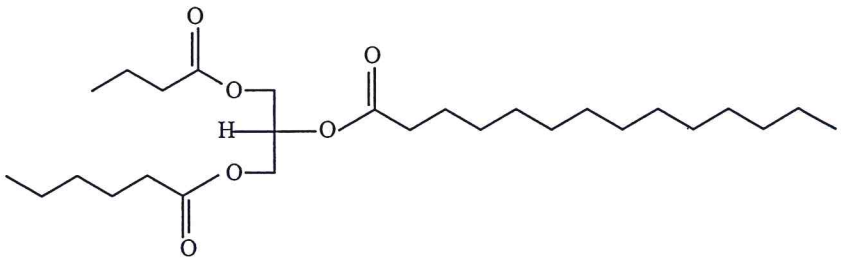
4.2.1 Olfactory response of *G. m. morsitans* to waterbuck sebum.

When the waterbuck sebum was analysed by GC (Fig.13) and by GC-EAD (Fig. 14) on female *G. m. morsitans*, two peaks (***I*** and ***II***) elicited EAG responses. The sebum was analysed using GC-MS and the tentative structure of the two EAG-active compounds identified from their mass spectra. For identification, the mass spectrum of the unknown compound was compared with the NIST and WILEY mass spectral data bases installed in a computer linked to the GC-MS. The mass spectrum which was closest to those of the EAG-active peaks from a library search of the mass spectral data

base was that of glyceryltritetradecanoate (Fig.15). Using the ionization pattern of glyceryltritetradecanoate as a reference, the structure of the compound in peak ***I*** was assigned as the diacylglycerol, glyceryl-1-butanoate-3-myristate (*32*) while that of compound in peak ***II*** was assigned as the triacylglycerol, glyceryl-1-butanoate-3-hexanoate-2-myristate (*33*). The identification of these compounds was achieved through a rationalised accounting of fragments resulting to peaks in their mass spectra (Fig. 15, Scheme-5, Scheme-6) and by comparing with mass spectra of glycerols from literature. These two acylglycerols were not available commercially, thus authentication by GC co-injection and spectral comparisons needs to be carried out. Synthesis of these compounds, GC and GC-MS comparisons followed by GC-EAD tests on *G. m. morsitans* would be necessary for structural confirmation.



32



33

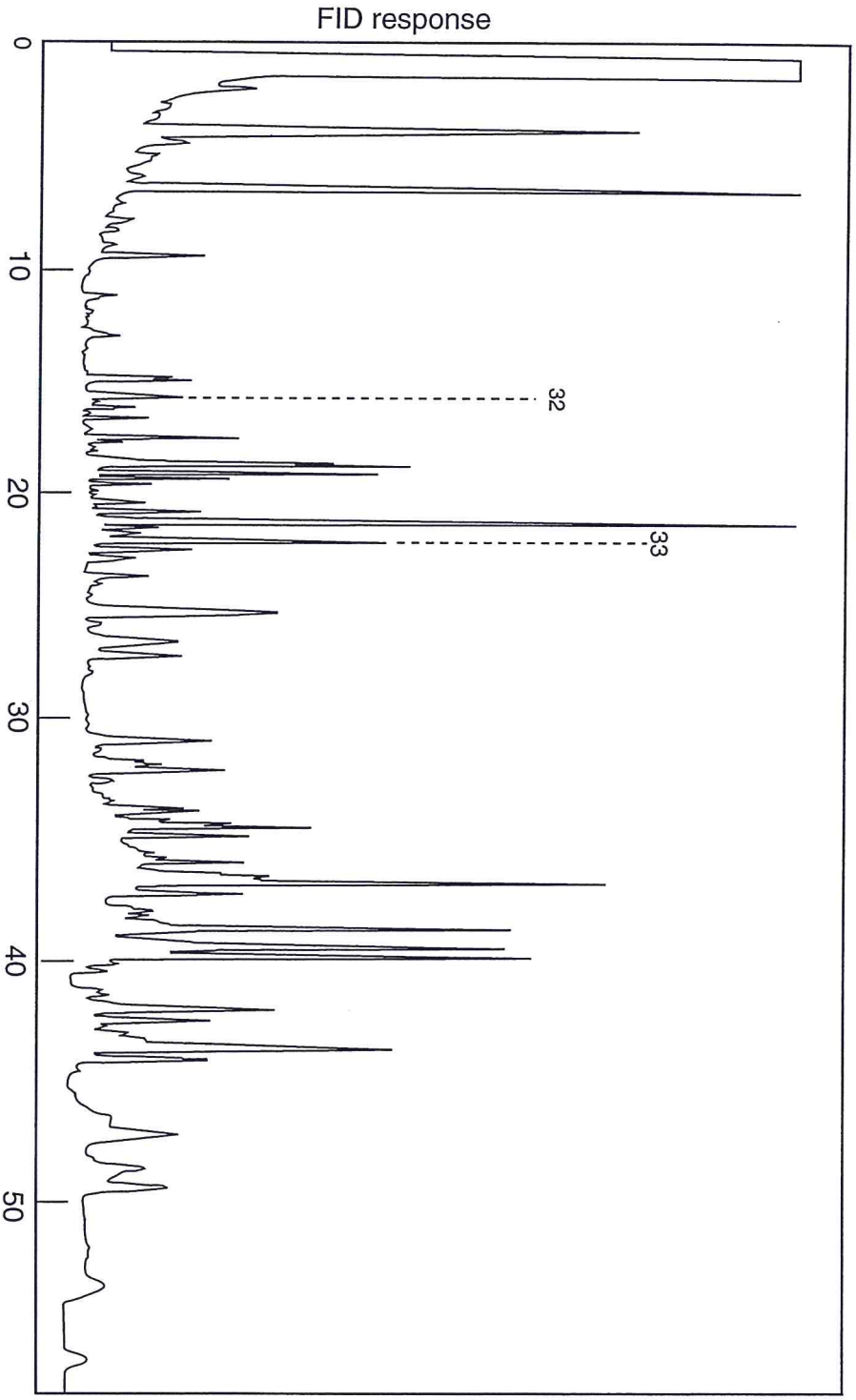


Fig. 13. Gas chromatogram of waterbuck body wash (sebum) showing EAG - active peaks (32 and 33) on *G. m. morstans*.

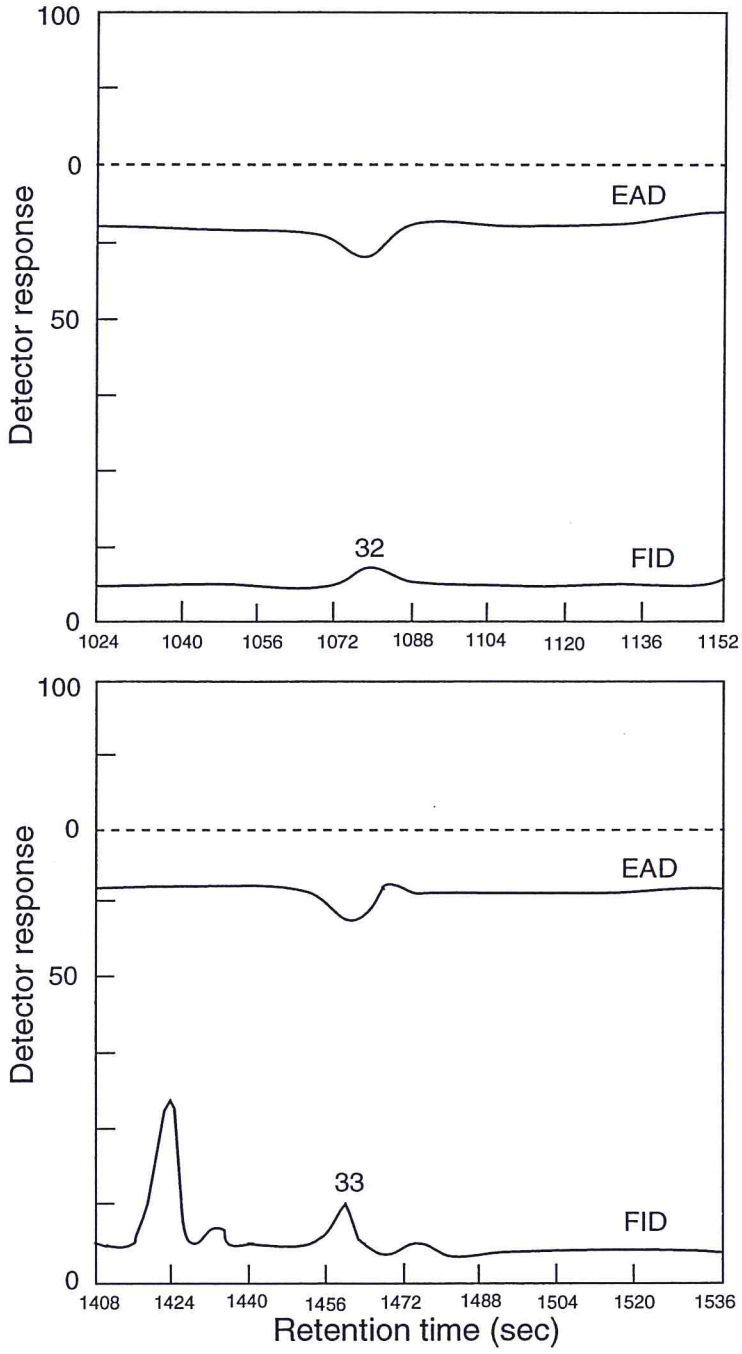


Fig. 14. GC - EAD chromatogram of waterbuck sebum showing EAG responses from antenna of *G. m. morsitans* to compounds 32 and 33.

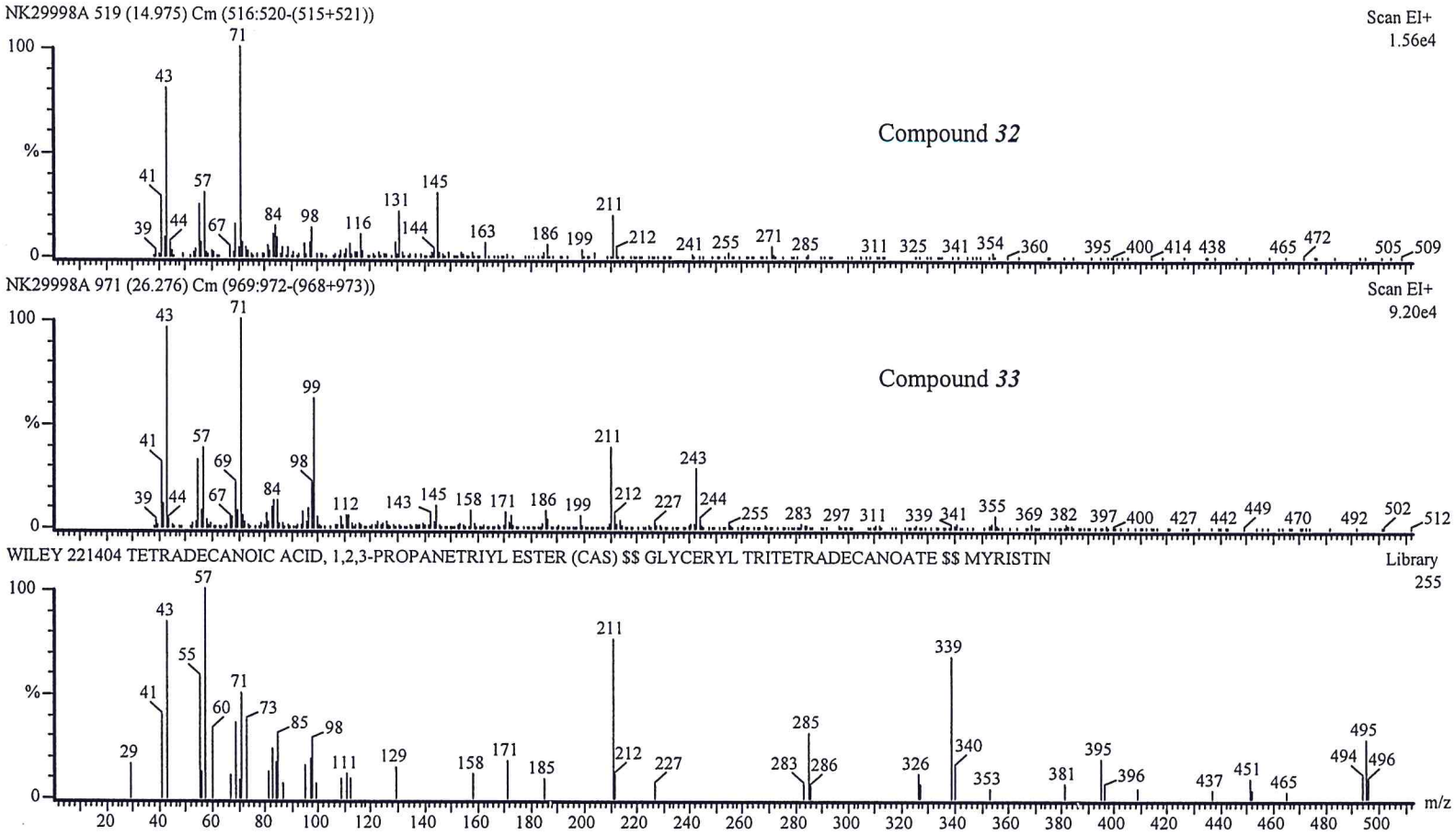
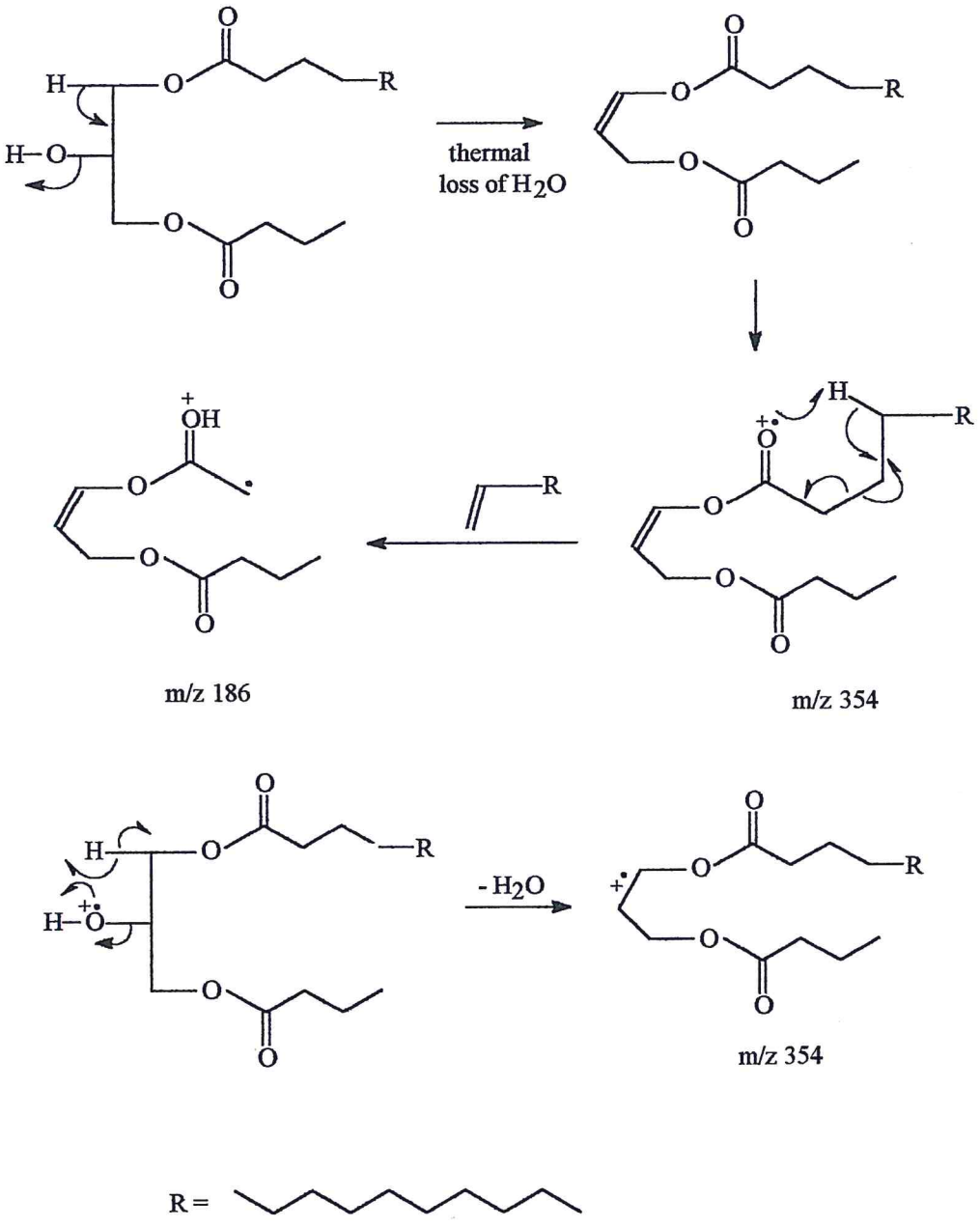
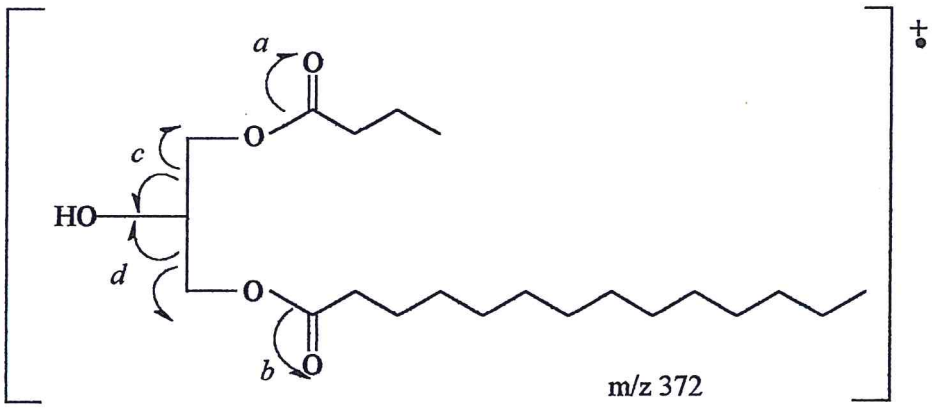
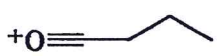


Fig. 15. Mass spectra of compound *32* and *33* compared to that of glyceryltritetradecanoate.



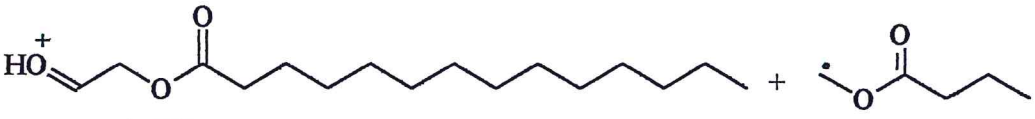
Scheme 5. Proposed fragmentation pattern of compound ***32***



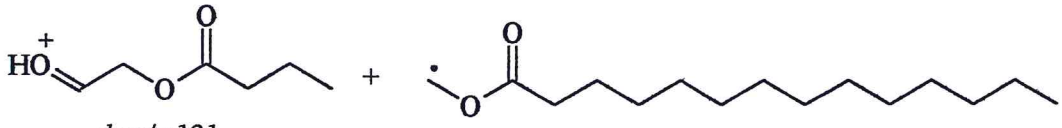


a. m/z 71

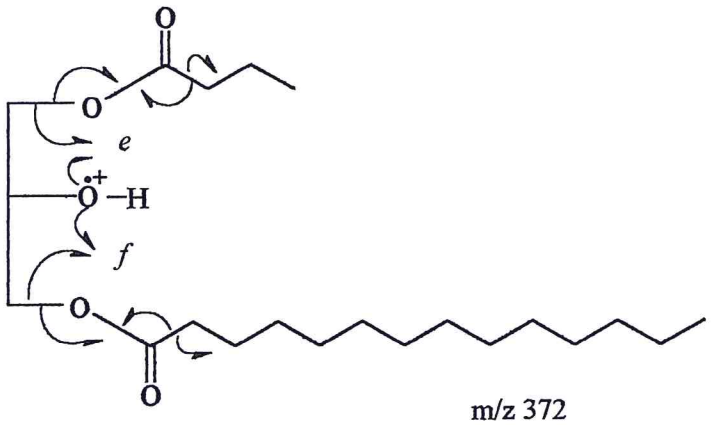
b. m/z 211



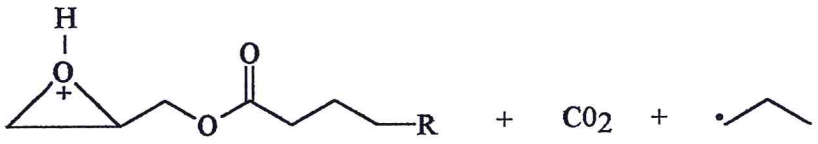
c. m/z 271



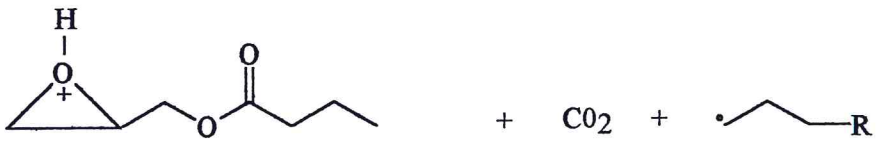
d. m/z 131



m/z 372

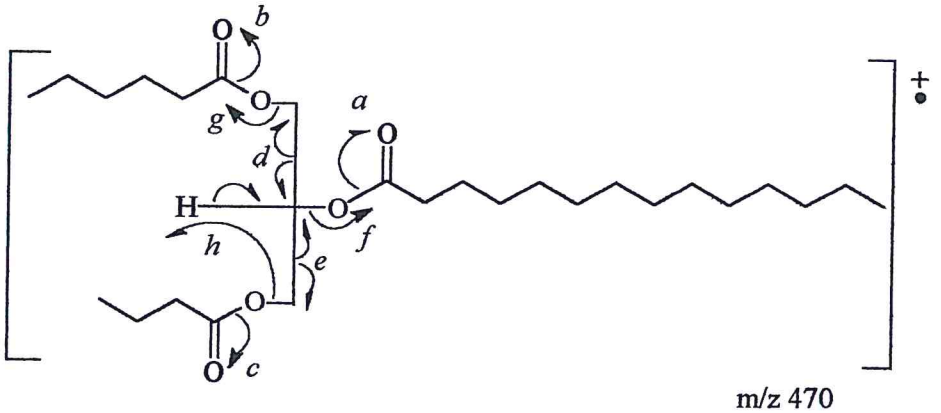


e. m/z 285



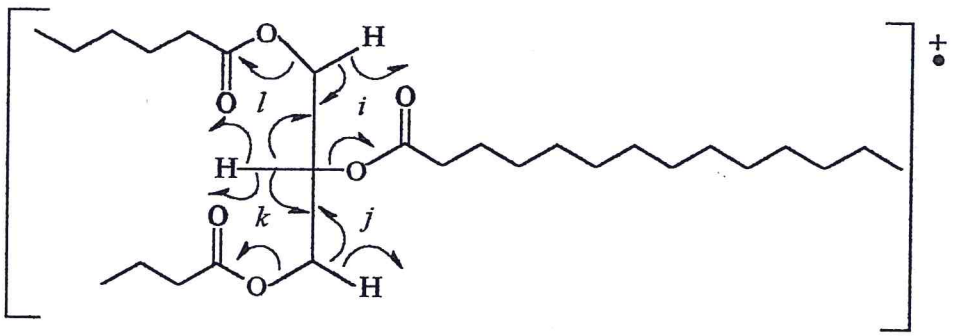
f. m/z 145

R = 



m/z 470

Generation of acyloxymethylene radicals and acylium ions.



m/z 470

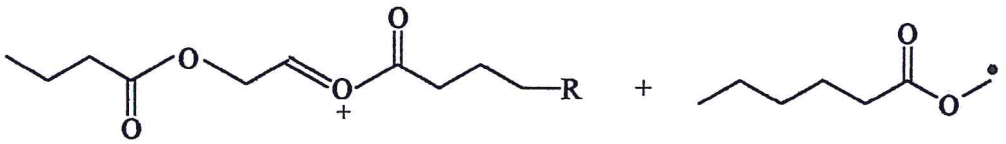
McLafferty rearrangements

Scheme 6. Proposed fragmentation pattern of compound *33*

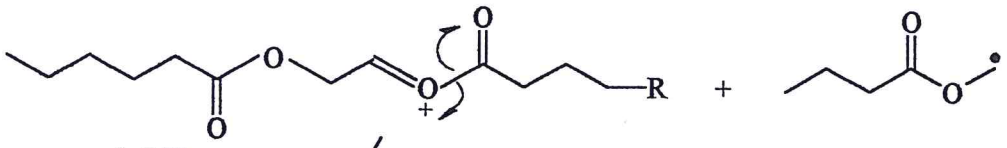
a. m/z 211

b. m/z 99

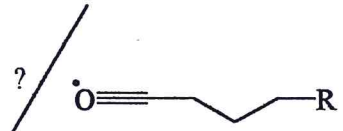
c. m/z 71

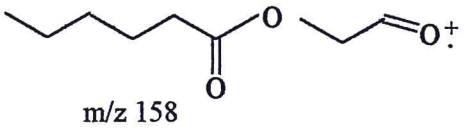


d. m/z 341



e. m/z 369

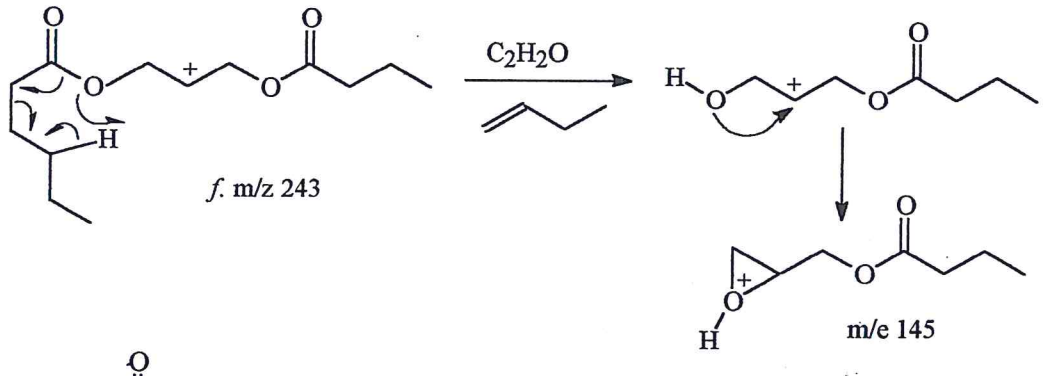




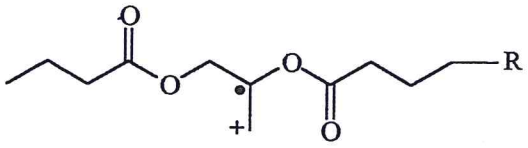
m/z 158

Ions *d* and *e* give diagnostic peaks

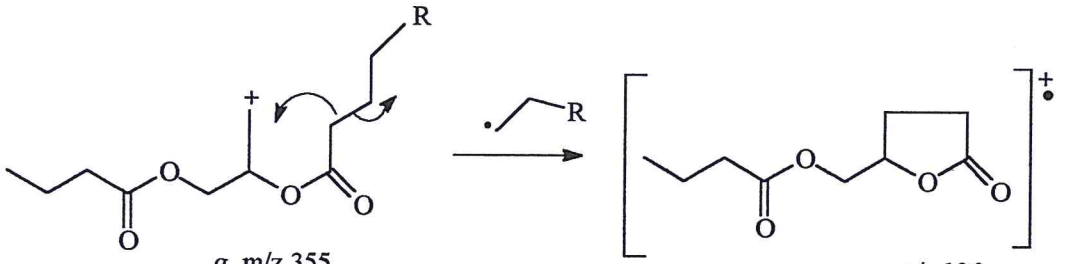
R =



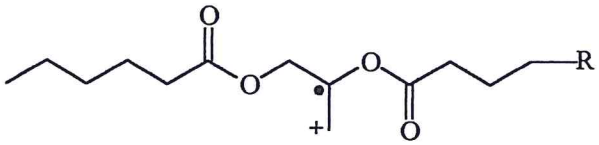
f. m/z 243



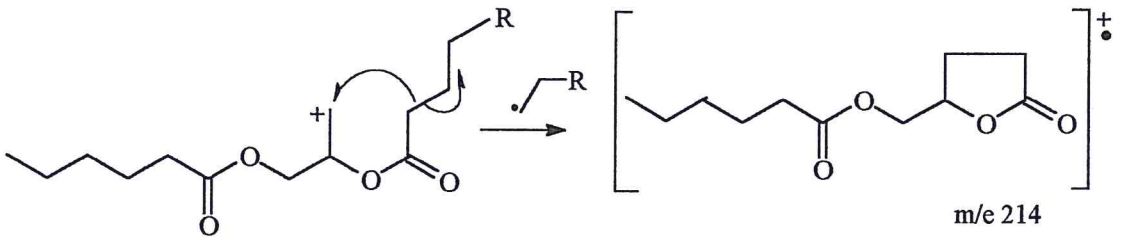
l. m/z 354



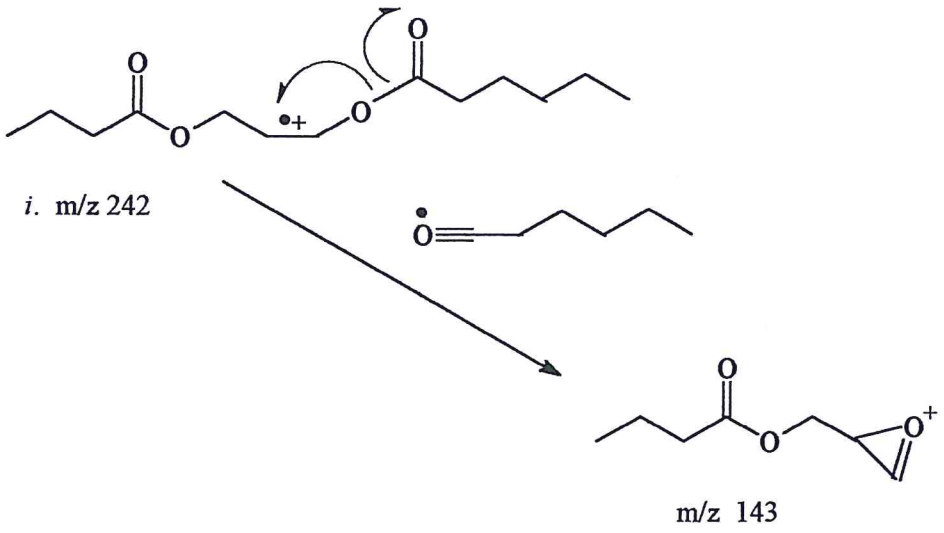
g. m/z 355



k. m/z 382

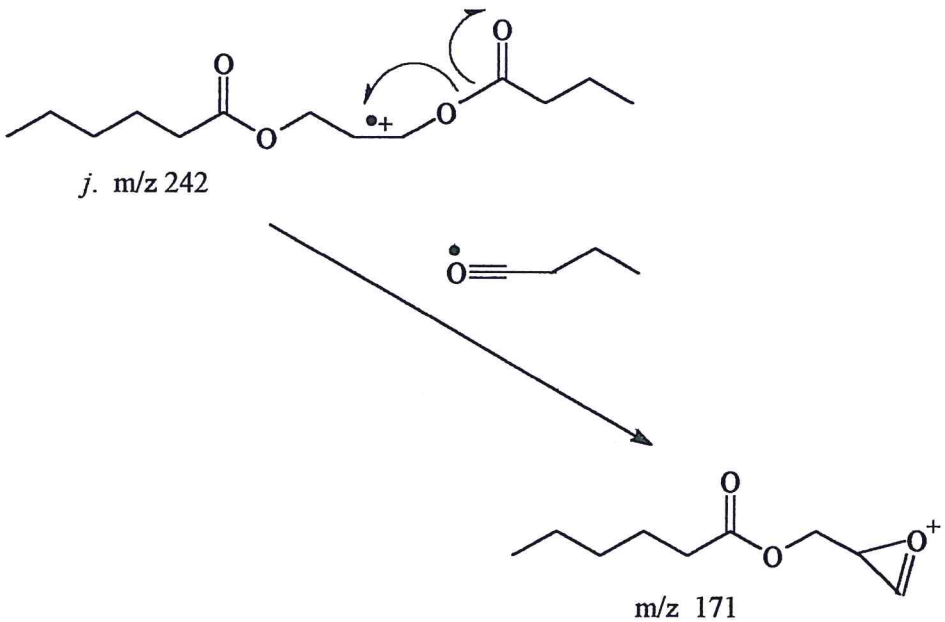


h. m/z 383



i. m/z 242

m/z 143

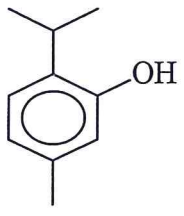


j. m/z 242

m/z 171

4.2.2 Fractionation of waterbuck sebum.

The fractionation of waterbuck sebum yielded (by weight): 1.85% of acidic, 0.52% of phenolic, trace amount of basic, and 97.53% of neutral fractions. GC and GC-MS analyses of the fractions showed that the acidic fraction contained all the saturated carboxylic acids from C_{14} to C_{22} plus trace amounts of oleic acid (Z,9-octadecenoic acid). The major carboxylic acids were tetradecanoic and hexadecanoic acids. The carboxylic acids having even numbered carbon atoms occurred in relatively higher amounts than those having odd numbers of carbon atoms (Fig. 16A). The GC and GC-MS analyses of the phenolic and basic fractions were carried out using a 50m capillary column (see **3.6.1** above) since no peaks were detected using the 15m wide bore column. The phenolic fraction contained one major compound forming about 80% (GC) identified as 2-isopropyl-5-methylphenol (***34***) using GC-MS and GC co-injection with an authentic standard. Other phenolic compounds in trace amounts were 4-methylphenol (***5***) and 4-ethylphenol (*7*) (Fig. 17). The neutral fraction contained the two EAG-active compounds (see **4.2.1** above) among several other high molecular weight compounds.



34

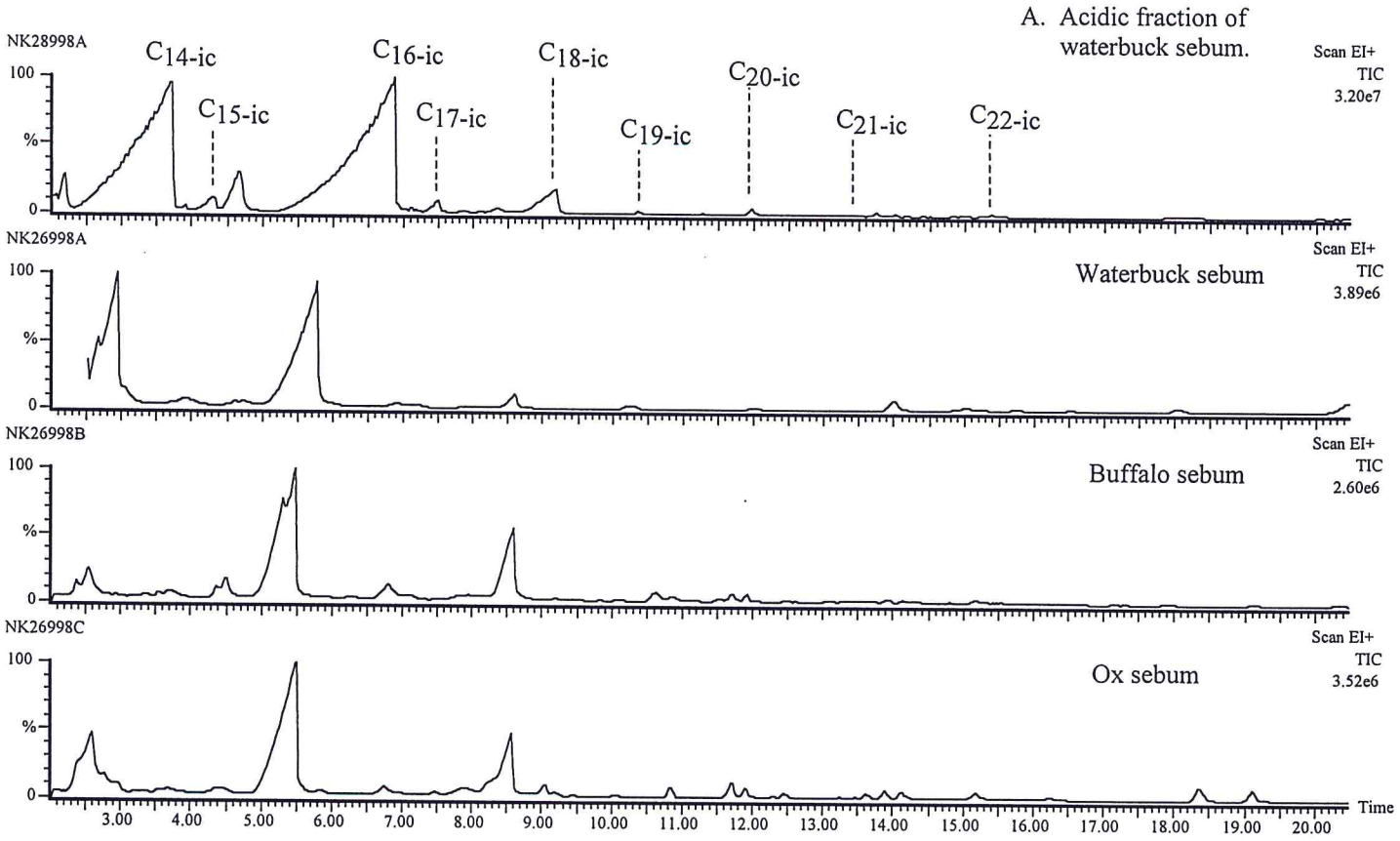


Fig. 16. GC-MS total ion chromatograms of sebum from waterbuck, buffalo and ox compared to the the acidic fraction of the waterbuck sebum.

$C_{14\text{-ic}}$ etc., refers to tetradecanoic acid etc.

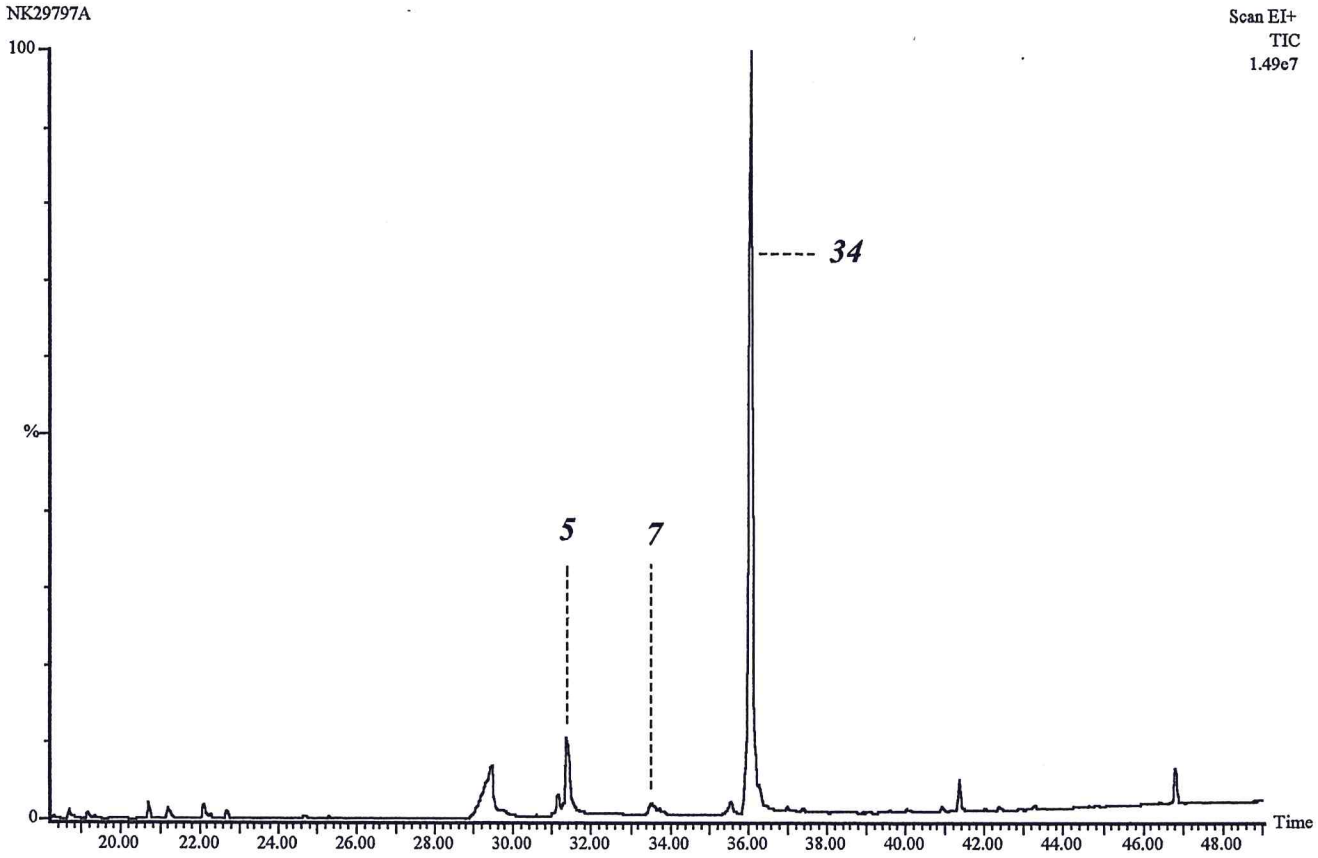


Fig. 17. GC-MS total ion chromatogram of the phenolic fraction of waterbuck sebum.

When sebum from ox or buffalo was analysed by GC and GC-MS, a series of long chain carboxylic acids similar to those in waterbuck sebum were found (Fig. 16). However, these tsetse hosts (ox and buffalo) lacked the two EAG-active compounds identified in the waterbuck sebum. Absence of these peaks was also confirmed by screening for the major diagnostic fragmentation ions of the EAG-active compounds from the mass spectra of sebum from ox (Fig. 18a; Fig. 18b; Fig. 18c; Fig. 18d) or buffalo (Fig.19a; Fig.19b; Fig.19c; Fig.19d). A comparison of GC profiles of the body wash from the three animals (Fig-20) shows that apart from the long chain carboxylic acids, the waterbuck has several other very different chemical constituents in its sebum that are absent in the sebum from ox and buffalo. On the other hand, the ox sebum has similar chemical constituents to the buffalo sebum.

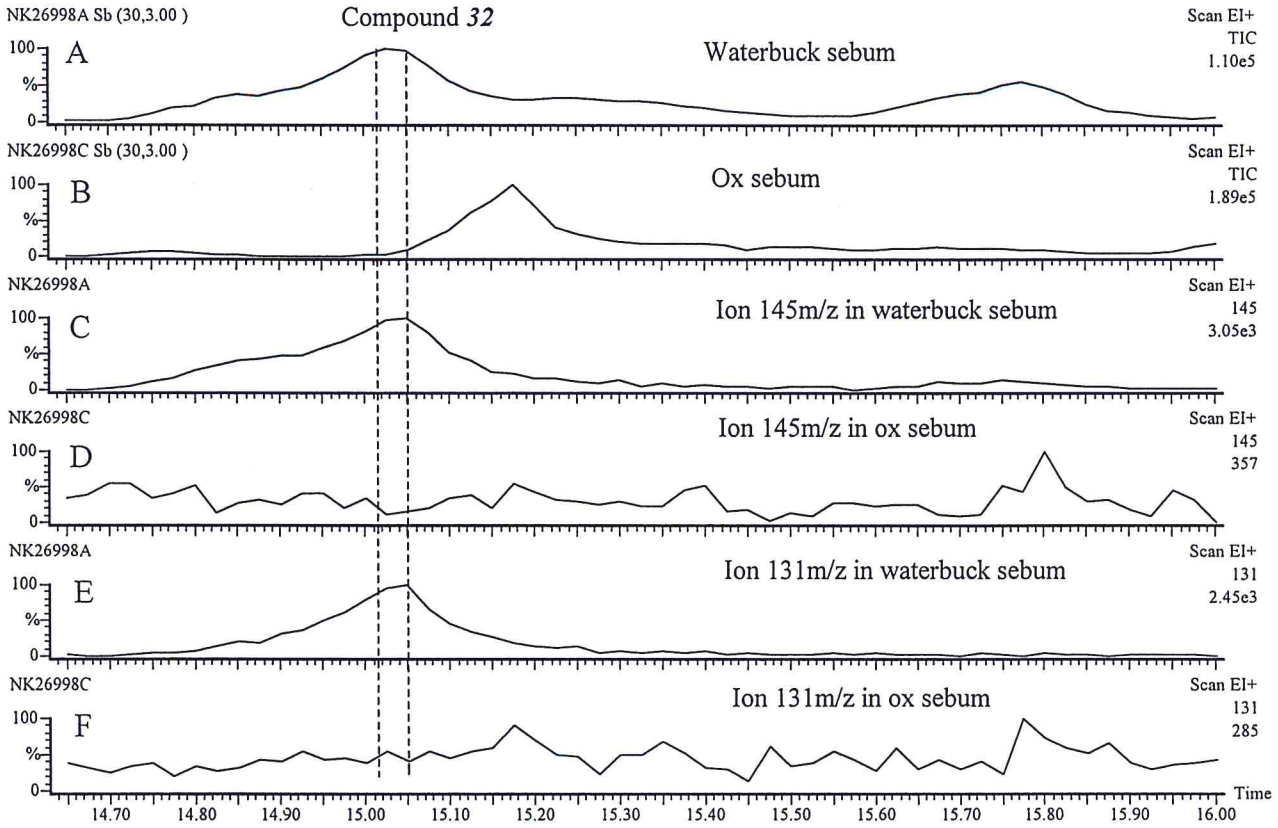


Fig. 18a Distribution of diagnostic fragmentation ions (m/z 131 and 145) of compound *32* in sebum from waterbuck (A,C,E) and ox (B,D,F).

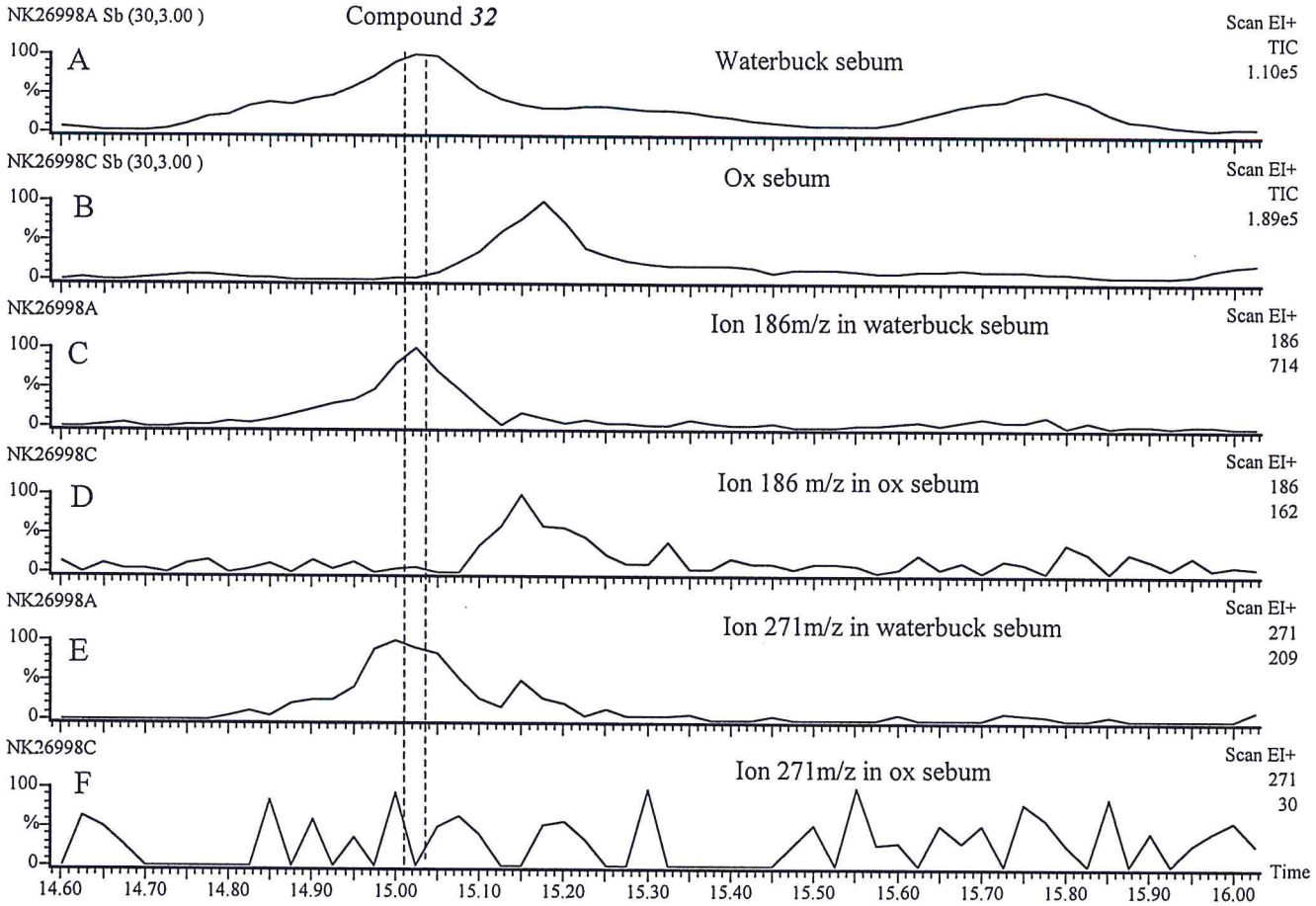


Fig. 18b. Distribution of diagnostic fragmentation ions (m/z 186 and 271) of compound *32* in sebum from waterbuck (A,C,E) and ox (B,D,F).

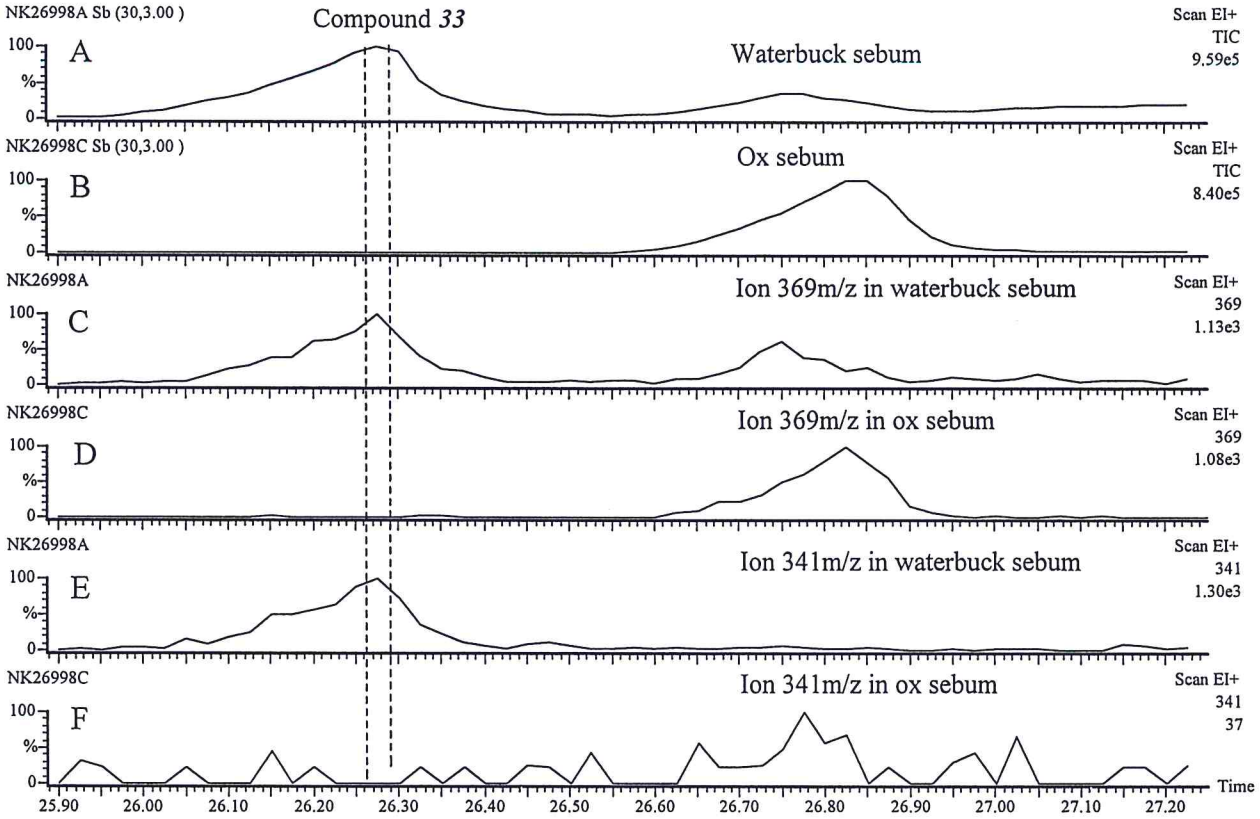


Fig. 18c. Distribution of diagnostic fragmentation ions (m/z 341 and 369) of compound *33* in sebum from waterbuck (A,C,E) and ox (B,D,F).

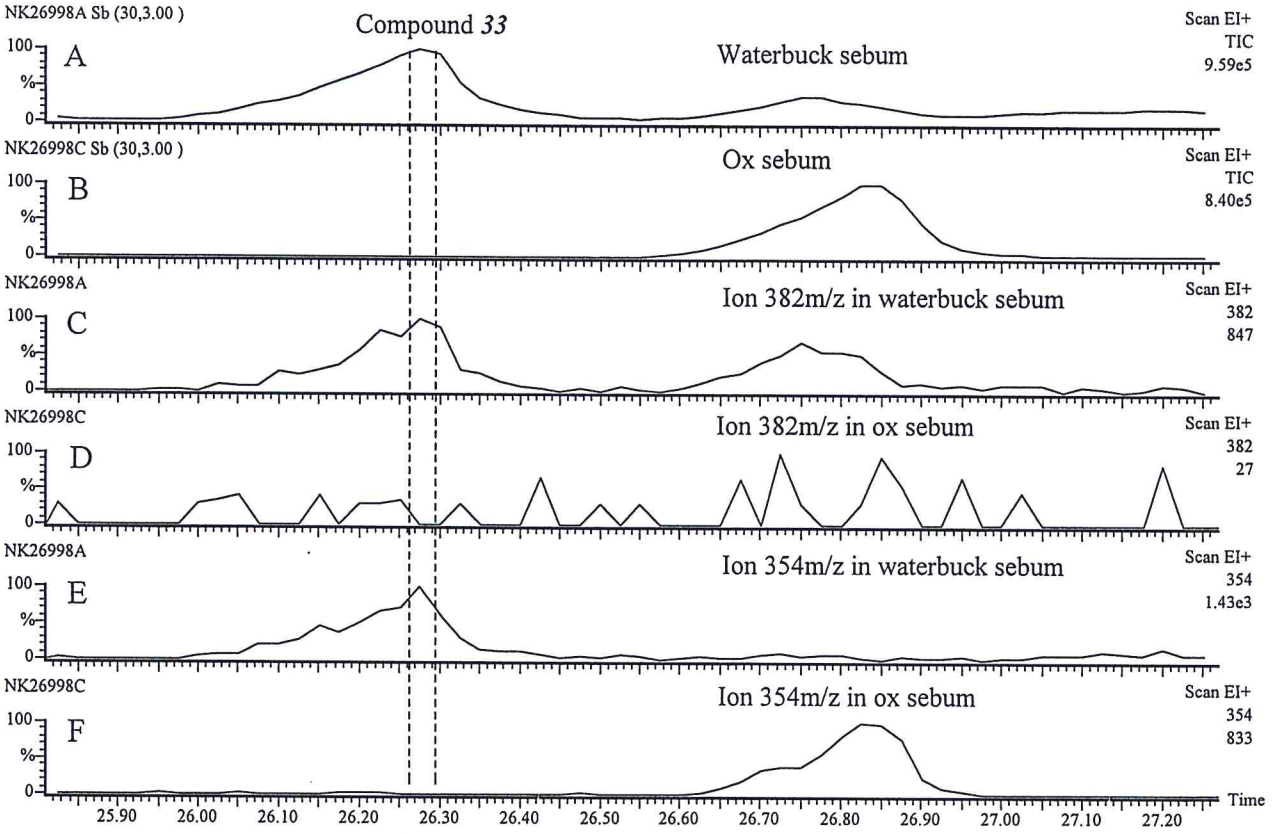


Fig. 18d. Distribution of diagnostic fragmentation ions (m/z 354 and 382) of compound ***33*** in sebum from waterbuck (A,C,E) and ox (B,D,F).

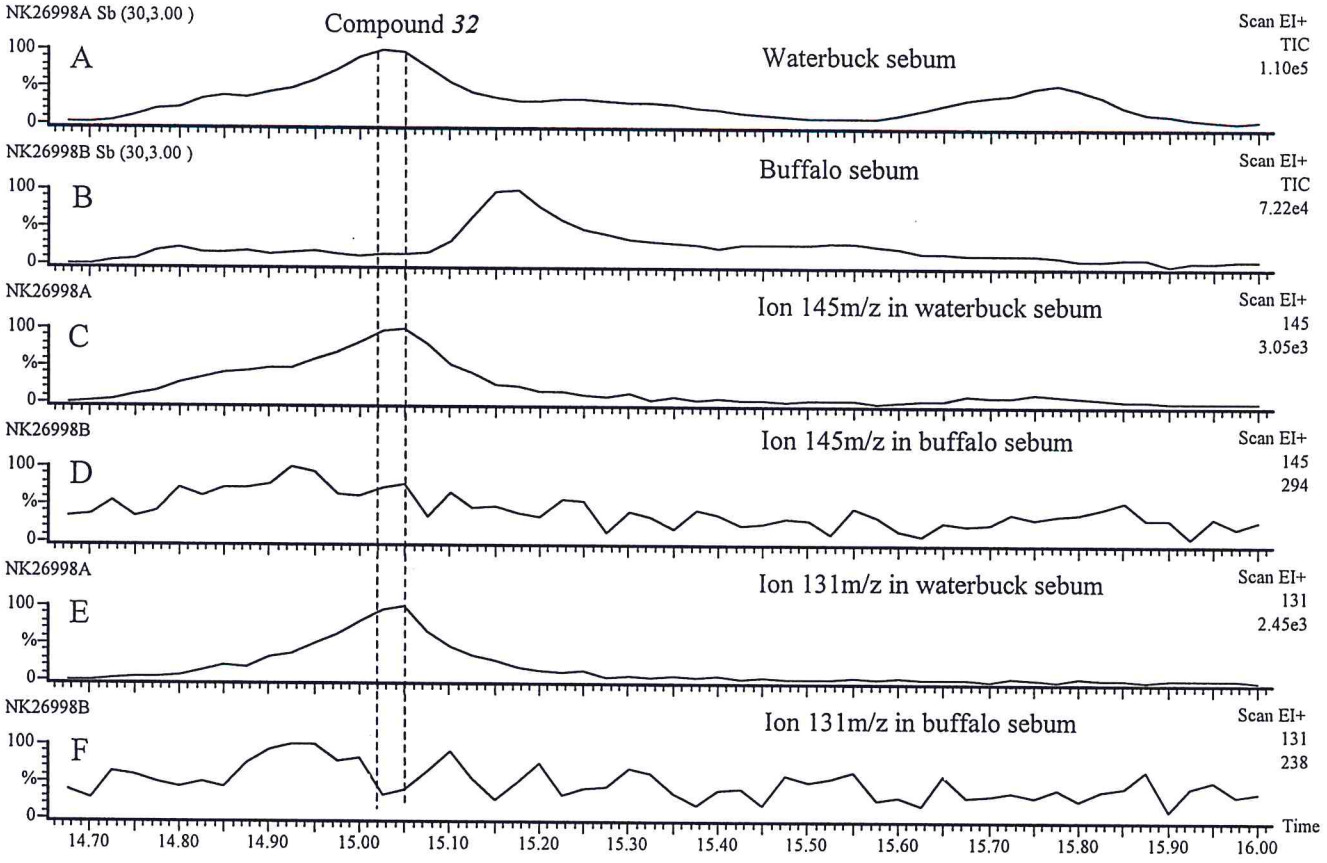


Fig. 19a. Distribution of diagnostic fragmentation ions (m/z 131 and 145) of compound *32* in sebum from waterbuck (A,C,E) and buffalo (B,D,F).

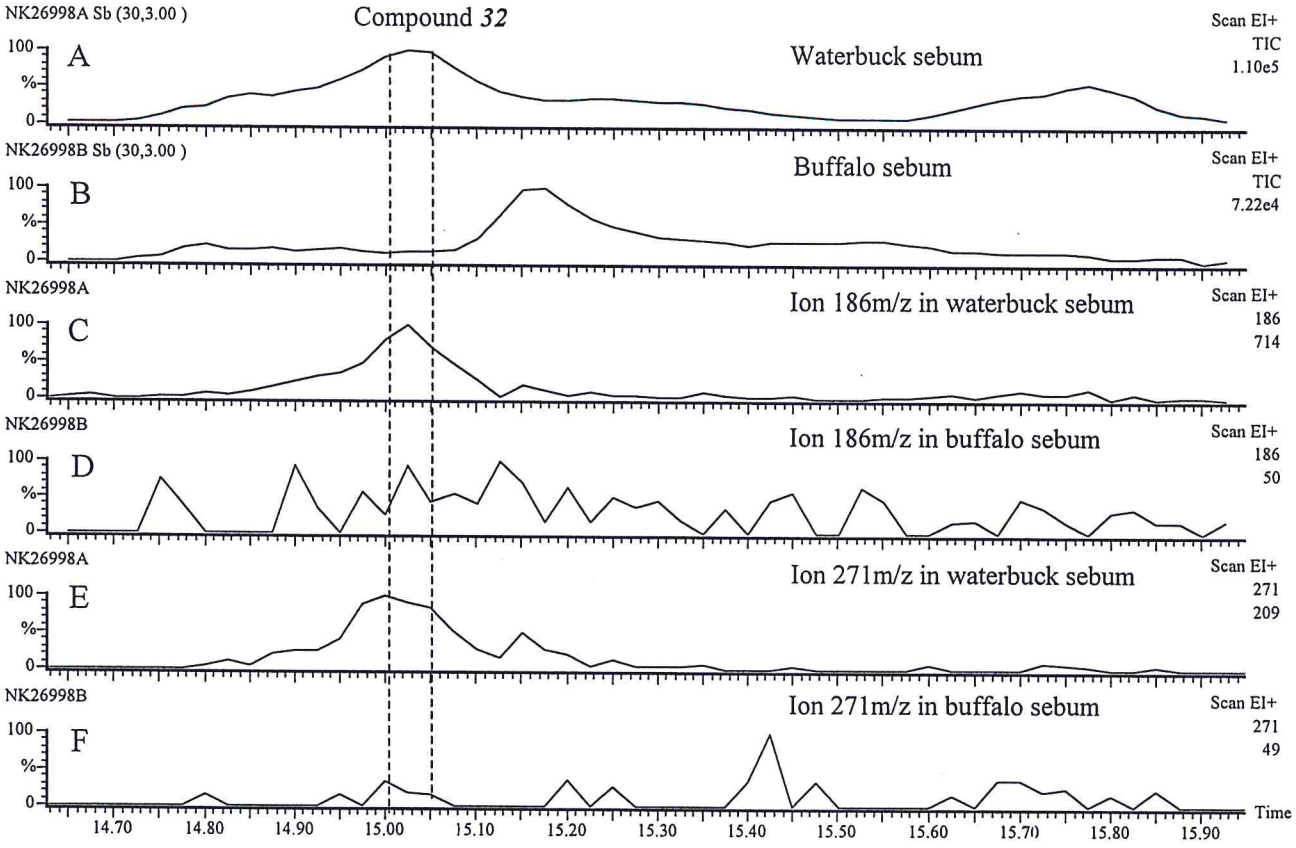


Fig. 19b. Distribution of diagnostic fragmentation ions (m/z 186 and 271) of compound *32* in sebum from waterbuck (A,C,E) and buffalo (B,D,F).

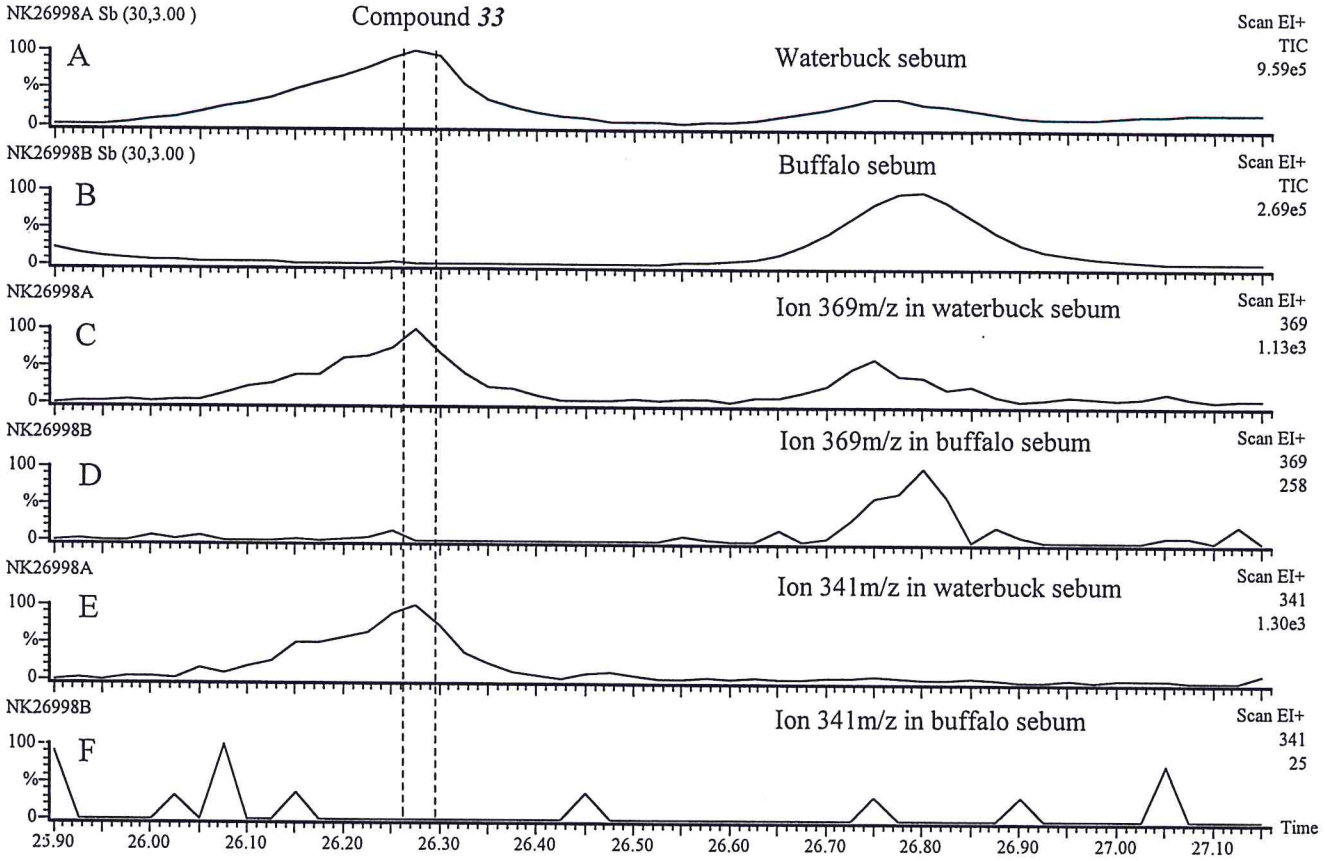


Fig. 19c. Distribution of diagnostic fragmentation ions (m/z 341 and 369) of compound *33* in sebum from waterbuck (A,C,E) and buffalo (B,D,F).

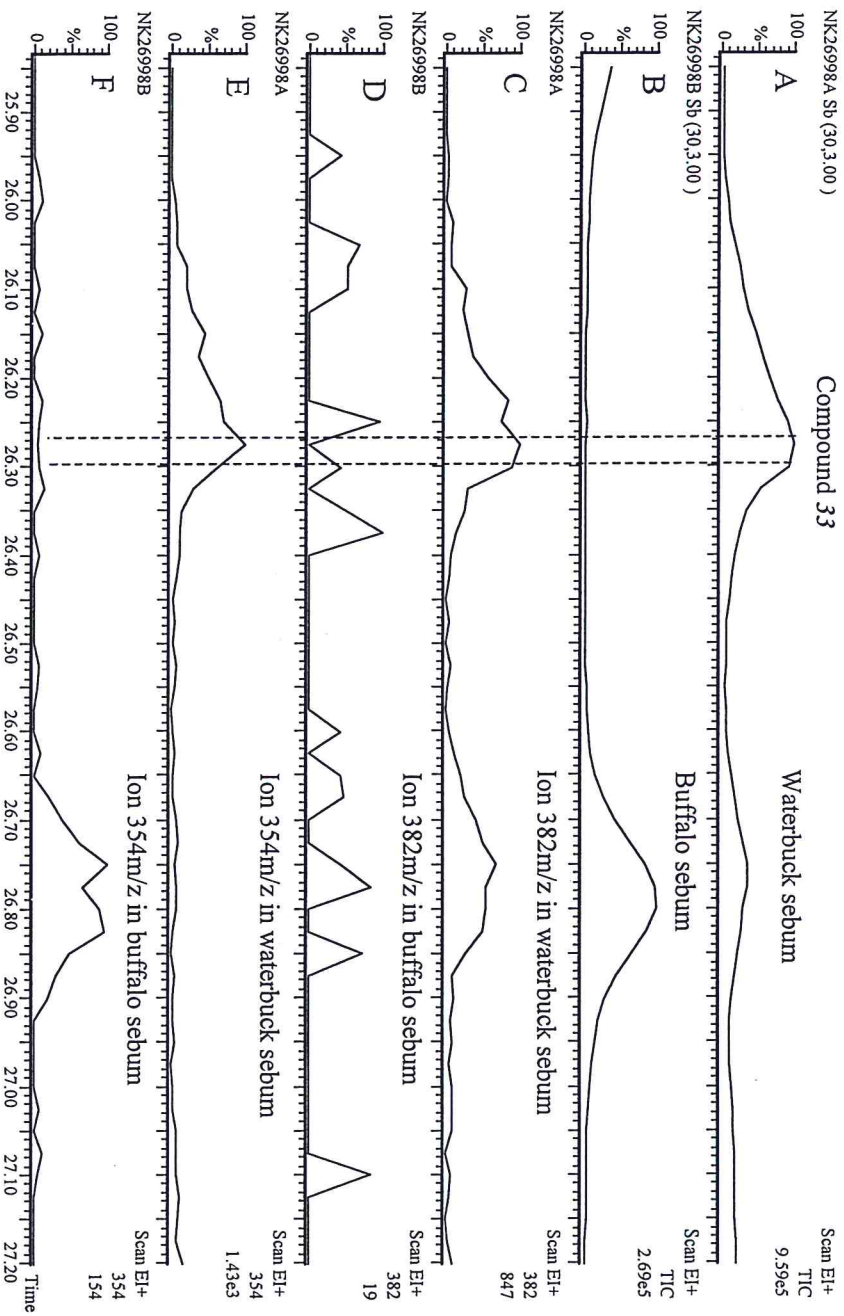


Fig. 19d. Distribution of diagnostic fragmentation ions (m/z 354 and 382) of compound *33* in sebum from waterbuck (A,C,E) and buffalo (B,D,F).

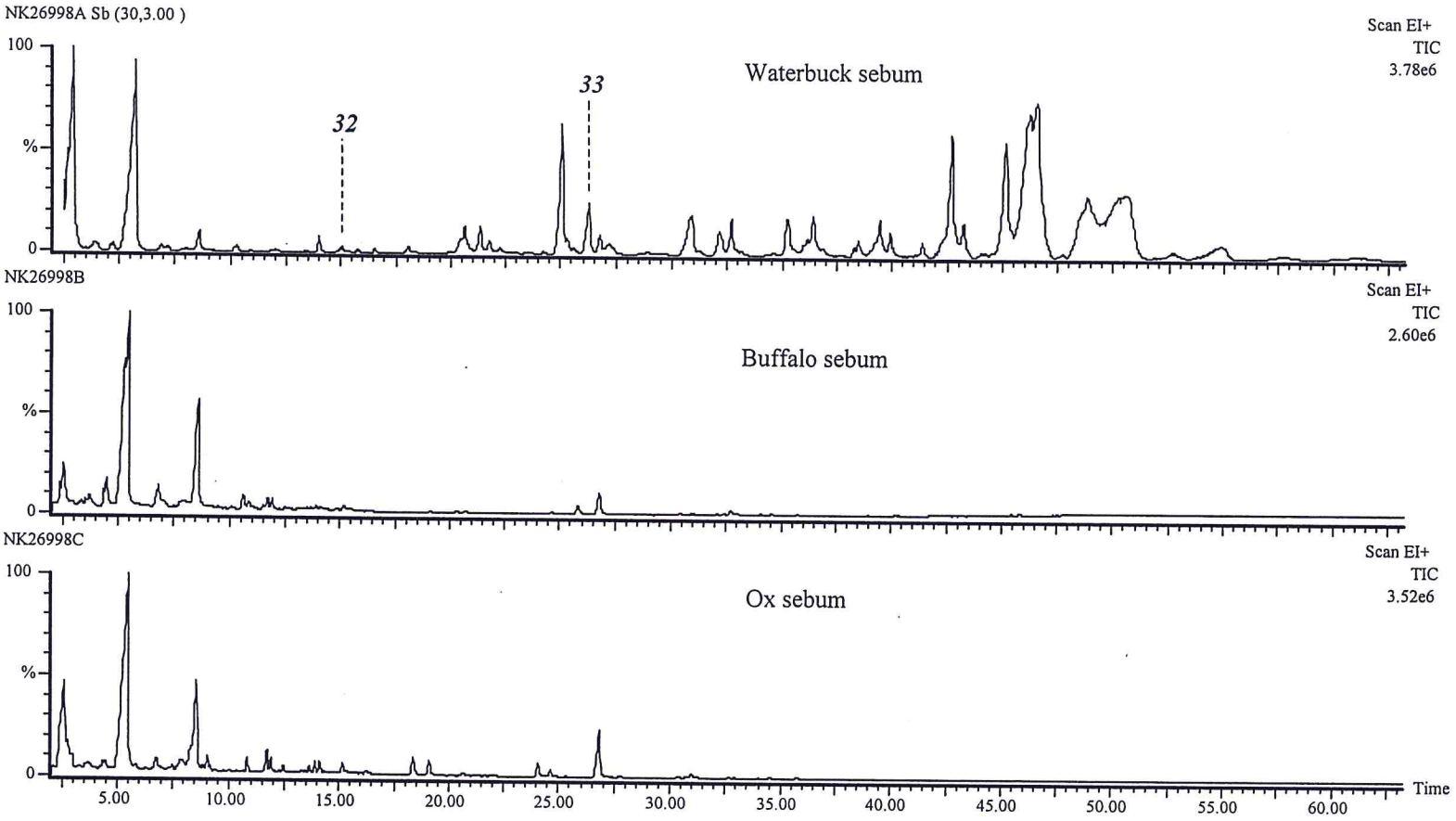


Fig. 20. GC-MS total ion chromatograms of sebum from waterbuck, buffalo and ox showing EAG-active peaks (***32*** and ***33***) on *G. m. morsitans* from the waterbuck sebum.

4.2.3 Volatile compounds from body surface of animals - waterbuck, ox and buffalo.

Odour from the waterbuck body was trapped on various adsorbents including activated charcoal, glass wool and reverse-phase silica (octadecyl bonded silica). GC analyses showed that activated charcoal and reverse-phase silica were equally effective for adsorbing the volatile compounds (volatiles), their GC profiles being similar. However, the relative proportions of some peaks in the two types of profiles were different (Fig. 21; Fig. 22). Subsequent trapping of volatiles from the waterbucks, oxen and buffaloes was carried out on the reverse phase.

Similar GC profiles of reverse phase-trapped volatiles from the waterbuck were obtained from the three body regions: neck, body near front legs and near hind legs. GC profiles of volatiles from three different waterbucks was also similar and did not change significantly over the four years when odour was trapped from them (Fig. 21; Fig. 23; Fig.24). The GC profiles of reverse phase-trapped body volatiles from the buffalo and ox were different from those of volatiles trapped from waterbuck (Fig. 25; Fig. 26).

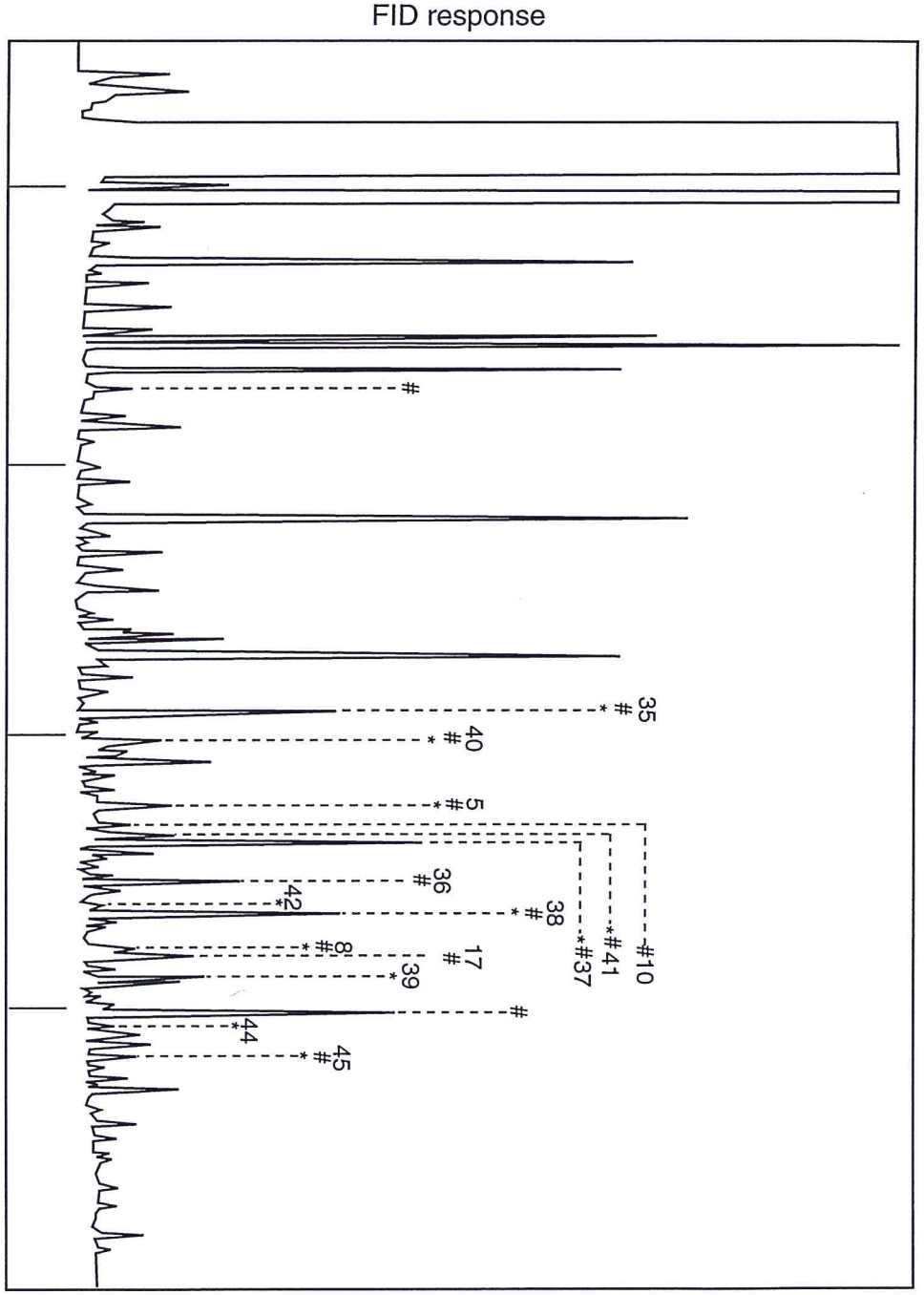


Fig. 21. Gas chromatogram of waterbuck (No. 7333; 1997) body volatiles, trapped on reverse phase showing EAG - active peaks (numbered) on *G. m. morsitans* and #G. *pallidipes*.

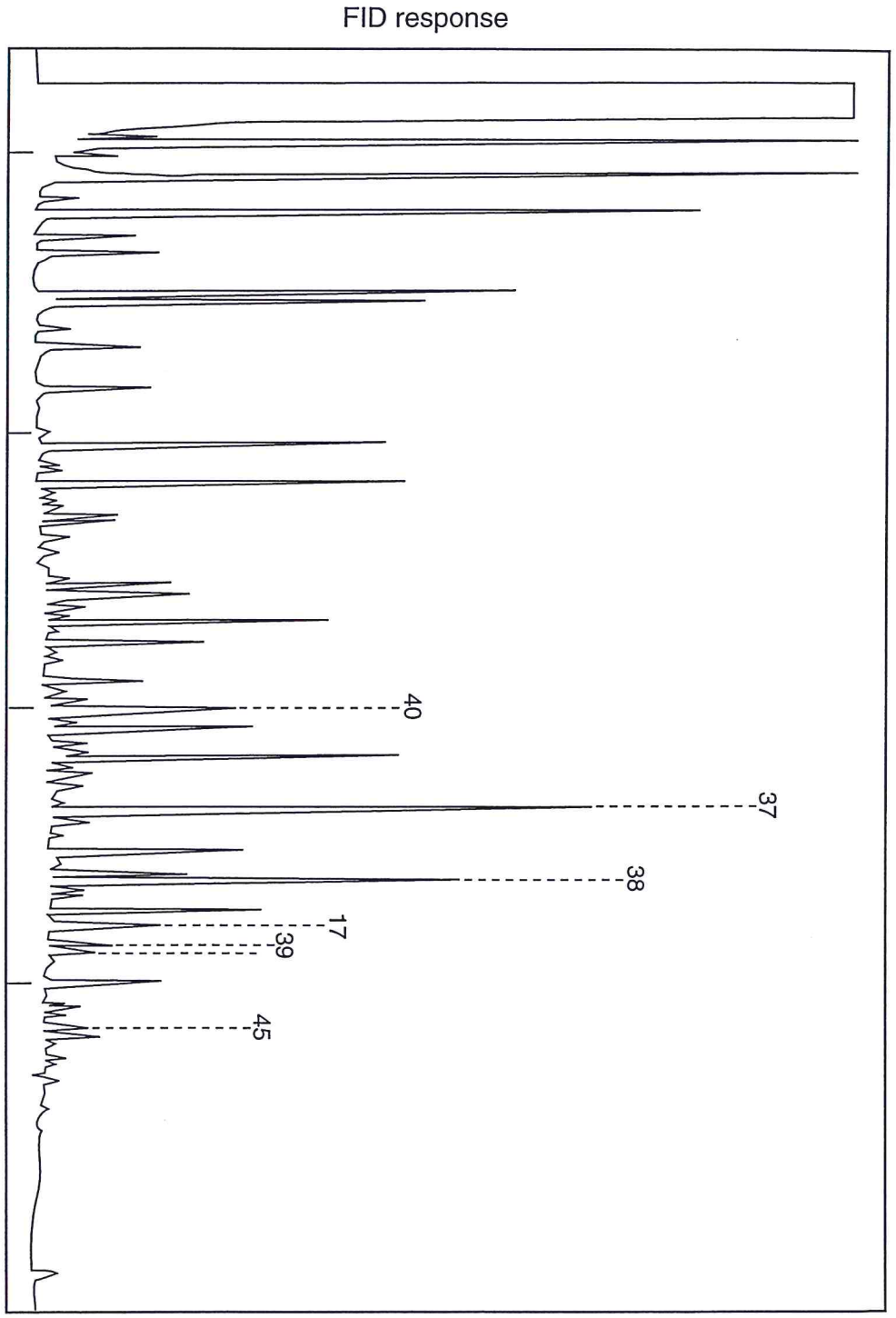


Fig. 22. Gas chromatogram of waterbuck (No. 7333; 1996) body volatiles trapped on activated charcoal showing EAG - active peaks (numbered) on *G. m. morsitans*.

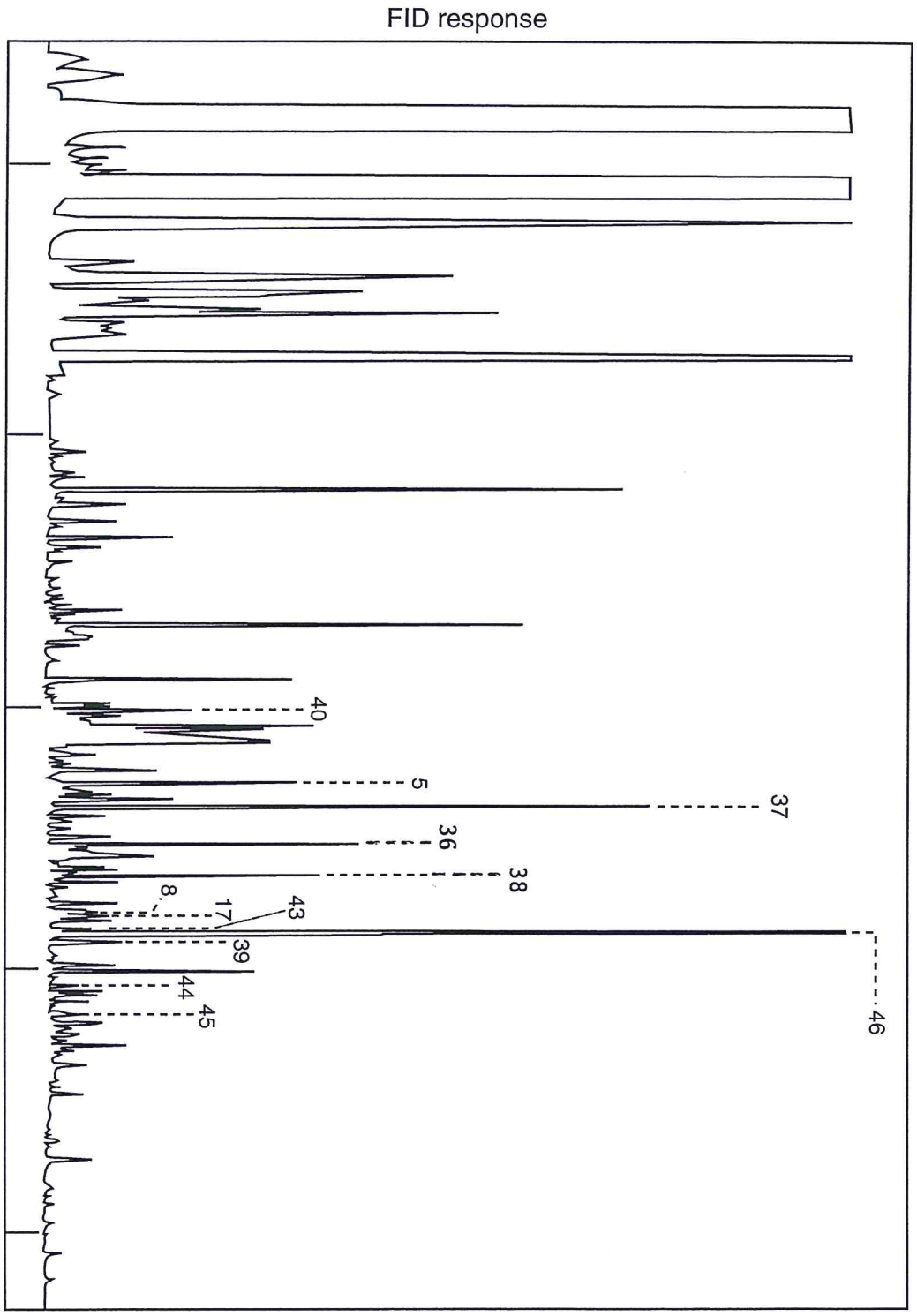


Fig. 23. Gas chromatogram of waterbuck (No. 7510, 1995) body volatiles trapped on reverse phase, showing EAG - active peaks (numbered) on *G. m. morsitans*.

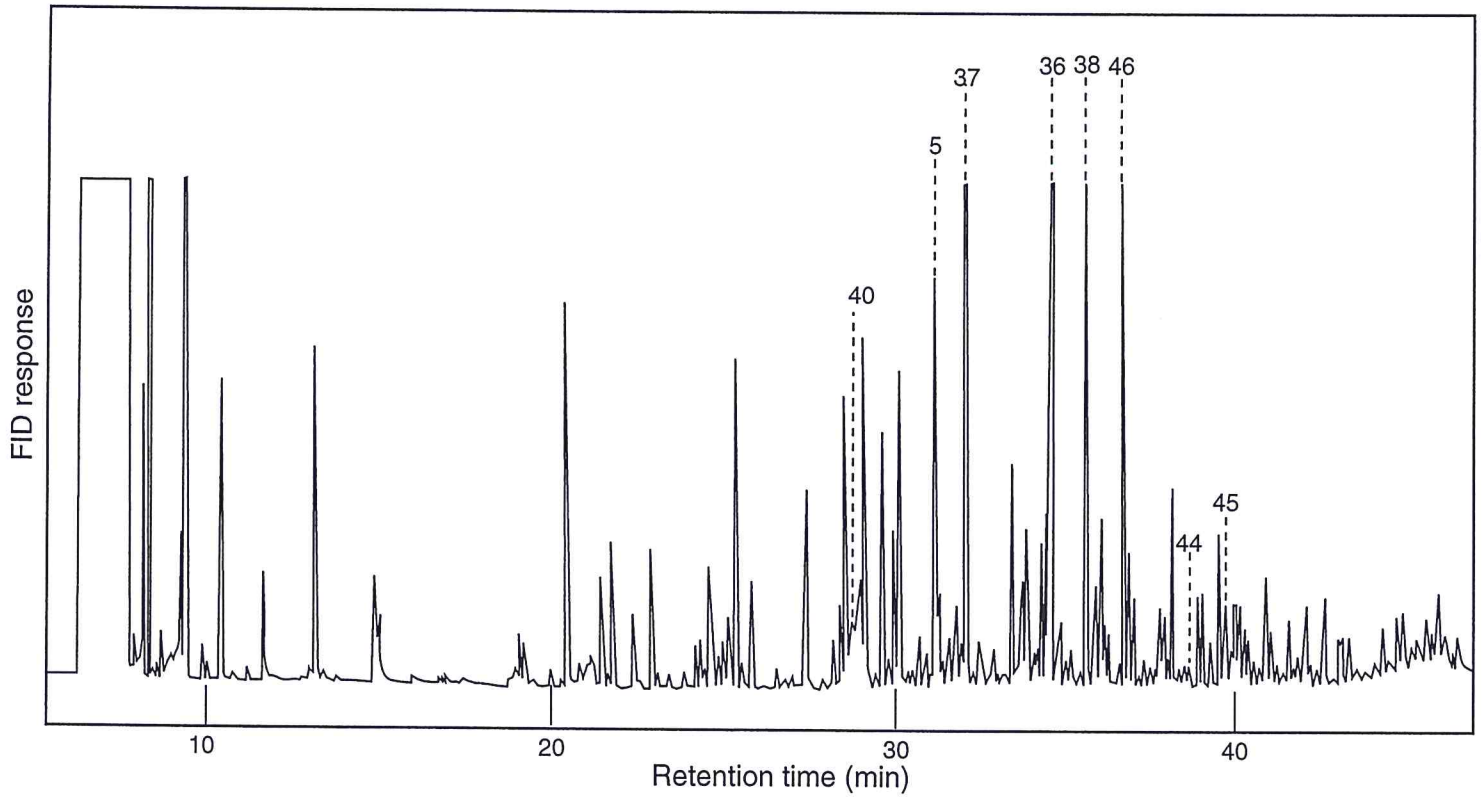


Fig. 24. Gas chromatogram of waterbuck (No. 7766; 1996) body volatiles trapped on reverse phase, showing EAG - active peaks (numbered) on *G. m. morsitans.*

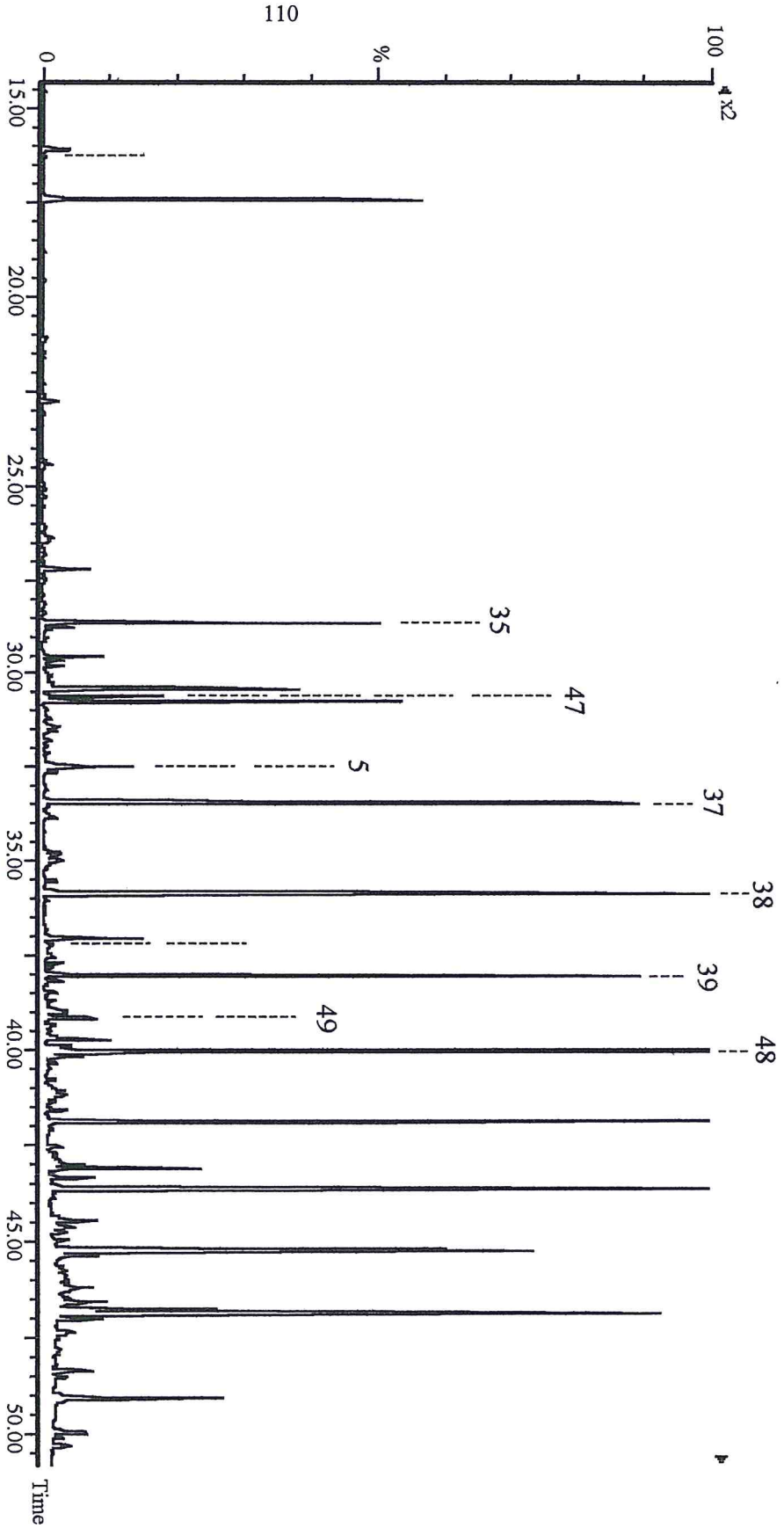


Fig. 25. Gas chromatogram of buffalo body volatiles trapped on reverse phase, showing EAG-Active peaks (numbered) on *G. m. morsitans*.

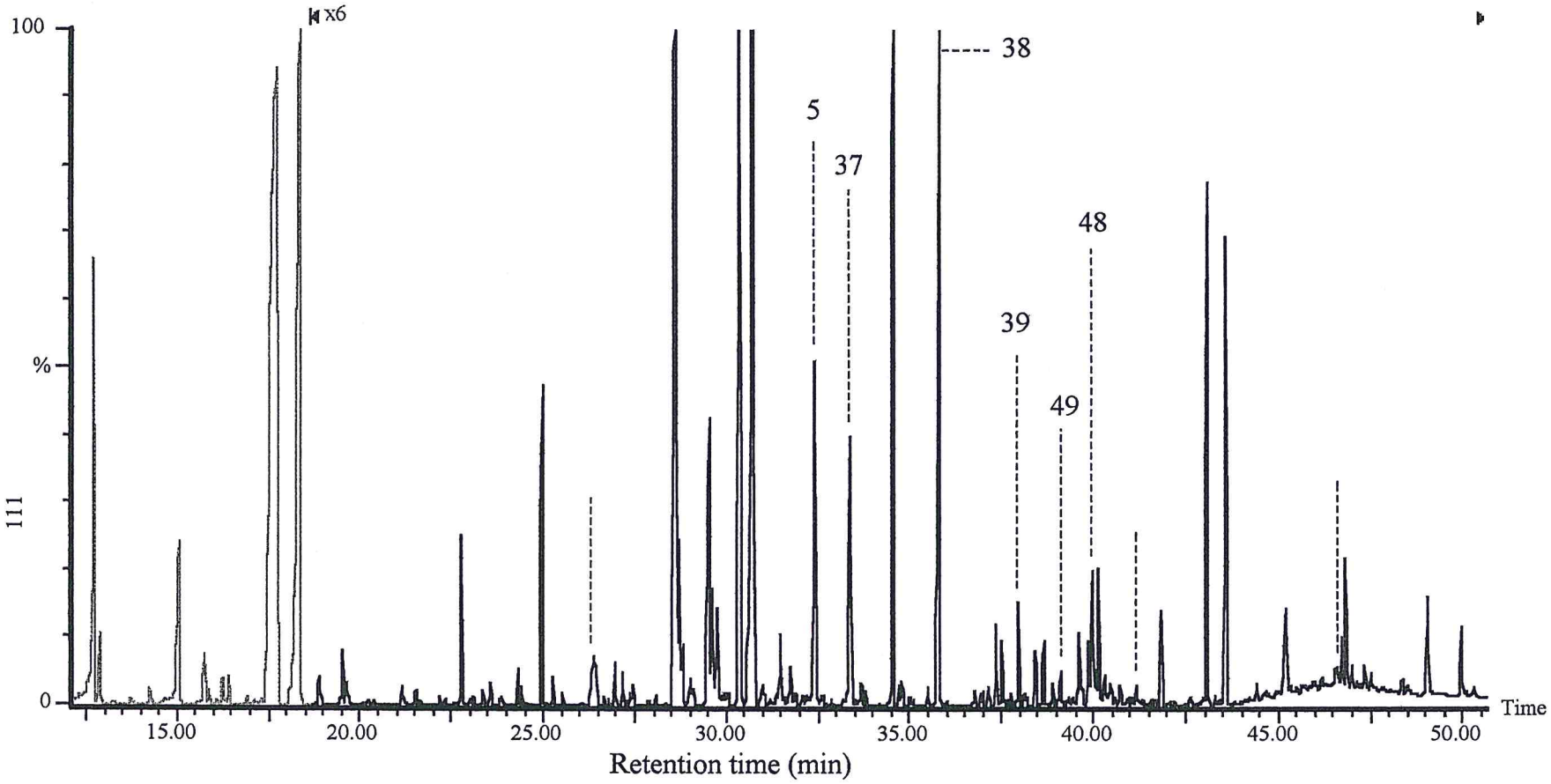


Fig. 26. Gas chromatogram of ox body volatiles trapped on reverse phase, showing EAG-active peaks (numbered) on *G. m. morsitans*.

4.2.4 Olfactory responses of tsetse flies to animal body volatiles.

GC-EAD analyses on waterbuck body volatiles were carried out with insectary-reared *G. m. morsitans* and on both insectary-reared and field-trapped *G. pallidipes*. Fourteen EAG-active peaks on *G. m. morsitans* were detected in the reverse phase and activated charcoal-trapped volatiles (Fig. 27; Fig. 28; Fig. 29). The insectary-reared *G. pallidipes* did not show EAG responses to any of the compounds in the waterbuck volatiles. However, thirteen EAG-active peaks on field-trapped *G. pallidipes* were detected in the reverse phase-trapped volatiles (Fig. 30; Fig. 31). Some of the peaks detected by *G. m. morsitans* antennae were the same as those detected by antennae of *G. pallidipes*. Ten EAG-active peaks on *G. m. morsitans* were detected in the reverse phase-trapped volatiles from the buffalo (Fig. 32) while nine were detected in the reverse phase-trapped volatiles from the ox (Fig. 33).

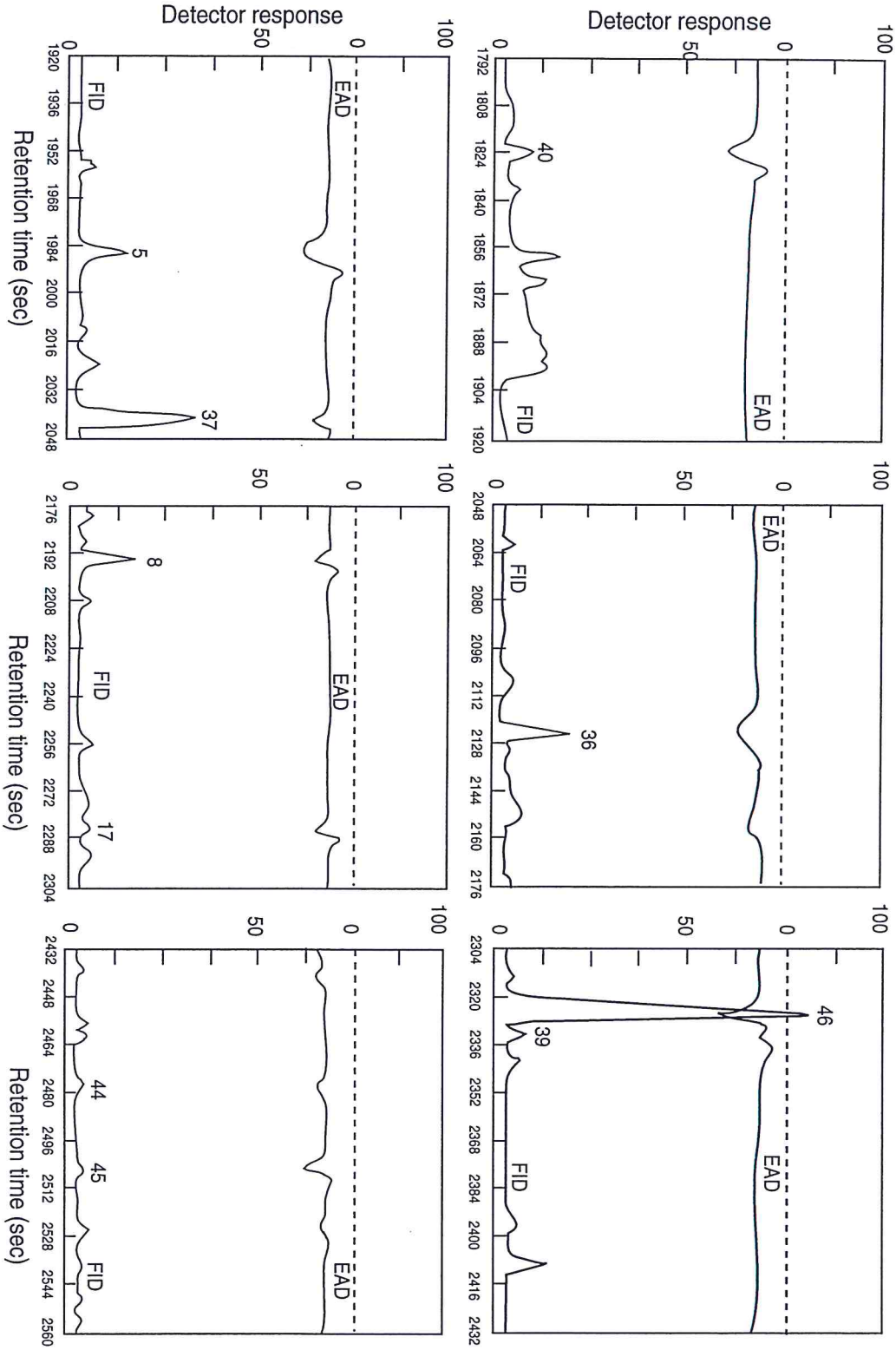


Fig. 27. GC - EAD chromatogram of waterbuck (No. 7510; 1995) body volatiles trapped on reverse phase showing EAG responses from antenna of *G. m. morstans* to compounds denoted by numbered peaks.

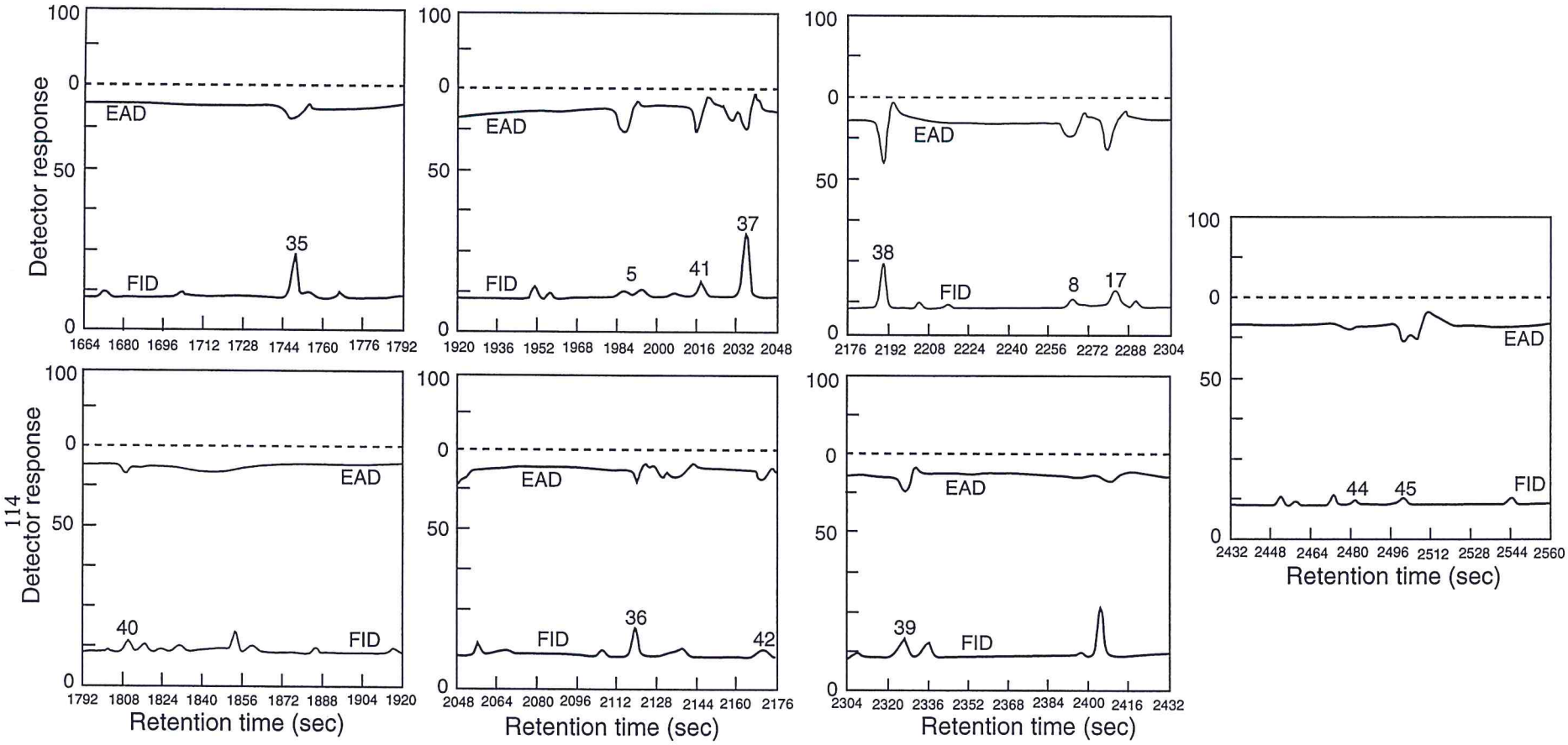


Fig. 28. GC - EAD chromatogram of waterbuck (No. 7333; 1997) body volatiles trapped on reverse phase showing EAG responses from antenna of *G. m. morstans* to compounds denoted by numbered peaks.

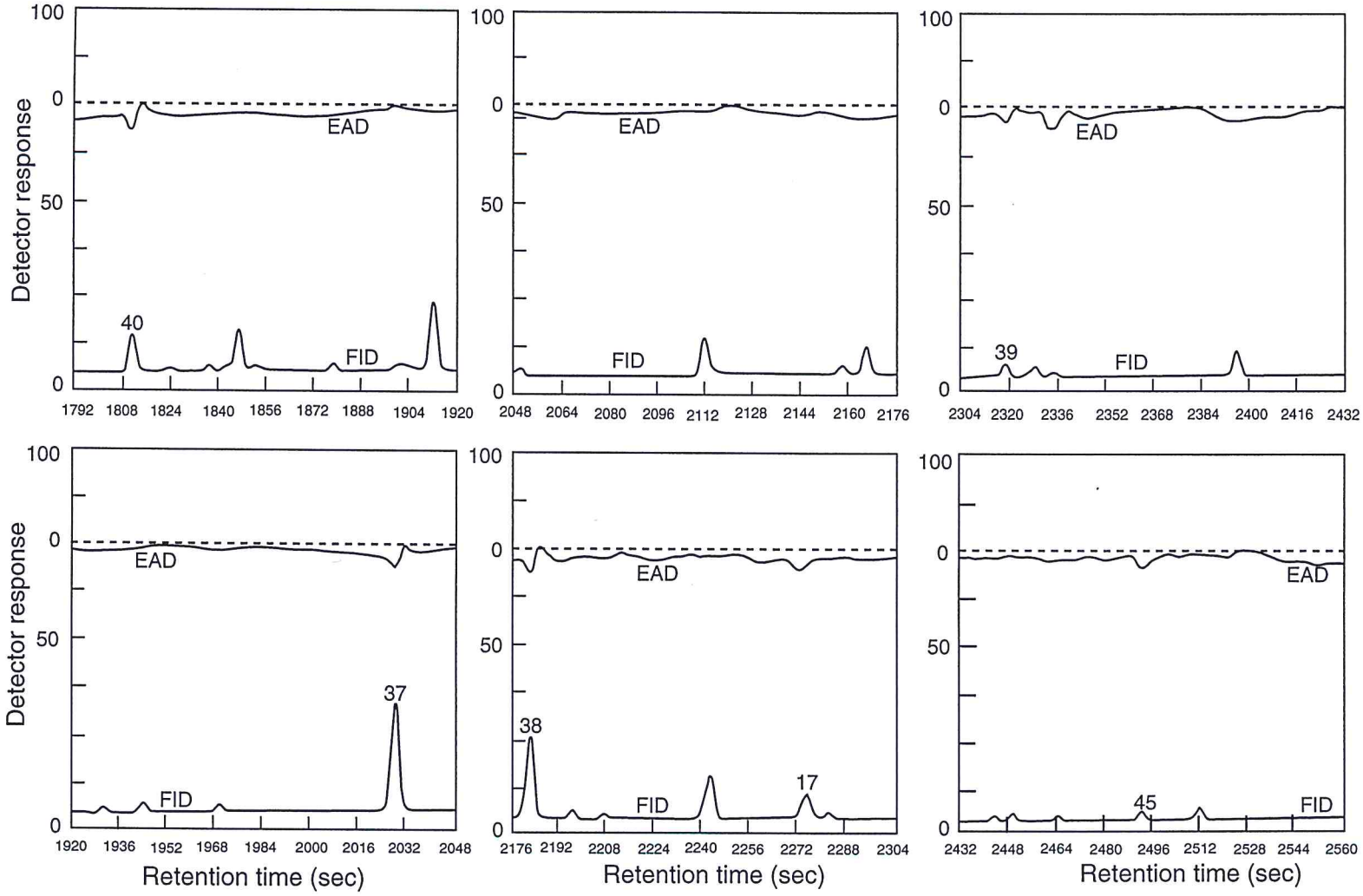


Fig. 29. GC - EAD chromatogram of waterbuck (No. 7333; 1996) body volatiles trapped on activated charcoal showing EAG responses from antenna of *G. m. morsitans* to compounds denoted by numbered peaks

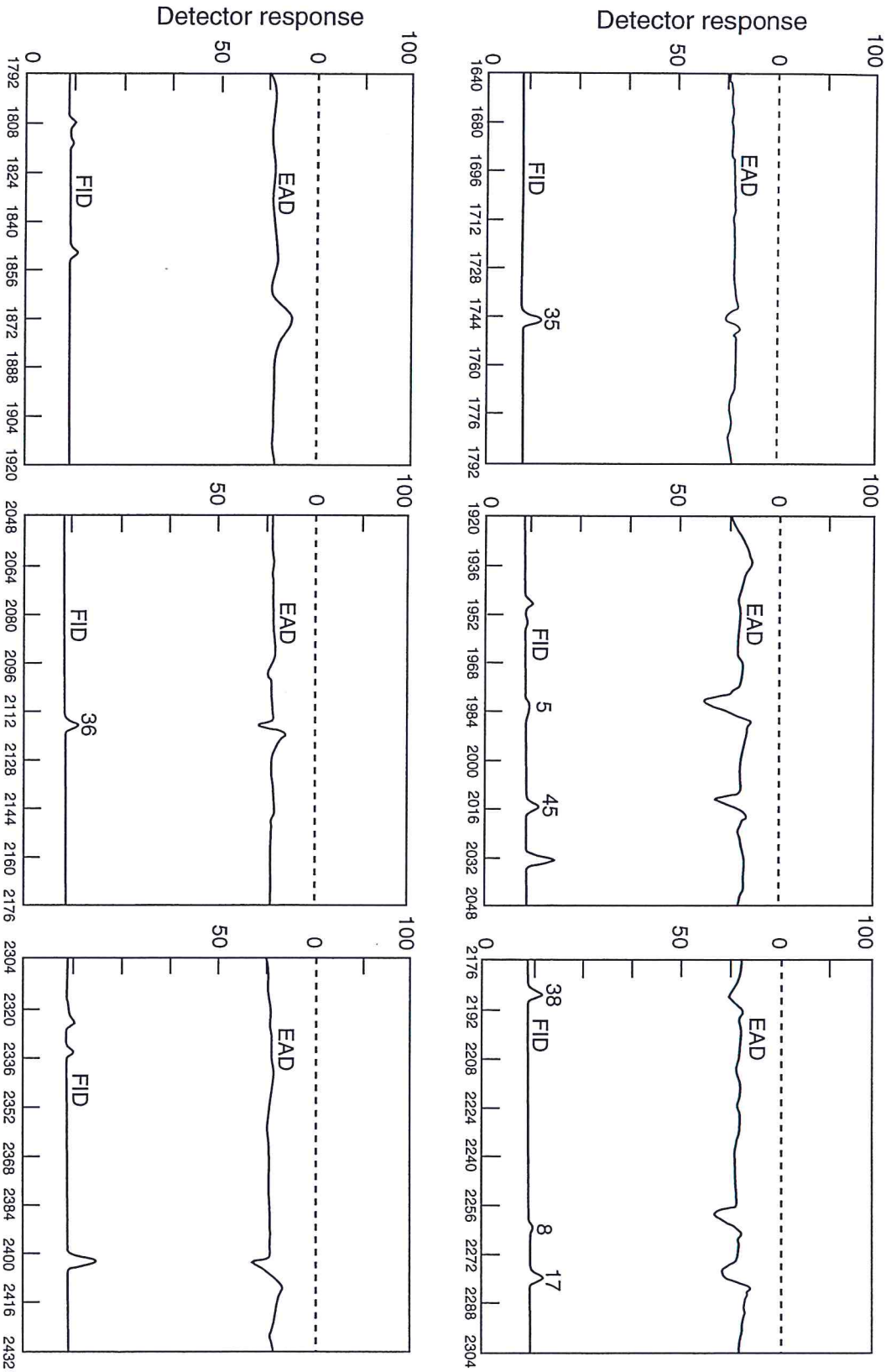


Fig. 30. GC - EAD chromatogram of waterbuck (No. 7333; 1997) body volatiles trapped on reverse phase (higher concentration) showing EAG responses from antenna of *G. pallidipes* to compounds denoted by numbered peaks.

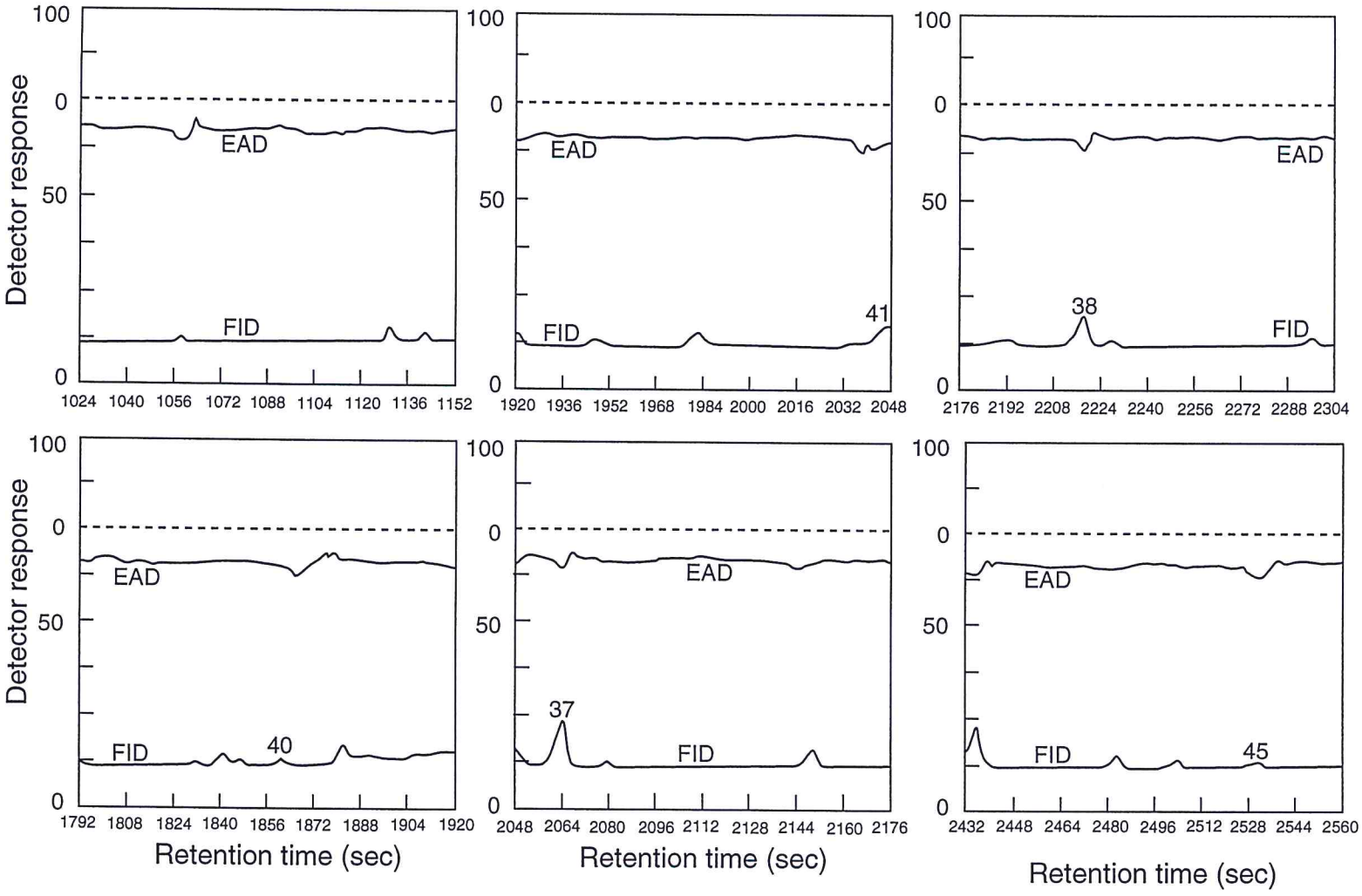


Fig. 31. GC - EAD chromatogram of waterbuck (No. 7333; 1997) body volatiles trapped on reverse phase (lower concentration) showing EAG responses from antenna of *G. pallidipes* to compounds denoted by numbered peaks.

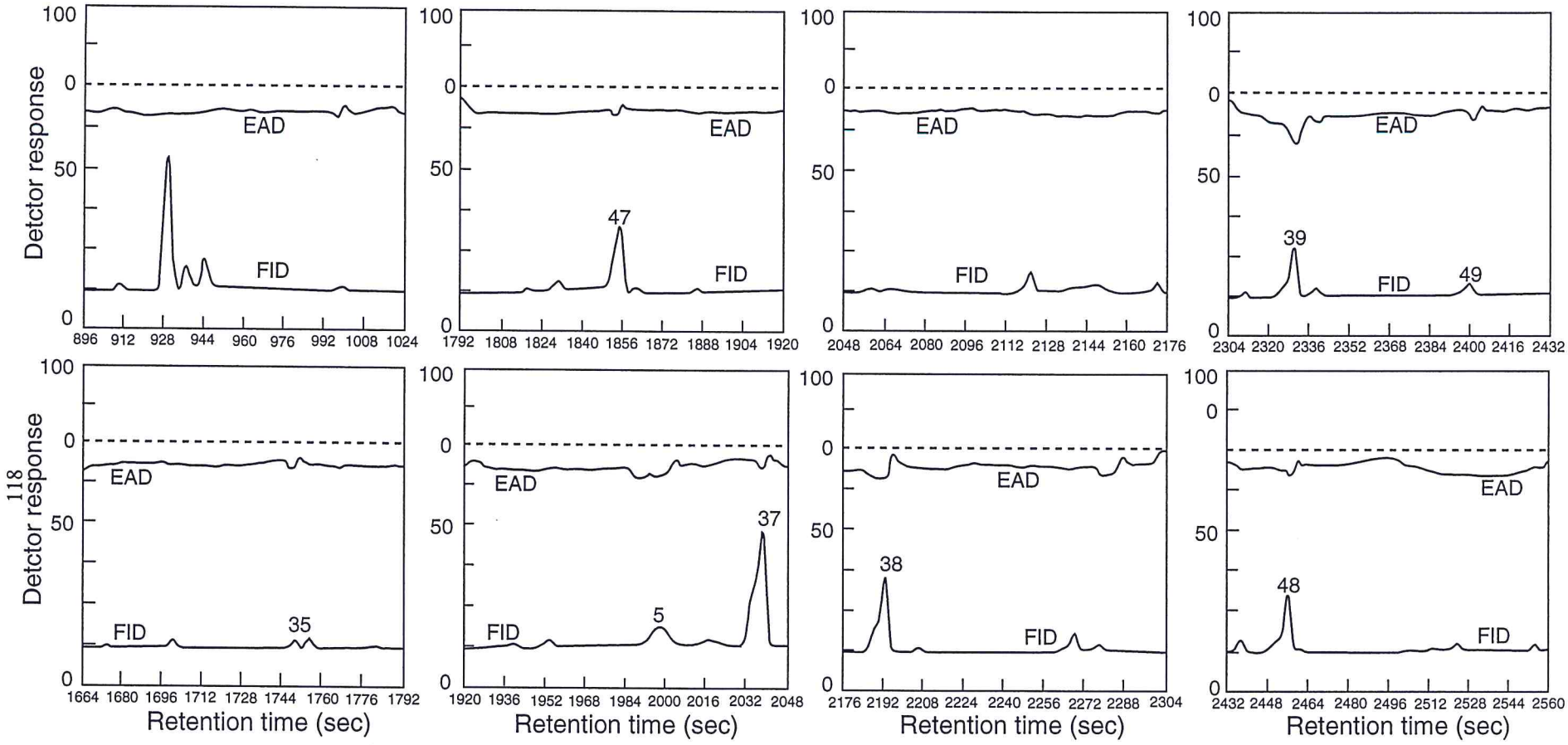


Fig. 32. GC - EAD chromatogram of buffalo body volatiles trapped on reverse phase showing EAG responses from antenna of *G. m. morsitans* to compounds denoted by numbered peaks.

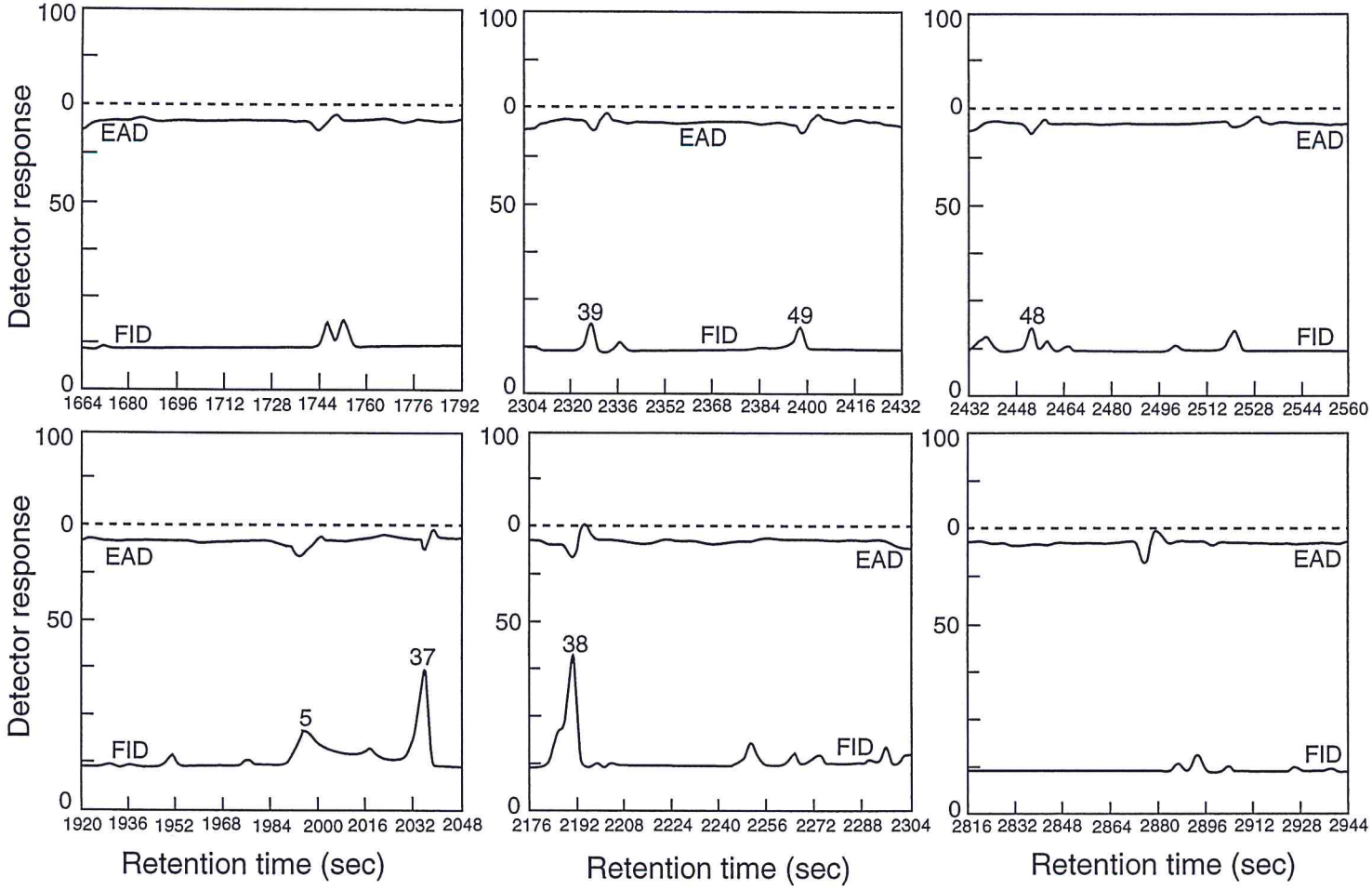


Fig. 33. GC - EAD chromatogram of ox body volatiles trapped on reverse phase showing EAG responses from antenna of *G. m. morsitans* to compounds denoted by numbered peaks.

4.2.5 Identification of EAG-active compounds from animal body volatiles.

The EAG-active compounds were initially identified from their mass spectra by comparing with the NIST and WILEY mass spectral data installed in a computer linked to the GC-MS. An authentic sample of the tentatively identified compound was co-injected with the animal body volatiles into GC for confirmation. If the compound co-eluted with the target peak (identified by peak enhancement), it was authenticated by carrying out GC-EAD tests on *G. m. morsitans* or on *G. pallidipes* and then by GC-MS. If the compound did not co-elute with the target peak or did not elicit an EAG-response on tsetse antenna, it was abandoned and the mass spectrum of the peak reanalysed for outlier fragmentation ions from neighbouring peaks. This was the case for most minor peaks that were EAG-active or those appearing as shoulders in other peaks. If the tentative compound was not available commercially, it was synthesized (see 3.7 above) and subjected to the same tests for authentication. The mass spectra of the identified compounds and those of the corresponding authentic samples are shown in Appendix - 8.1.

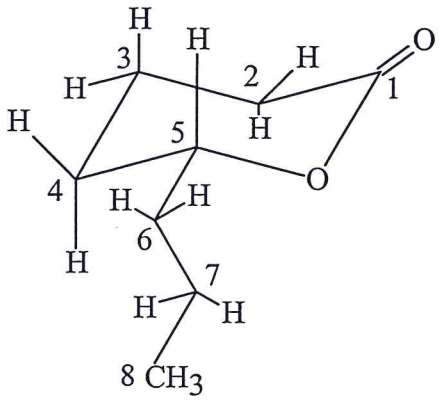
4.2.5.1 Spectral identification of δ-octalactone (*17*).

The structure of compound *17* was confirmed using mass spectrometry (Fig. 34; Scheme-7), ^{1}H and ^{13}C NMR spectrometry. The ^{13}C NMR DEPT (Fig. 35), revealed the presence of one methyl, five methylenes and one methine carbon atoms. The ^{13}C broad band decoupled spectrum (Fig. 36) showed a signal at 172.27 ppm due to the carbonyl carbon of the lactone (Kemp, 1987). Other ^{13}C signals occurred at 80.58 ppm for the

methine carbon (C-5), 38.12 ppm (C-2), 29.70 ppm (C-4), 28.02 ppm (C-6), 18.73 ppm (C-3), 18.41 ppm (C-7) and 14.08 ppm for the methyl carbon (C-8).

The ^{1}H NMR spectrum (Fig. 37) showed an unresolved multiplet at low field, 4.27 ppm (1H) which was assigned to the most deshielded proton, C-5. The lack of resolution in most signals in the ^{1}H NMR spectrum was attributed to the conformation of the molecule, making any pair of protons on one carbon atom unequal in their chemical shifts.

Compound *17*



The signal at 2.50 ppm (2H) was assigned to the second most deshielded protons at C-2. The assignment of the chemical shifts to C-2 and C-5 is consistent with that of δ-lactone, which gives these shifts at 2.27 ppm and 4.06 ppm respectively (Silverstein *et al.*, 1981). The signal at 0.92 (triplet, J = 7.0Hz, 3H) was assigned to the protons in the methyl carbon (C-8) observed in ^{13}C NMR DEPT.

The ^{1}H - ^{1}H correlation spectrum (COSY) (Fig. 38), shows a cross peak 'a' which was attributed to the interaction between C-8 and C-7 protons. The multiplet at around 1.49 ppm was partly assigned to C-7 protons. The COSY spectrum depicted a cross peak 'b' due to the interaction between C-2 and C-3 protons and another cross peak 'c' due to further interaction between C-3 and C-4 protons. The C-4 protons

interact with the C-5 proton giving cross peak 'd', and C-5 proton further interacts with C-6 protons giving cross peak 'e'. The signal at about 1.87 ppm (2H) was assigned to C-3 protons, 1.67 ppm to C-6 protons while the multiplet at about 1.50 ppm assigned to both C-4 and C-7 protons. The region between 1.38 - 1.72 ppm in the ^{1}H NMR spectrum integrated for 6H belonging equally to C-4, C-6 and C7.

These results, together with GC co-injection with the waterbuck body volatiles and GC-EAD analysis on antenna of *G. m. morsitans* confirmed the structure of the synthesized δ-octalactone (*17*).

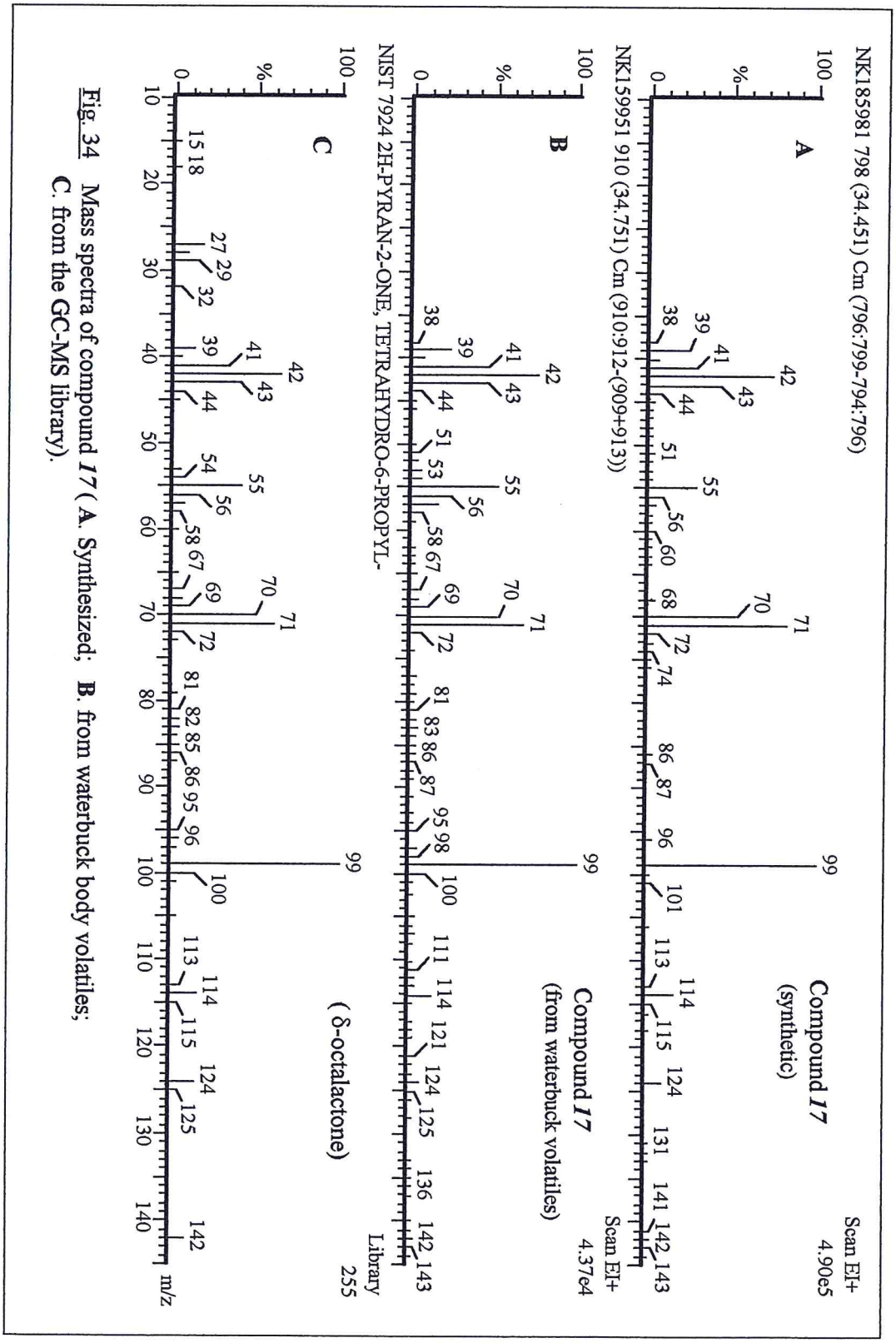
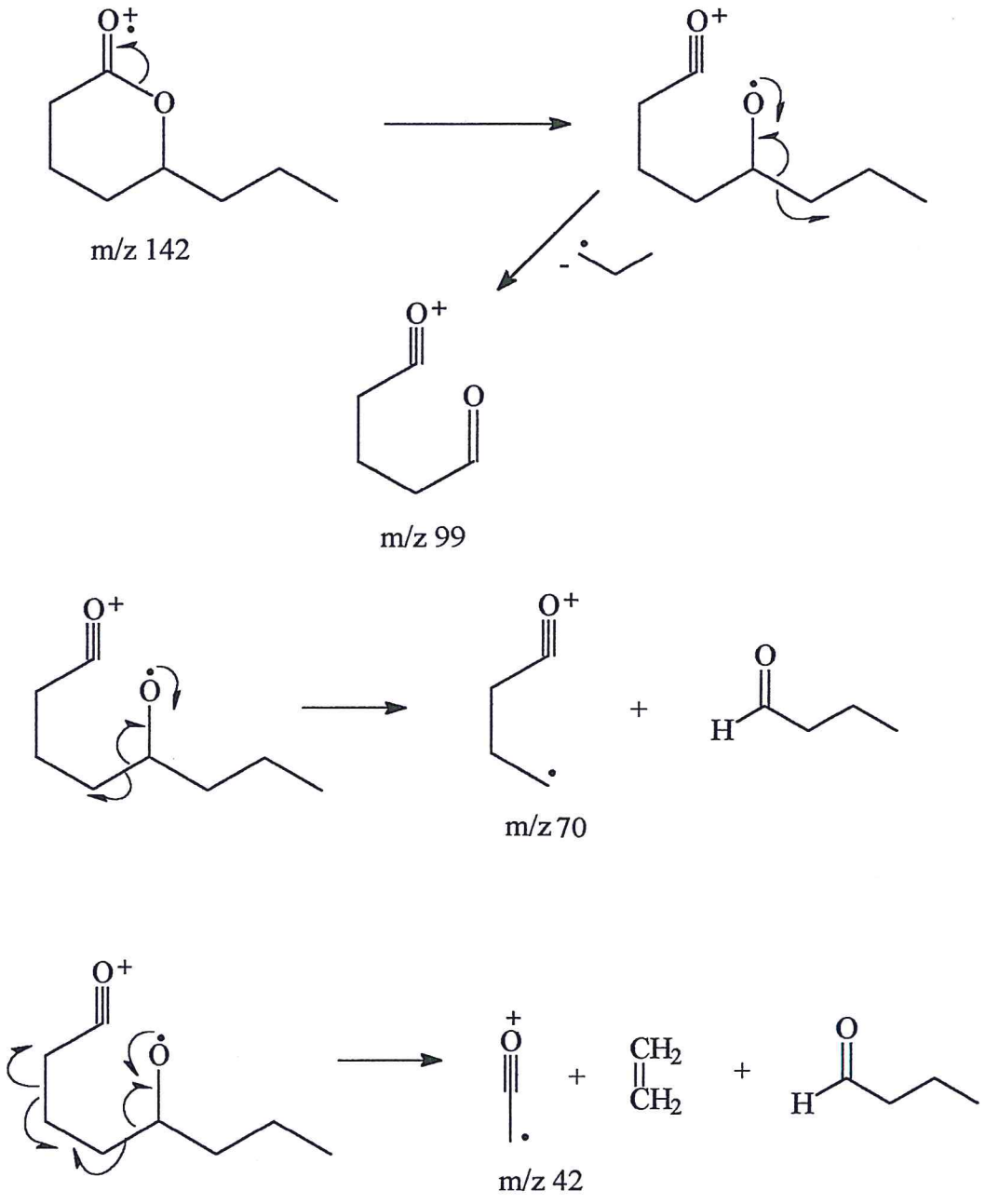
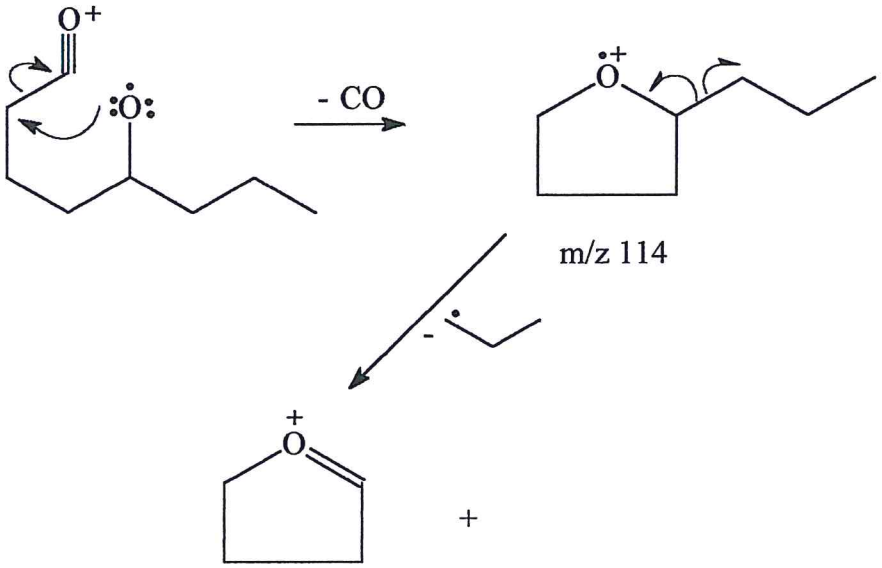
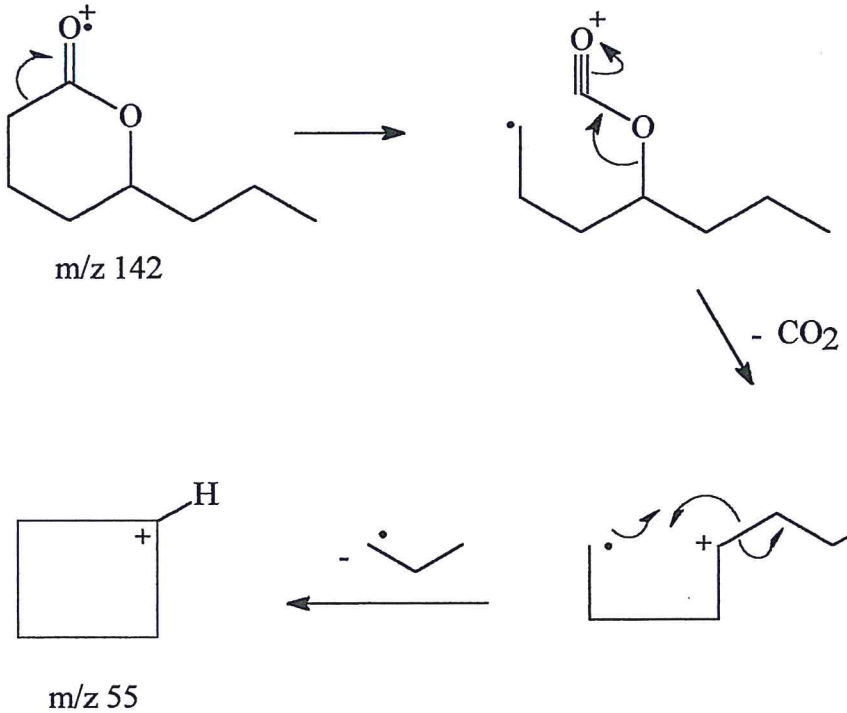


Fig. 34 Mass spectra of compound *17* (**A**. Synthesized; **B**. from waterbuck body volatiles; **C**. from the GC-MS library).



Scheme 7. Proposed fragmentation pattern of compound *17*.


- CO
m/z 114
m/z 71


m/z 142
- CO2
m/z 55

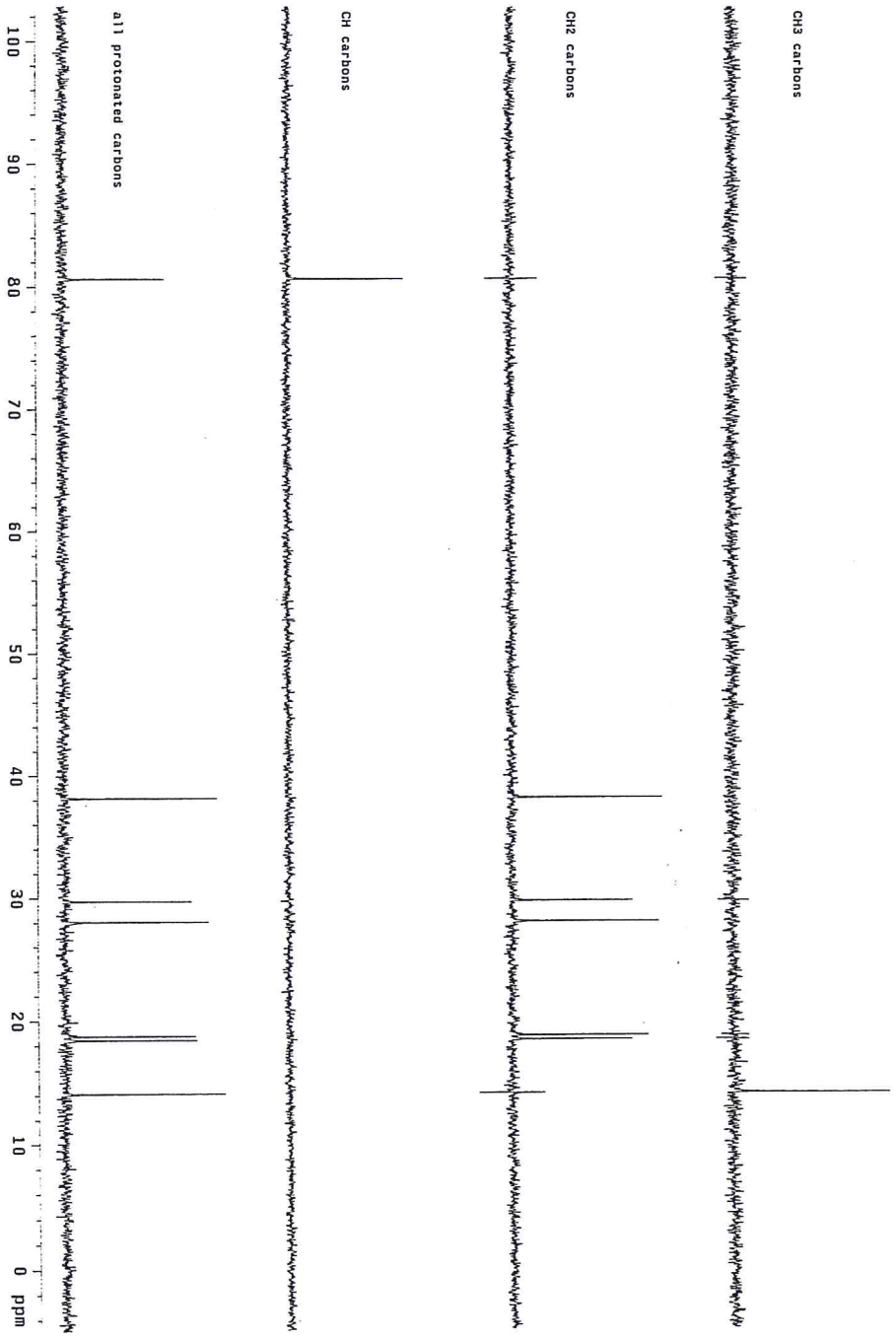


Fig. 35. ^{13}C NMR (DEPT) spectrum of compound *17*

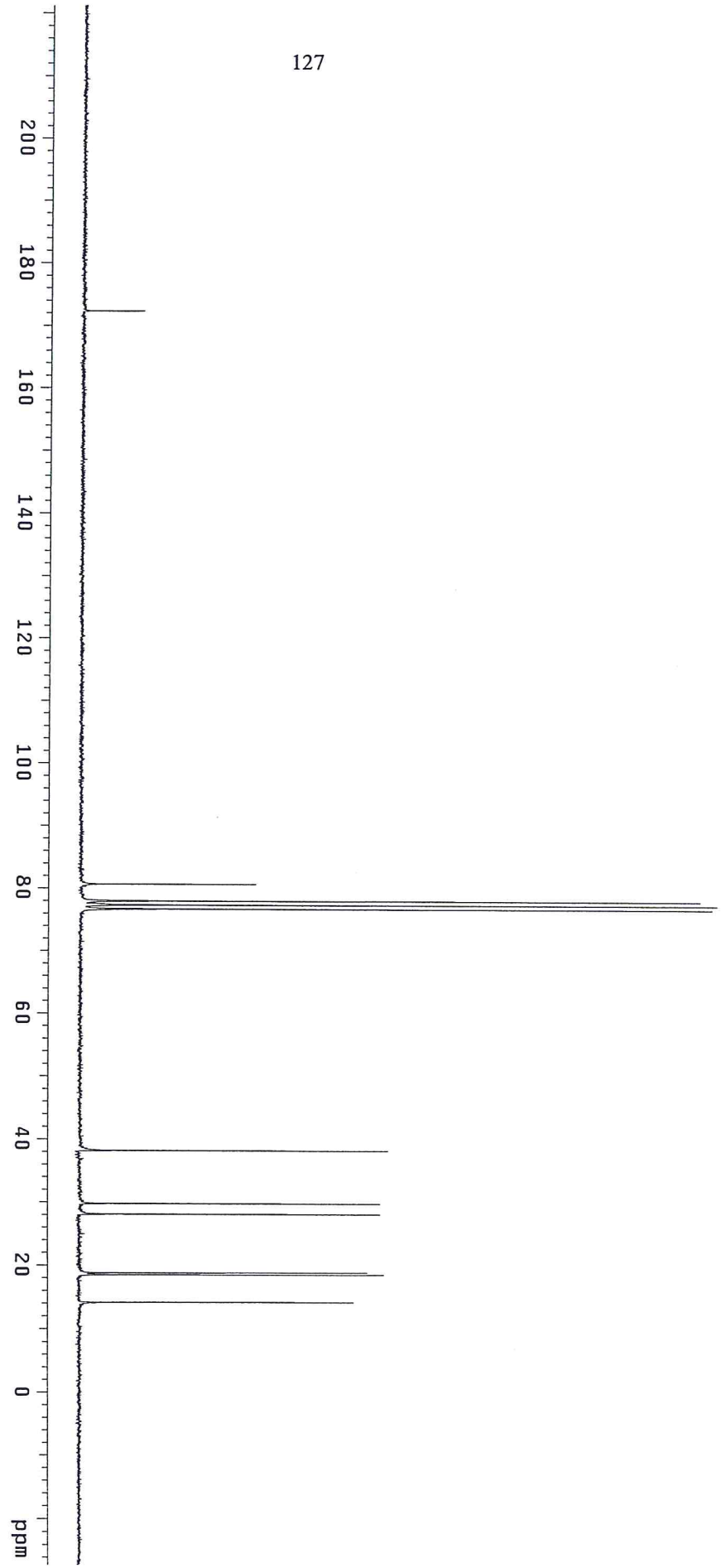


Fig. 36 ^{13}C NMR spectrum of compound *17*

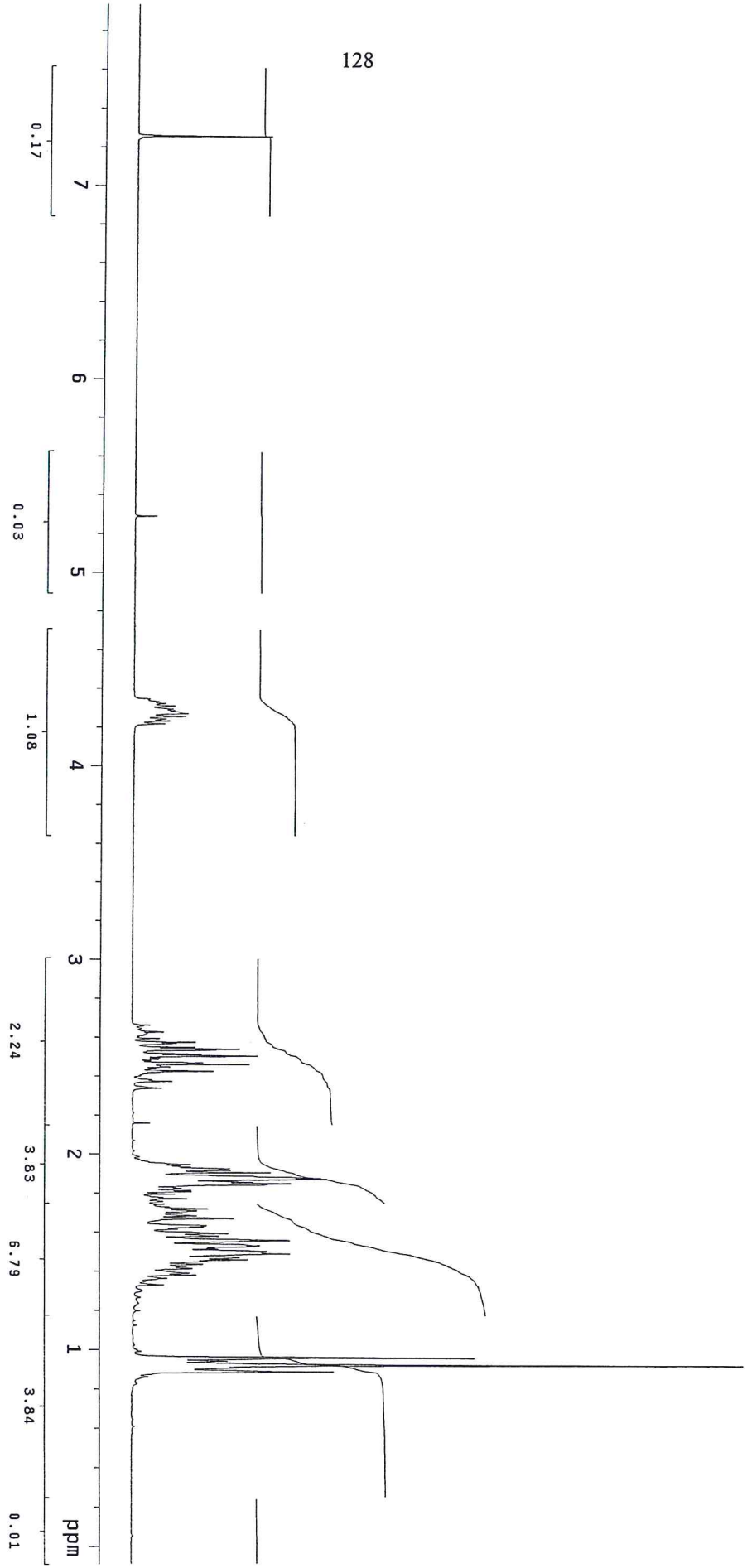


Fig. 37 ^{1}H NMR spectrum of compound *17*

SYN-A5-COSY

Pulse Sequence: COSY
Solvent: CDCl3
Ambient temperature
File: COSY
Mercury-200 "uonnmr200"

PULSE SEQUENCE: COSY
Relax. delay 1.000 sec
Acq. time 0.160 sec
Width 3200.9 Hz
2D Width 3200.9 Hz
2 repetitions
128 increments
OBSERVE H1, 200.0557687 MHz
DATA PROCESSING
Sq. sine bell 0.080 sec
F1 DATA PROCESSING
Sq. sine bell 0.040 sec
FT size 1024 x 1024
Total time 6 min, 36 sec

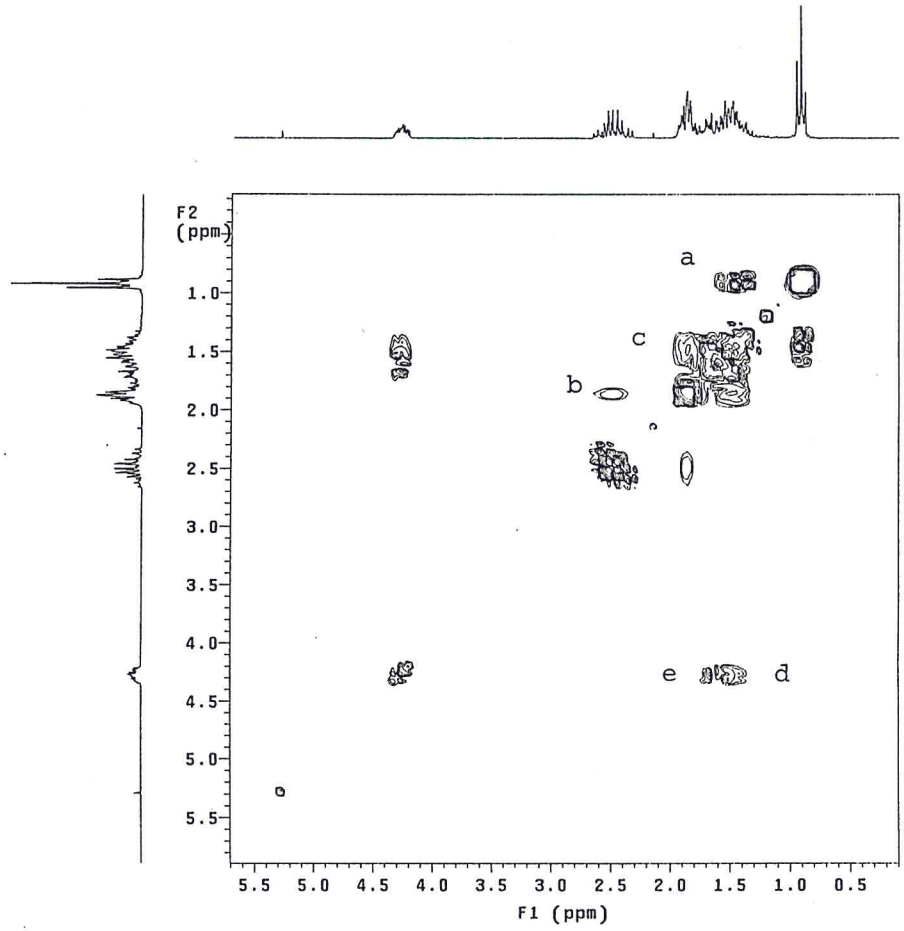


Fig. 38 ^{1}H NMR (COSY) spectrum of compound *17*

4.2.6 EAG-active compounds from waterbuck body volatiles on *G. m. morsitans* and *G. pallidipes*.

All the fourteen peaks associated with waterbuck volatiles that were EAG-active with respect to *G. m. morsitans* were identified and authenticated. Eleven EAG-active peaks on *G. pallidipes* detected in the waterbuck volatiles were also identified and authenticated. The *G. m. morsitans* active compounds were identified as: (E)-2-heptenal (***35***), nonanal (***37***), decanal (***38***), undecanal (***39***), 2-octanone (***40***), 2-nonanone (***41***), 2-decanone (***42***), 2-undecanone (***43***), 2-dodecanone (***44***), (E)-6,10-dimethyl-5,9-undecadien-2-one (***45***), 4-methylphenol (***5***), 3-n-propylphenol (***8***), δ-octalactone (***17***), and 3-isopropyl-6-methylphenol (***46***). The compounds identified for *G. pallidipes* were: (E)-2-heptenal (***35***), (E)-2-nonenal (***36***), nonanal (***37***), decanal (***38***), 2-octanone (***40***), 2-nonanone (***41***), (E)-6,10-dimethyl-5,9-undecadien-2-one (***45***), 4-methylphenol (***5***), 3-n-propylphenol (***8***), 2-methoxyphenol (***10***), and δ-octalactone (***17***) [Fig.39]. The compounds belong to four chemical categories: aldehyde, ketone, phenol and lactone. Out of the EAG-active compounds, nine were detected by both *G. m. morsitans* and *G. pallidipes*.

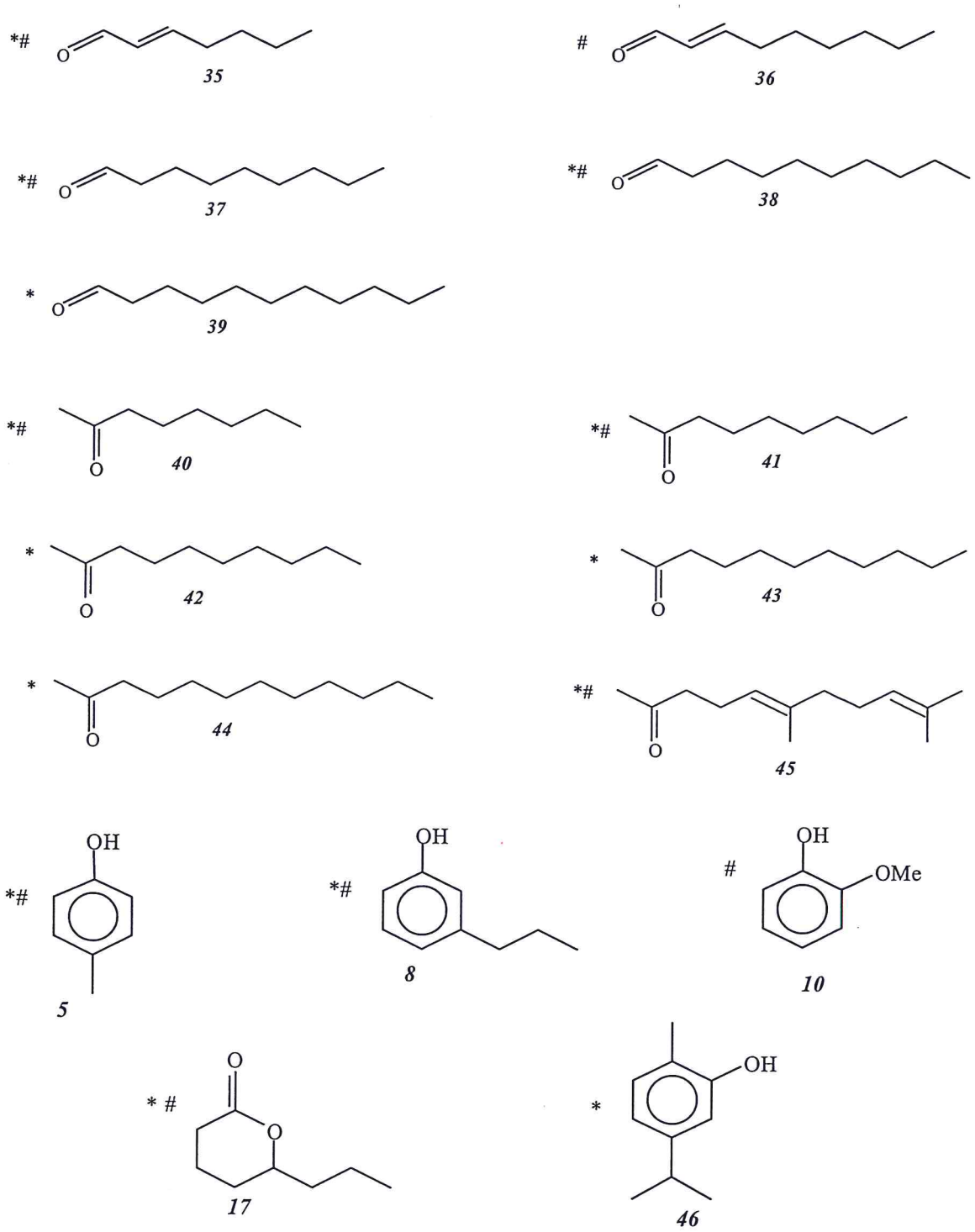


Fig.39 EAG-active compounds from waterbuck body volatiles on antenae of:
* *G.m. morsitans* and # *G. pallidipes*

4.2.7 EAG-active compounds from body volatiles of buffalo and ox on *G. m. morsitans*.

Eight of the ten EAG-active peaks on *G. m. morsitans* in buffalo body volatiles were identified and authenticated as: (E)-2-heptenal (*35*), nonanal (*37*), decanal (*38*), undecanal (*39*), 4-methylphenol (*5*), octanal (*47*), dodecanal (*48*) and (E)-2-undecenal (*49*). Out of the nine *G. m. morsitans* EAG-active peaks detected in the body volatiles of ox, six were identified and authenticated as: nonanal (*37*), decanal (*38*), undecanal (*39*), 4-methylphenol (*5*), dodecanal (*48*) and (E)-2-undecenal (*49*) [Fig. 40].

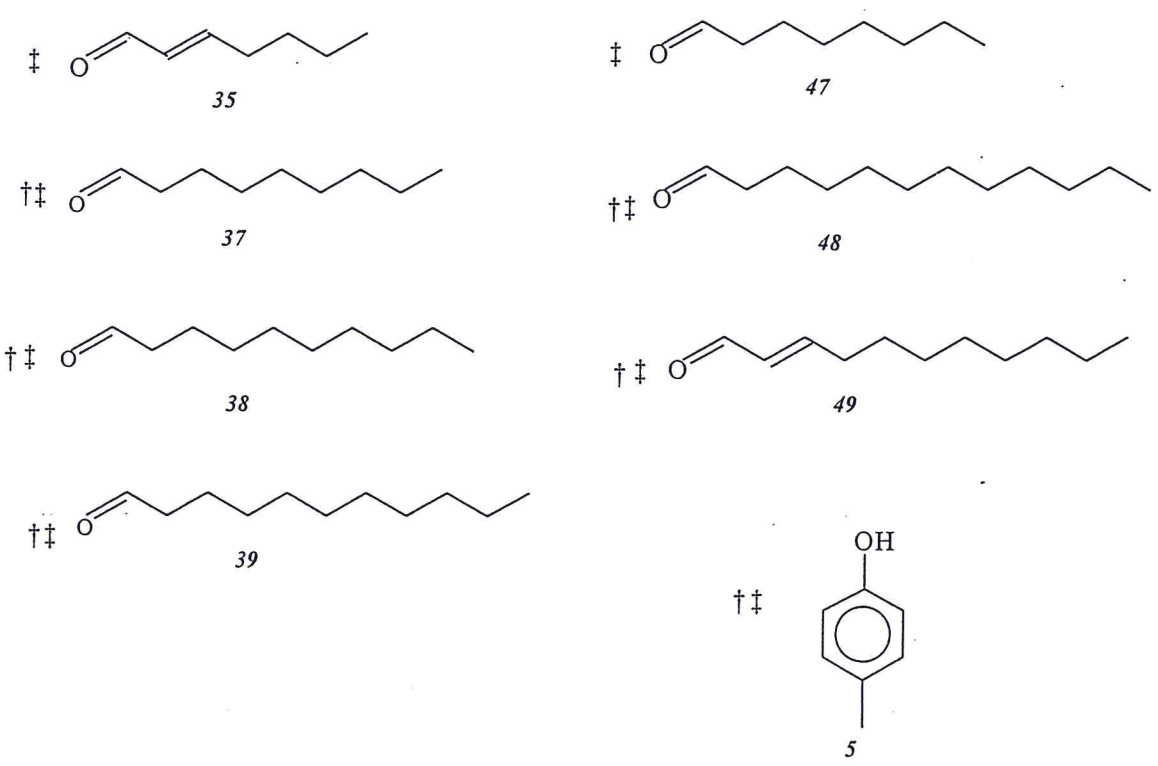


Fig. 40 EAG-active compounds on antenae of *G. m. morsitans* from ‡ buffalo and †ox body volatiles

Unlike the EAG-active compounds on *G. m. morsitans* from the waterbuck, those identified from both buffalo and ox were aldehydes and a phenol. All the EAG-active compounds identified in the ox volatiles were also present in the buffalo volatiles. Six of the EAG-active compounds in both ox and buffalo volatiles were also present in the waterbuck volatiles. The unidentified EAG-active peaks on *G. m. morsitans* from ox and buffalo volatiles had different retention times from the identified EAG-active peaks in the waterbuck volatiles.

4.3 Responses of *G. m. morsitans* to EAG-active compounds from animal body volatiles in the choice wind-tunnel

4.3.1 Activation behaviour.

Over 90% of the 3-day teneral *G. m. morsitans* released in the middle of the choice wind-tunnel in which no odour was dispensed were activated in that they walked or hoped out of the release cage three quarters of which flew upwind. When a blend of known attractants (see section **3.8.2.2**): acetone, 4-methylphenol, 3-n-propylphenol and 1-octen-3-ol (ATT) was dispensed from a low dose of 1 mg in 200 µl of paraffin oil, fewer flies (80%) got activated and even fewer, less than half, flew upwind (Table-8a; Table-8b; Table-9). The odour stimuli from host animals: waterbuck, buffalo and ox, dispensed in the tunnel elicited varying activation responses by the flies. Whereas the number of flies activated by any blend of stimuli (treatment) did not depend on the dose, this behaviour was significantly dependent on the treatment ($\chi^2_{(3)}$, 10.189; $P < 0.05$).

Table-8a. Behaviour of 3-day teneral *G.m.morsitans* in a choice wind-tunnel in which olfactory stimuli from waterbuck were dispensed.

Test blend	Dose (mg)§	N	% of flies activated	% of flies landing after flight in the tunnel: Initial direction of flight: Control	Middle	Treated	*P	Final resting position: Control	Middle	Treated	*P
Blank (BNK)	0.00	50	94.0	38.0	18.0	38.0	> 0.05	34.0	24.0	36.0	> 0.05
Whole waterbuck volatiles (WBV)	10.00	71	88.7	22.5	45.1	21.1	< 0.01	18.3	52.1	18.3	< 0.001
	1.00	50	82.0	28.0	26.0	28.0	> 0.05	34.0	34.0	14.0	> 0.05
	0.10	55	83.6	23.6	32.7	27.3	> 0.05	25.5	36.4	21.8	> 0.05
Waterbuck volatiles excluding common compounds with buffalo and ox (REP)	10.00	57	93.0	29.8	26.3	36.8	> 0.05	36.8	40.4	15.8	< 0.05
	1.00	59	88.1	33.9	25.4	28.8	> 0.05	32.2	42.4	13.6	< 0.01
	0.10	59	96.6	30.5	40.7	25.4	> 0.05	30.5	52.5	13.6	< 0.001
	0.01	57	94.7	35.1	26.3	33.3	> 0.05	31.6	35.1	28.1	> 0.05

*P. significance level in χ^2 test, comparing the distribution between the control, middle and treated sections.

§. in 200 µl of paraffin oil.

Table-8b. Behaviour of 3-day teneral *G.m.morsitans* in a choice wind-tunnel in which olfactory stimuli from animals: waterbuck, buffalo and ox, were dispensed.

Test blend	Dose (mg)§	N	% of flies activated	% of flies landing after flight in the tunnel: Initial direction of flight: Control	Middle	Treated	*P	Final resting position: Control	Middle	Treated	*P
Common compounds in waterbuck, ox and buffalo volatiles (WOB)	10.00	55	85.5	12.7	41.8	30.9	< 0.05	18.2	45.5	21.8	< 0.01
	1.00	40	90.0	12.5	35.0	42.5	< 0.05	15.0	37.5	37.5	> 0.05
	0.10	47	91.5	27.7	34.0	29.8	> 0.05	21.3	40.4	29.8	> 0.05
Buffalo and ox volatiles excluding mp (BUX)	10.00	53	81.1	11.3	34.0	35.8	< 0.05	15.1	52.8	13.2	< 0.001
	1.00	59	81.4	16.9	27.1	37.3	> 0.05	18.6	37.3	25.4	> 0.05
	0.10	49	81.6	16.3	40.8	24.5	> 0.05	10.2	46.9	24.5	< 0.01
Known attractants (a+mp+pp+oct) (ATT)	1.00	85	80.0	11.8	35.3	32.9	< 0.01	14.1	40.0	25.9	< 0.01

*P. significance level in χ^2 test, comparing the distribution between the control, middle and treated sections.

§. in 200 µl of paraffin oil.

a = acetone, mp = 4-methylphenol, pp = 3-n-propylphenol, oct = 1-octen-3-ol

Table-9. Behaviour of 3-day teneral *G.m.morsitans* after leaving mid-section of the wind-tunnel in which olfactory stimuli from waterbuck, buffalo and ox, were dispensed.

Test blend	Dose (mg)[§]	N	% of flies leaving mid-section	% of flies landing after flight in the tunnel: Initial take off Control	Initial take off Treated	Final resting position Control	Final resting position Treated
Blank (BNK)	0.00	50	76	50.0	50.0	48.6	51.4
Whole waterbuck volatiles (WBV)	10.00	71	43.6	51.6	48.4	50.0	50.0
	1.00	50	56.0	50.0	50.0	70.8	29.2 *
	0.10	55	50.9	46.4	53.6	53.8	46.2
Waterbuck volatiles excluding common compounds with buffalo and ox (REP)	10.00	57	66.6	44.7	55.3	70.0	30.0 *
	1.00	59	62.7	54.1	45.9	70.4	29.6 *
	0.10	59	55.9	54.5	45.5	69.2	30.8 *
	0.01	57	68.4	51.3	48.7	52.9	47.1
Common compounds in waterbuck, ox and buffalo volatiles (WOB)	10.00	55	43.6	29.2	70.8 *	45.5	54.5
	1.00	40	55.0	22.7	77.3 *	28.6	71.4 *
	0.10	47	57.5	48.1	51.9	41.7	58.3
Buffalo and ox volatiles excluding mp (BUX)	10.00	53	47.1	24.0	76.0 **	53.3	46.7
	1.00	59	54.2	31.2	68.8 *	42.3	57.7
	0.10	49	40.8	40.0	60.0	29.4	70.6
Known attractants (a+mp+pp+oct) (ATT)	1.00	85	44.7	26.3	73.7 **	35.3	64.7

*, **. indicate significant differences at $P < 0.05$ and $P < 0.01$, (χ^2 test), respectively, between the control and treated sections.

§ = in 200 µl of paraffin oil

a = acetone, mp = 4-methylphenol, pp = 3-n-propylphenol, oct = 1-octen-3-ol

Table-8a and Table-8b show that the strongest activating stimulus on the flies was the blend of EAG-active compounds from the waterbuck body volatiles that were absent in both buffalo and ox volatiles (REP). Nearly all the doses tested in this blend activated over 90% of the flies. The blend of EAG-active compounds that were common to the three animals, waterbuck, buffalo and ox (WOB), activated about 90% while the blend of all EAG-active compounds from waterbuck volatiles (WBV) activated less than 90% of the flies. The least activating stimulus was the blend of EAG-active compounds from buffalo and ox volatiles excluding 4-methylphenol (BUX). All the doses of this blend activated about 80% of the flies, a proportion similar to that elicited by ATT.

4.3.2 Upwind flight

Of the flies exhibiting activation behaviour, some flew upwind towards the treatment or the control while others remained in the mid-section. The flies that could not make a directional choice remained in the middle throughout the observation time while some that initially flew upwind finally returned to the mid-section. The initial distribution of flies in the tunnel (control, treated or mid-section), showed that whereas the proportions were not significantly different when no odour (BNK), REP and lower doses of WBV and BUX were dispensed, significantly more flies ($P < 0.05$ - $P < 0.01$) settled towards the treated and mid-section when WOB, BUX and ATT were dispensed (Table-8a; Table-8b). The proportions of *G. m. morsitans* flying upwind from mid-section followed a similar pattern to that of activation behaviour. The number of flies flying upwind was significantly dependent on the treatment ($\chi^2_{(3)}$, 8.088; $P < 0.05$) but not on the dose. The strongest stimulus eliciting upwind flight was REP (over 60%), followed by WOB (over 50%), while the weakest were WBV and BUX eliciting 50%

or less (Table-9). The initial choice of direction of flight (to either treated or control side) was significantly dependent on the treatment ($\chi^2_{(3)}$, 10.917; $P < 0.01$) but not on the dose. A direct comparison of the proportions of flies that flew upwind showed that the initial direction of choice by flies exposed to WBV and REP stimuli was not statistically different and was similar to that in the tunnel when no odour was dispensed. However, when WOB and BUX were dispensed, significantly more flies flew towards the treatment compared to the control ($P < 0.05$ - $P < 0.01$), a choice similar to that when ATT was dispensed (Table-9).

At the end of the observation time (3 minutes), the resting position was significantly dependent on the treatment ($\chi^2_{(3)}$, 18.782; $P < 0.001$) and not on the dose. Table-8a and Table-8b show that the final distribution of flies in the tunnel when REP, WOB and lower doses of BUX were dispensed, was biased towards the middle and odour source. The direct comparison of the proportions of flies resting in either control or treated zones, revealed that significantly fewer flies rested in the treated zone when REP was dispensed compared to the control zone ($P < 0.05$). Similarly, fewer flies rested in the treated zone when WBV was dispensed. On the contrary, higher proportions rested in the treated zone when WOB, BUX or ATT were dispensed (Table-9).

4.3.3 Upwind-flight progression and dispenser contact.

Flies released in the tunnel when no odour was dispensed moved similar distances towards both dispensers (Table-10). When ATT was dispensed, flies moved significantly longer distances towards the odour source compared to the control ($P < 0.01$). WBV did not elicit any significant differences in upwind progression between the control and the treated arms of the tunnel. Although upwind distances covered were not statistically different at the higher or lower doses of REP, doses of 1.0 and 0.1 mg elicited significantly lower upwind distances towards the treated compared to the control chamber/arm ($P < 0.05$). Flies exposed to lower doses of WOB and BUX (1.0 and 0.1 mg, respectively) showed significantly higher upwind progression in the treated compared to the control chamber/arm ($P < 0.05$ and $P < 0.01$, respectively). Whereas more flies hovered around or contacted dispensers treated with WOB, BUX or ATT compared to the controls, no fly contacted the dispensers when REP was dispensed. Similarly, there was hardly any fly contacting dispensers when WBV was dispensed (Table-10).

4.3.4 Escape behaviour of flies exposed to REP.

Flies that approached the REP dispenser exhibited an escape behaviour characterised by either turning at 180^0 while in flight, attempting to fly through the wall of the tunnel, or both. Table-11 shows that this behaviour was significantly higher for the treated compared to the control arms of the tunnel. The escape or avoidance behaviour to REP was stronger for the lower than higher doses.

Table-10. Upwind-flight distance and dispenser contact by 3-day teneral *G.m.morsitans* in the wind-tunnel in which olfactory stimuli from waterbuck, buffalo and ox, were dispensed.

Test blend	Dose (mg)§	N	Average upwind distance (cm) covered during flight in the tunnel (± s.e.)		Number of flies hovering around or contacting the dispenser	
			Control	Treated	Control	Treated
Blank (BNK)	0.00	38	26.74 ± 4.28	28.24 ± 4.56	0	1
Whole waterbuck volatiles (WBV)	10.00	31	20.42 ± 5.11	30.39 ± 4.76	0	1
	1.00	28	25.43 ± 5.08	20.32 ± 4.78	0	0
	0.10	28	18.82 ± 4.62	30.36 ± 6.13	0	0
Waterbuck volatiles excluding common compounds with buffalo and ox (REP)	10.00	39	24.15 ± 4.13	23.08 ± 4.02	0	0
	1.00	35	31.80 ± 4.12	19.97 ± 4.14 *	0	0
	0.10	33	33.48 ± 4.87	22.61 ± 4.43 *	0	0
	0.01	39	23.26 ± 4.42	20.82 ± 3.69	0	0
Common compounds in waterbuck, ox and buffalo volatiles (WOB)	10.00	23	20.13 ± 5.70	32.22 ± 5.89	0	6
	1.00	30	13.53 ± 3.08	24.33 ± 4.32 *	0	1
	0.10	27	24.22 ± 6.11	23.56 ± 5.00	1	3
Buffalo and ox volatiles excluding mp (BUX)	10.00	25	14.16 ± 4.94	18.60 ± 4.93	0	2
	1.00	32	18.31 ± 4.45	23.47 ± 4.31	1	4
	0.10	20	10.90 ± 3.41	31.95 ± 6.66 **	0	4
Known attractants (a+mp+pp+oct) (ATT)	1.00	38	17.95 ± 4.12	35.76 ± 4.75 **	0	5

*, **. indicate significant differences at $P < 0.05$ and $P < 0.01$ (χ^2 test), respectively, between the control and treated sections.

§ = in 200 µl of paraffin oil

a = acetone, mp = 4-methylphenol, pp = 3-n-propylphenol, oct = 1-octen-3-ol

Table-11. Escape behaviour of 3-day teneral *G. m. morsitans* females in the choice wind tunnel in which a blend of the repellent component of the the waterbuck volatiles (REP) was dispensed.

Dose (mg)§	N		Number of flies responding		
			X	**E**	**EX**
10.00	57	Treated	6	15 *	18 *
		Control	2	7	9
1.00	59	Treated	8 *	12	17
		Control	2	7	9
0.10	59	Treated	12 ***	10 **	19 ***
		Control	1	2	3
0.01	57	Treated	10 **	13 ***	19 ***
		Control	2	1	3
0.00	50	Treated	1	8	9
		Control	1	8	9

*, **, ***. indicate significant differences at $P < 0.05$, $P < 0.01$ or $P < 0.001$, respectively between the control and treated sections.

X. Turning at 180^0 in flight

E. Escape (Dragging against tunnel wall in flight or while fanning wings)

EX. Avoidance behaviour (escaped, turned or both)

§. in 200 µl of paraffin oil.

4.3.5 Responses of flies to olfactory stimuli dispensed from both sides of the wind-tunnel (push-pull).

The complete blend of EAG-active compounds from the waterbuck volatiles (WBV) did not elicit any specific response to teneral *G. m. morsitans* with regard to being attractive or repellent. It was interesting, however, that REP and WOB, the two components of WBV, elicited repellence and attractance behaviours respectively, to the flies. WOB was dispensed against either WBV or REP in a push-pull bioassay. The proportions of flies activated and those that flew upwind when WBV was dispensed against WOB were similar to those observed when WBV was dispensed against a control (Table-12; Table-13). Similarly, when REP was dispensed against WOB, the proportions activated and those flying upwind were not different from those observed when WOB was dispensed against the control. There were no significant differences in the proportions of flies that remained in the middle, those that flew towards WBV dispenser or those that approached the WOB dispenser when equal quantities of WOB and WBV were dispensed. This applied to both initial choice of flight direction and final resting position (Table-12). By doubling the quantity of WBV, the proportion of flies leaving mid-section was reduced and the distribution of flies was significantly biased towards the WOB dispenser and mid-section for both initial ($P < 0.05$) and final resting position ($P < 0.001$). However, the distribution of flies between WBV and WOB dispensers was not statistically different (Table-13). Surprisingly, when equal quantities of REP and WOB were dispensed, the distribution of flies in the tunnel at the end of observation period was significantly biased towards the REP dispenser and mid-section (Table-12). However, the proportions that rested towards the REP and WOB dispensers were not statistically different (Table-13). When the quantities of REP and

WOB were varied by doubling either of them, the proportions of flies finally resting towards the WOB dispenser was significantly higher compared to the REP dispenser ($P < 0.05$) (Table-13). There were no significant differences between upwind-flight distance towards WBV or WOB dispensers and there were hardly any flies contacting the dispensers. When REP was dispensed against twice the amount of WOB, the flies covered a significantly longer distance towards WOB compared to REP dispenser ($P < 0.001$). More flies contacted or hovered around the WOB dispenser compared to the REP dispenser (Table-14).

Table-12. Behaviour of 3-day teneral *G. m. morsitans* in a choice wind-tunnel in which different olfactory stimuli from waterbuck were dispensed from both sides (Push-Pull).

Test blends	Dose (mg)[§] r / a	N	% of flies activated	% of flies landing after flight in the tunnel: Initial direction of flight a	Middle	r	*P	Final resting position a	Middle	r	*P
Waterbuck volatiles (WBV = **r**) vs waterbuck attractants (WOB = **a**)	1.0 / 1.0	44	79.5	29.5	27.3	22.7	> 0.05	20.5	34.1	25.0	> 0.05
	2.0 /1.0	58	84.5	25.9	43.1	15.5	< 0.05	20.7	50.0	13.8	< 0.001
Waterbuck repellents (REP = **r**) vs waterbuck attractants (WOB = **a**)	1.0 / 1.0	81	88.9	23.5	43.2	22.2	< 0.05	13.6	51.9	23.5	< 0.001
	1.0 / 2.0	48	87.5	31.3	39.6	16.7	> 0.05	33.3	43.8	10.4	< 0.01
	2.0 / 1.0	39	92.3	35.9	28.2	28.2	> 0.05	38.5	38.5	15.4	> 0.05

*P. significance level in χ^2 test, comparing the distribution between the control, middle and treated sections.

§. in 200 µl of paraffin oil.

Table-13. Behaviour of 3-day teneral *G. m. morsitans* after leaving mid-section of the wind-tunnel in which different olfactory stimuli from waterbuck were dispensed from both sides (Push-Pull).

Test blends	Dose (mg)[§] **r / a**	N	% of flies leaving mid-section	% of flies landing after flight in the tunnel: Initial direction of flight **a**	Initial direction of flight **r**	Final resting position **a**	Final resting position **r**
Waterbuck volatiles (WBV = **r**) vs waterbuck "attractants" (WOB = **a**)	1.0 / 1.0	44	52.2	56.5	43.5	45.0	55.0
	2.0 /1.0	58	41.4	62.5	37.5	60.0	40.0
Waterbuck "repellents" (REP = **r**) vs waterbuck "attractants" (WOB = **a**)	1.0 / 1.0	81	45.7	51.4	48.6	36.7	63.3
	1.0 / 2.0	48	48.0	65.2	34.8	76.2	23.8 *
	2.0 / 1.0	39	64.1	56.0	44.0	71.4	28.6 *

* indicate significant differences at $P < 0.05$ (χ^2 test) between the two directions.

[§] = in 200 µl of paraffin oil

Table-14. Upwind-flight distance and dispenser contact by 3-day teneral *G.m.morsitans* in the wind-tunnel in which different olfactory stimuli from waterbuck were dispensed from both sides (Push-Pull).

Test blends	Dose (mg)[§] r/a	N	Average upwind distance (cm) covered during flight in the tunnel (± s.e.) a	r	Number of flies hovering around or contacting the dispenser a	r
Waterbuck volatiles (WBV = **r**) vs waterbuck "attractants" (WOB = **a**)	1.0 / 1.0	23	22.13 ± 5.57	34.09 ± 6.68	0	0
	2.0 /1.0	24	23.21 ± 5.56	19.83 ± 5.63	1	0
Waterbuck "repellents" (REP = **r**) vs waterbuck "attractants" (WOB = **a**)	1.0 / 1.0	37	19.81 ± 4.00	30.65 ± 4.77 *	3	1
	1.0 / 2.0	23	43.78 ± 5.13	18.61 ± 4.85 ***	5	0
	2.0 / 1.0	26	32.00 ± 6.07	26.23 ± 5.96	2	0

*, ***. indicate significant differences at $P < 0.05$ and $P < 0.001$ (χ^2 test), respectively, between the two directions.
§ = in 200 µl of paraffin oil

CHAPTER 5.

5.0 DISCUSSION

5.1 Feeding responses of *G. m. morsitans* on hosts and on membranes treated and untreated with waterbuck sebum.

The behaviour of teneral *G.m.morsitans* confined in small experimental cages on waterbuck and ox, and on feeding membranes with and without smears of waterbuck sebum were examined. There were no significant differences between the times the flies took to land on the two animals on one hand and between smeared and unsmeared parts of the feeding membranes on the other. Similarly, there were no significant differences between the times taken by 2- and 3-day teneral flies to land on the membranes indicating that the hunger status of the flies and olfactory cues were surpassed by other factors such as thermal gradient in eliciting this behaviour. These results are consistent with previous reports that the identification of a feeding site by tsetse flies requires the heat stimulus (Van der Goes van Naters and Den Otter, 1998). When working on *G. fuscipes fuscipes* Van der Goes van Naters *et al,* (1998) reported that the flies probed on blank heated paper discs while uric acid, which had elicited electrophysiological responses from taste hairs on the flies' legs, failed to evoke probing when heat was not applied. The flies perceive this heat stimulus via receptors on the antennae (Dethier, 1954) and thermoreceptors located in the tarsi (Reinouts van Haga and Mitchell, 1975), and it induces the insect not only to land but also to initiate probing. This important fact has been exploited in tsetse rearing using membranes (Bauer and Wetzel, 1976; Moloo and Pimley, 1978; Moloo *et al.*, 1988), where the thermal gradient is the only stimulus.

Caged flies placed on the animals in the field took a shorter time to land on the skin compared to those placed on the membranes under laboratory conditions. Although it was difficult to ensure that a fly was tranquil at the top of the cage when placing it on hosts compared to membranes, other factors may have predisposed the insects to respond faster to the thermal gradient on animals compared to membranes. Specifically, the flies may have been activated by host breath constituents such as carbon dioxide, acetone and 1-octen-3-ol (Vale and Hall, 1985) and/or phenolic compounds from excretory products around the crush (Hassanali *et al.*, 1986; Bursell *et al.*, 1988; Saini *et al.*, 1993; Madubunyi *et al.*, 1996).

The behaviours of flies that contacted the body of the waterbuck or areas of the membrane treated with different doses of its sebum were significantly different from those of flies that contacted the ox or untreated (control) areas of the membrane. All flies that landed started probing immediately, but they showed a significant reluctance to feed on the waterbuck or on parts of membrane treated with its sebum. The reluctance to feed on the waterbuck or on areas of membranes treated with its sebum was manifested by high proportions of flies escaping, changing probing sites, and general delays in the initiation of feeding compared to ox or untreated zones of the membrane.

Although the sebum concentration on the skin surface of waterbuck was estimated at 0.7 mg/cm^2, flies that landed on parts of membranes treated with this dose did not exhibit any significant differences in behaviour compared to the control. The concentration of waterbuck sebum was clearly underestimated as evidenced by GC analysis of sequential extracts from a marked out portion of the waterbuck. The results showed that secretion of sebum was continuous and its chemical composition was

retained. However, environmental factors such as temperature and humidity are known to affect the quantity of sebum produced by an animal (Jenkinson and Mabon, 1973; Smith *et al.*, 1975), which could also have affected the amount of sebum on the waterbuck extracted at the time of taking swabs. The responses of *G. m. morsitans* on parts of membranes treated with 1.0 mg/cm^2 of waterbuck sebum rather than 1.4 mg/cm^2 were very similar to those on a live waterbuck. This suggested that the concentration of compounds responsible for these behaviours on the waterbuck skin was very close to that in the sebum at a dose of 1.0 mg/cm^2.

After piercing the skin, engorgement by the flies is known to be stimulated by blood constituents such as ATP (Galun, 1987; Galun and Margalit, 1969). Since blood from the two bovids had been reported to provide a suitable meal for survival and reproduction of *G. m. morsitans* (Moloo *et al.*, 1988), the behavioural discrepancies of the flies on the animals or on the membranes is consistent with the presence of aversive constituents (allomones) in the waterbuck sebum. The escape behaviour after landing on waterbuck or its sebum and frequent changes of probing sites suggest the presence of a deterrent signal of low volatility perceived by tarsi receptors or olfaction at very close range (Van der Goes van Naters and Rinkes, 1993; Van der Goes van Naters and Den Otter, 1998). Contact chemoreception via tarsi is an excellent mode for host discrimination during inspection of a feeding site by flies (Van der Goes van Naters and Den Otter, 1998). Additionally, significant demonstration of similar elements of behaviour by 2-day unfed flies that landed on the control zones of treated membranes compared with those on untreated (double control) membranes, also implicated a more volatile signal.

Examination of waterbuck sebum by gas chromatography linked to electroantennographic detector (GC-EAD) revealed the presence of two electrophysiologically active constituents of relatively high molecular weights (compounds ***32*** and ***33***) which may have a role in the close-range/contact effects. The more volatile component of the waterbuck sebum (phenolic fraction) comprised mainly of 2-isopropyl-5-methylphenol (thymol) (***34***) and traces of 4-methylphenol (***5***) and 4-ethylphenol (*7*). 4-Methylphenol and 4-ethylphenol were previously detected in the ox sebum alongside other phenols (Warnes, 1990; Saini *et al.*, 1993) and had earlier been shown to be attractive to *G. m. morsitans* and *G. pallidipes* in the field (Hassanali *et al.*, 1986; Owaga *et al.*, 1988; Bursell *et al.*, 1988). Thymol (***34***), the major component of this fraction, was not detected in the ox sebum and is known to have antimold, antifungal and antiseptic properties (The Merck index, 1996), implying that this compound could have deterrent properties against the tsetse flies. Another possibility is that thymol (***34***) may inhibit the bacteria responsible for converting precursors of attractive phenols such as 4-methylphenol and 3-n-propylphenol (Okech and Hassanali, 1990), and limit their relative amounts.

These results provide an interesting insight into the behavioural plasticity of tsetse flies that find themselves in a chemically hostile environment of a refractory host. Two-day teneral flies were clearly more sensitive to the airborne volatiles associated with sebum on feeding membranes compared to their 3-day counterparts (compare the responses of 2- and 3-day teneral flies that landed on control zones of treated membranes, Table-2; -3; -4; -5; -7). Thus, the degree of hunger of a fly is likely to affect somewhat the aversive effects of non-host chemical constituents. Moreover, at above-normal dose (1.4 mg/cm^2), 3-day flies spent less time probing compared to flies

at the same state of hunger feeding at lower sebum doses (Table-5; Fig.12), suggesting the operation of a behavioural adaptation mechanism in the fly. This plasticity may account for occasional feeding on waterbuck and other refractory animals by the fly in the field (Weitz 1963; Moloo, 1993; Clausen *et al.*, 1998), despite the existence of allomone barriers implicated in the present results.

5.2 Volatile compounds from the body surface of animals.

An effective novel technique for trapping odour from a specific area on the body surface of a live animal was developed in this study. This technique relied on the fact that odour volatilises from the skin surface of the animal and diffuses into the air above the skin. Since different compounds have different diffusion rates into the air stream, placing an adsorbent on the skin ensured that all compounds had a chance of being adsorbed. The adsorbent placed on the skin therefore trapped most of the volatile compounds emanating from the animal without allowing them to escape, as is normally the case with conventional methods of volatile trapping where air is forced through an adsorbent (Hall *et al.*, 1984). In the conventional methods, the adsorbent is packed in a column and the volatile compounds from an organism, carried in a gas (air or nitrogen), are pumped through the column. Whereas some of these compounds are trapped in the adsorbent, the more volatile compounds with lower molecular weights have a high tendency of breaking through the column, and this leads to incorrect conclusions about the chemical composition and ratios in the volatiles. In the present technique, the alluminium foil covering the backside of the adsorbent belt (Fig. 4) acted not only to prevent the escape of the trapped volatiles and to prevent adsorption of chemical compounds from the environment, but also to prevent possible degradation of adsorbed

compounds by UV radiation from the sun. This technique can therefore be utilised to trap odour (of high or low volatility) from other animals and also from plants.

Whereas taste and thermal receptors are located on the tarsi of tsetse flies (Reinouts van Haga and Mitchell, 1975; Van der Goes van Naters and Rinkes, 1993; Van der Goes van Naters and Den Otter, 1998; Van der Goes van Naters *et al.,* 1998), odours are detected by antennal receptors (Hall *et al.*, 1984; Den Otter and Saini, 1985; Saini, 1986; Saini *et al.*, 1989). Using electroantennographic recordings (GC-EAD), several compounds from the waterbuck body volatiles were detected and identified as EAG-active on laboratory-reared *G. m. morsitans* and field-trapped *G. pallidipes*. The failure by laboratory-reared *G. pallidipes* to show any EAG-activity with volatiles from the waterbuck remains unclear; but it could be related to species specific factors associated with removal from the natural environment. Whereas the two species of flies responded to nine common compounds, implying that they utilise similar olfactory cues in host seeking strategy, each species responded uniquely to other compounds. This indicates the complexity of discrimination by the flies and is consistent with the observed gradation of a particular host as a source of bloodmeal by various species and subspecies of tsetse flies (Moloo, 1993). In his report, Moloo (1993) indicated that the waterbuck, though generally an unpreferred host of *Glossina,* is comparatively more palatable to *G. pallidipes* than *G. m. morsitans*. If the identified EAG-active compounds (Fig. 39) are all utilised by the flies in host location, then the compounds unique to each fly species may be responsible for the observed preference by either species. *G. pallidipes* responded uniquely to two compounds: 2-methoxyphenol (***10***) and (E)-2-nonenal (***36***), while *G. m. morsitans* responded uniquely to five compounds: undecanal (***39***), 3-isopropyl-6-methylphenol (***46***), 2-decanone (***42***), 2-undecanone (***43***)

and 2-dodecanone (***44***). 2-Methoxyphenol (***10***) which was previously detected as a minor component of cattle urine was reported to evoke a low EAG response to *G. m. morsitans* and *G. pallidipes* (Bursell *et al.*, 1988). This compound elicited upwind flight in wind-tunnel experiments but reduced trap catches of the two species in the field (Bursell *et al.*, 1988; Vale *et al.*, 1988; Green, 1993; Torr *et al.*, 1996). On the other hand, this study has demonstrated that 2-decanone, 2-undecanone and 2-dodecanone, alongside other compounds are repellent to *G. m. morsitans* in wind-tunnel experiments. This suggests that *G. m. morsitans* detects more repellent compounds than *G. pallidipes* from the waterbuck, and these may be directly linked to the observed variation in this animal being a source of bloodmeal for the flies.

Unlike the *G. m. morsitans* EAG-active compounds from the waterbuck, those identified from both buffalo and ox were mainly aldehydes. All the EAG-active compounds identified in the ox volatiles were also present in the buffalo volatiles (Fig. 40). The buffalo and ox are both preferred hosts of most *morsitans* group of *Glossina* while the waterbuck is unpreferred. *G. m. morsitans* derives about 2 and 26 times more bloodmeals from buffalo than from the ox and waterbuck, respectively (Moloo, 1993). The level of preference as a source of bloodmeal by *G. m. morsitans* is reflected in the nature of the EAG-active compounds identified from these animals. The two EAG-active aldehydes: (E)-2-heptenal (***35***) and octanal (***47***) identified in the buffalo and not in ox volatiles, may be among the compounds responsible for the differences in attractiveness of this animal to flies and eventually, as food source. The unidentified *G. m. morsitans* EAG-active peaks from ox and buffalo volatiles had different retention times (GC) from the identified EAG-active peaks in the waterbuck volatiles. However, six of the EAG-active compounds in both ox and buffalo volatiles were also present in

the waterbuck volatiles (Figs. 39 and 40). Eight EAG-active compounds were detected only in the waterbuck body volatiles and these may be the ones that are responsible for the specific identity of waterbuck to tsetse.

The role played by the volatile EAG-active compounds in attraction of *G. m. morsitans* to these animals was investigated in a choice wind-tunnel. The EAG-active compounds detected only in the waterbuck volatiles (REP) elicited repellent behaviours on *G. m. morsitans* in the tunnel. These behaviours were characterised by turning at 180^0 while in flight and/or attempting to escape through the tunnel wall after perceiving the odour and, eventually, significantly more flies settling on the untreated arm of the tunnel than on the treated arm. On the other hand, the EAG-active compounds common to all three animals (WOB) or EAG-active compounds detected from volatiles of buffalo and ox excluding 4-methylphenol (BUX), elicited attraction responses on *G. m. morsitans*.

In wind-tunnel experiments where WOB or BUX were dispensed, responses were characterised by a specific initial reaction of the flies. After detecting the odour, significantly more flies responded by flying upwind towards the odour source and some hovered around or contacted the dispenser. These flies later moved downwind probably due to the absence of near-host cues such as heat, thus making the final resting position unimportant. On the contrary, the final resting position and not the initial reaction was the most important response when flies perceived the repellent odour.

EAG-active compounds from the waterbuck volatiles comprise both attractants and repellents (WBV). When these compounds were dispensed in the wind-tunnel, there was no directional preference in the initial response by the flies. Whereas equal proportions of flies settled on the treated and control sides of the tunnel at the higher

dose of 1.0 mg/200µl, higher proportions settled on the control side at lower doses, implying, on one hand, that the blend of EAG-active compounds from the waterbuck volatiles was repellent to *G. m. morsitans* at the appropriate dose, and, on the other hand, a state of confusion at the higher dose. The presence of these repellents on the body surface of waterbuck and their absence on the buffalo and ox explains why few flies were seen attracted to waterbuck compared to buffalo and ox in the field (Grootenhuis, 1986; Grootenhuis and Olubayo, 1993). Moreover, the buffalo and ox attracted roughly the same numbers of flies in the field (Grootenhuis, 1986; Grootenhuis and Olubayo, 1993) which is consistent with similar attractants emanating from these animals as confirmed in this study.

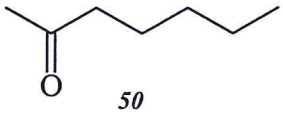
4-Methylphenol (***5***) and 3-n-propylphenol (***8***) are known tsetse attractants previously detected in the urine of buffalo (Hassanali *et al.*, 1986; Owaga *et al.*, 1988), cattle (Bursell *et al.*, 1988), waterbuck (Madubunyi *et al.*, 1996) and from the body wash of cattle (Warnes, 1990; Saini *et al.*, 1993). These compounds when dispensed in combination with other attractants such as 1-octen-3-ol (***2***) and acetone (***1***) have been used to enhance trap catches in the field. Although no other attractants have been identified from hosts in the recent past, it has always been suspected that there are other unidentified attractants from hosts' odour (Torr *et al*, 1995; Hargrove *et al*, 1995). Vale (1980), suggested that aldehydes, which had been suspected to be present in odours of ox, were likely to contribute to the attractiveness of its odour to tsetse. Whereas aldehydes have been confirmed as some of the missing attractants of tsetse in the present study, a few other compounds which had elicited EAG activity on *G. m. morsitans* from both buffalo and ox are yet to be identified (Fig. 25; Fig. 26).

Long chain ketones especially 2-octanone (***40***) and short chain carboxylic acids such as pentanoic, hexanoic, heptanoic, and octanoic acids were reported to be repellent to tsetse in the field (Vale, 1980; Torr *et al.*, 1996). All these short chain carboxylic acids including nonanoic acid were detected in the body volatiles of the waterbuck in relatively large amounts (Appendix-8.2), but had little or no EAG response on *G. m. morsitans* and *G. pallidipes*. Although some of these acids are known to be present in ox odour (Torr *et al.*, 1996) they were below detection level in volatiles of the ox and buffalo in the present study. These short chain carboxylic acids together with 2-isopropyl-5-methylphenol (***34***) and 3-isopropyl-6-methylphenol (***46***) are responsible for the cloying, musky smell emitted by the waterbuck (Estes, 1991; Mihok *et al.*, 1991). They may also contribute to the refractoriness of this animal not only to tsetse flies but also to other predators like the lion, leopard and hunting dogs (Haltenorth and Diller, 1996).

Constituents of volatiles from these animals are mainly simple natural product chemicals and whereas several hundreds of them volatilise from the skin surface (Figs. 21, 23, 25, 26), only a few play a role in the insect-host interaction (Figs. 27 - 33). Some of the physiologically important compounds identified from the waterbuck body volatiles have been isolated from plant parts (Torto and Hassanali, 1997). For example, 3-isopropyl-6-methylphenol (***46***) and 2-dodecanone (***42***) were detected in the essential oils of aerial parts of the Capparidaceae family including; *Gyandropsis gynandra, Boscia mossambicensis, B. angustifolia, Cabada farinosa, Thylachium africunum* and *Cleome monophylla* (Ndungu *et al.*, 1995; Torto and Hassanali, 1997). These compounds were reported to be repellent to the brown ear tick *Rhipicephalus appendiculatus* and the maize weevil *Sitophilus zeamais*. 3-Isopropyl-6-methylphenol

(***46***) was also isolated from the sawdust of *Thujopsis dolabrata* and found to have high insecticidal, acaricidal, and termiticidal activity (Ahn *et al.,* 1998). 2-Octanone (***40***), 2-nonanone (***41***), 2-decanone (***43***), 2-dodecanone (***44***) were isolated from the resinous exudate of the stem bark of *Commiphora rostrata* (McDowell *et al.,* 1988) and shown to be repellent to *S. zeamais* (Lwande *et al.,* 1992).

Whereas several plants are known to produce repellents and feeding deterrents against insects (Torto and Hassanali, 1997), many insect species produce compounds (mainly carbonyls) which serve as effective deterrents to other arthropods (Blum, 1996). For example, 2-heptanone (***50***) which is similar in structure to compounds ***40, 41, 42, 43*** and ***44,*** identified in the waterbuck volatiles, is an alarm pheromone as well as a defensive allomone released by the dolichoderine ant *Iridomyrnex pruinosus* (Blum *et al.*, 1966). This compound is also an effective defensive allomone for cockroaches in the genus *Platyzosteria* (Wallbank and Waterhouse, 1970), beetles in the genus *Dyschirius* (Moore and Brown, 1979) and the honeybee (Shearer and Boch, 1965). The α,β-unsaturated aldehydes, (E)-2-hexenal (***51***) and (E)-2-octenal (***52***) which are similar in structure to compounds ***35***, ***36*** and ***49,*** identified in volatiles from the animals, are secreted by immature heteropterans and are used as dispersion pheromones as well as defensive allomones against adversaries (Blum, 1996). Other ketones used as defense allomones include: 3-octanone (***53***), 4-methyl-3-heptanone (***54***) by the formicids and ants, 6-methyl-5-hepten-2-one (***55***) by ants, termites and cockroaches in the genus *Neostylopyga* (Blum, 1996).



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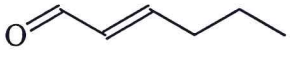

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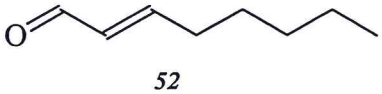

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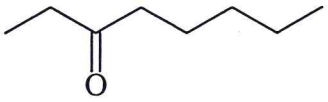

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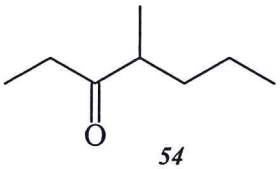

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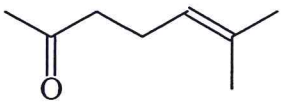

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Structure activity studies on releasers of alarm for *I. pruinosus* established that straight chain 2-ketones containing 6 to 10 carbon atoms were the most effective with maximum activity being expressed in the chains containing 7 to 9 carbons (Blum *et al.*, 1966). This suggests that the 2-ketones identified in the waterbuck body volatiles are actual defensive allomones for tsetse flies and, together with 3-isopropyl-6-methylphenol (***46***), 2-methoxyphenol (***10***) and the δ-octalactone (***17***), may also be effective defensive allomones against other arthropods like ticks.

Compounds used for defense by insects and marine organisms such as mollusks and some fishes are known to be sequestered from food plants or synthesized *de novo* (Harborne, 1989; Blum, 1996; Cimino and Ghiselin, 1998; Gavagnin *et al.*, 1999). Although the herbivores under this study naturally feed on green grass and browse on common plants, the waterbuck is known to feed on plants from the genus *Capparis* (Capparidaceae), especially *C. tomentosa* when green grass is in short supply during the dry season (Kuchar, 1981; Estes, 1991). Most *Capparis* species are known to be highly poisonous when eaten by other herbivores like cattle and camels (Kuchar, 1981; ITDG and IIRR, 1996). Interestingly, compounds like 2-dodecanone (***42***) and 3-isopropyl-6-

methylphenol (***46***) which have high repellency activity against arthropods have been isolated from the family Capparidaceae (Ndungu *et al.*, 1995; Torto and Hassanali, 1997). The animals used in this study (waterbucks, buffaloes and oxen) were, however, removed from their natural food sources and maintained on the same artificial diet for a couple of years and in the absence of biochemical mechanisms specific to one species, it would be expected that they would emit the same compounds from their skin surface. The presence of aversive compounds in the waterbuck volatiles and their absence in both ox and buffalo volatiles suggests that these compounds may have been produced from fatty acid metabolism through the mediation of additional enzymes. Furthermore, microbial activity on the skin surface of these animals play an important role in the breakdown of sebaceous and sweat components or even in production of other compounds (Jenkinson and Mabon, 1973; Smith *et al.*, 1975; Okech and Hassanali, 1990; Mihok *et al.*, 1991). This suggests that the waterbuck may be having specific microbes on its skin surface which are not present on either buffalo or ox that are responsible for the production of the deterrent compounds.

CHAPTER 6.

6.0 Conclusions and suggestions for further studies.

6.1 Conclusions.

1. An effective technique for trapping odour from a specific area on the body surface of a live animal was developed in this study. This new method can be used to determine chemical compounds that volatilise from surfaces of other animals or plants with minimum modifications.

2. Electrophysiologically-active compounds from volatiles and non-volatile skin secretions of animals (waterbuck, ox and buffalo) on tsetse antennae (*G. m. morsitans* and *G. pallidipes*) were detected using GC-EAD and identified using GC-MS, GC-coinjection, synthesis and confirmed using GC-EAD.

3. EAG-active compounds identified from volatiles of buffalo and ox were similar in structure and elicited attraction responses in *G. m. morsitans* when bioassayed in a choice wind-tunnel. On the other hand, EAG-active compounds identified from volatiles of the waterbuck comprised mostly of compounds identified in both ox and buffalo in addition to a series of other compounds that were shown to elicit aversive behaviour in *G. m. morsitans* in the wind-tunnel bioassays.
All the EAG-active compounds in the body volatiles from these animals except 4-methylphenol and 3-n-propylphenol are being reported as behaviour modifying chemicals (semiochemicals) in tsetse flies for the first time, in this study.

4. This study has confirmed that whereas 4-methylphenol and 3-n-propylphenol are known tsetse attractants, but not host specific (Madubunyi *et al.*, 1996), their kairomonal role stems from the fact that they volatilise from the skin surface of hosts rather than emanate from their urine as previously thought (Chorley, 1948; Owaga, 1984, 1985; Vale *et al.*, 1986; Hassanali *et al.*, 1986; Owaga *et al.*, 1988; Bursell *et al.*, 1988; Vale *et al.*, 1988; Gouteux *et al.*, 1995; Mihok *et al.*, 1996; Spath, 1997).

5. Results of this study allude that hosts of tsetse flies emit semiochemicals from their skin surface that give each host a *signature* (a blend of electrophysiologically active compounds specific to each host) which are recognized by tsetse flies and these are the basis of host preference and gradation as sources of bloodmeal.

6. Contrary to previous beliefs that the basis of feeding nonpreference in hosts by tsetse flies was due to mechanical defensive behaviours such as tail switch, leg kicks, body twitch (Vale, 1974b; Vale, 1977; Torr, 1994; Baylis, 1996; Torr and Hargrove, 1998), or host body mass (Vale, 1974b,c; Hargrove, 1976), unpreferred animals like the waterbuck actively produce defensive allomones to repel biting flies as demonstrated in this study.

7. The waterbuck emits both allomones and kairomones from its skin surface. The combined blend of allomones and kairomones (as in the waterbuck volatiles) was repellent to tsetse flies as demonstrated in wind-tunnel experiments. This explains why few flies were seen attracted to the waterbuck compared to ox and buffalo in the field (Grootenhuis, 1986; Grootenhuis and Olubayo, 1993).

8. The feeding experiments on live animals (waterbuck and ox) and on membranes treated and untreated with smears of waterbuck sebum has demonstrated that thermal gradient is the dominant factor in influencing the landing behaviour of tsetse flies at close range.

9. This study has demonstrated that in addition to emission of volatile allomones, the waterbuck has other less-volatile allomones in its sebum some of which are detected by receptors located in the tarsi and others that are detected by olfactory receptors in the antennae that deter the tsetse flies from feeding.

10 The production and use of defensive allomones in higher mammals against tsetse flies is being reported for the first time, in this study.

11 Results from this study have shown that the waterbuck utilises several allomones for defense against tsetse flies. These allomones probably act both synergistically and additively at three levels: long-range olfactory repellence, short-range olfactory repellence and contact deterrents. Therefore, any repellent(s) being developed for tsetse control must borrow a leaf from the waterbuck strategy to achieve meaningful results.

In summary, results of this study provide a clear evidence that the relative refractoriness of waterbuck to tsetse flies is mediated by allomones. The allomones, (both volatile and less-volatile) would account for low numbers of the flies that are attracted to and eventually feed on the waterbuck in the wild. The waterbuck may represent a group of wild game in Africa that may have successfully evolved a first line defense mechanism against tsetse (and the disease it transmits) based on secondary metabolites similar to what is commonly found in plants and some insects.
The allomones identified from the waterbuck may be used in developing protectants for cattle and humans against tsetse fly attack.

6.2 Suggestions for further studies.

The studies outlined here are necessary for the development and optimisation of a push-pull control strategy for tsetse flies, based on the chemical ecology of their host preference in the wild.

1) Evaluation of efficacy of the identified repellents in protecting cattle in tsetse infested areas.

2) Field-testing of the new attractants to determine their potency in increasing trap catches.

3) Identification of compounds from waterbuck sebum that deterred teneral *G. m. morsitans* females from feeding on waterbuck or on membranes treated with its sebum. These compounds are suspected to have been detected by tarsal gustatory receptors.

4) Synthesis and evaluation of behaviour modulating activity of the two EAG-active acylglycerols, ***32*** and ***33***, identified from the waterbuck sebum in *G. m. morsitans*. These compounds are suspected to play a role in feeding deterrence of teneral *G. m. morsitans* females on live waterbuck or on membranes treated with its sebum.

5) Identification of the remaining EAG-active compounds from waterbuck, buffalo and ox body volatiles and determination of the effect of these compounds on the behaviour (repellence/attractance) of *G. m. morsitans* and *G. pallidipes*

6) Investigation on the extent of use of defensive allomones against tsetse flies by other unpreferred wild animals e.g. zebra, wildebeest and impala. This may provide an array of other more potent repellent/deterrent compounds which could be incorporated into management strategies for the tsetse flies.

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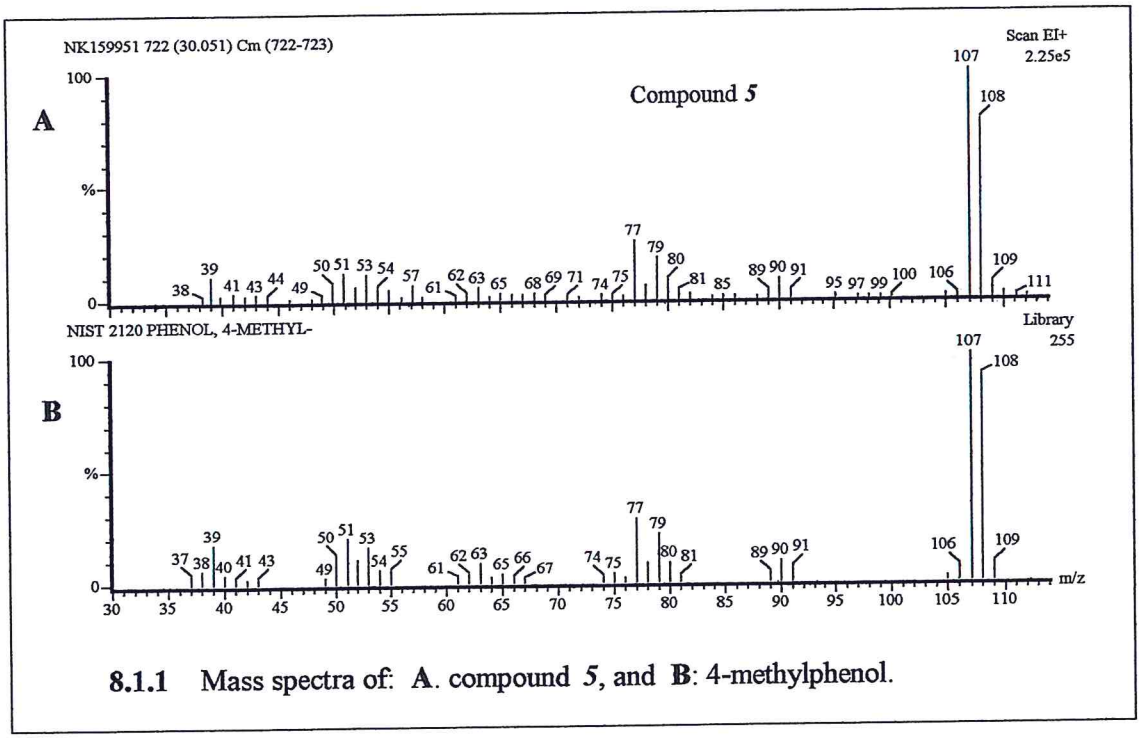
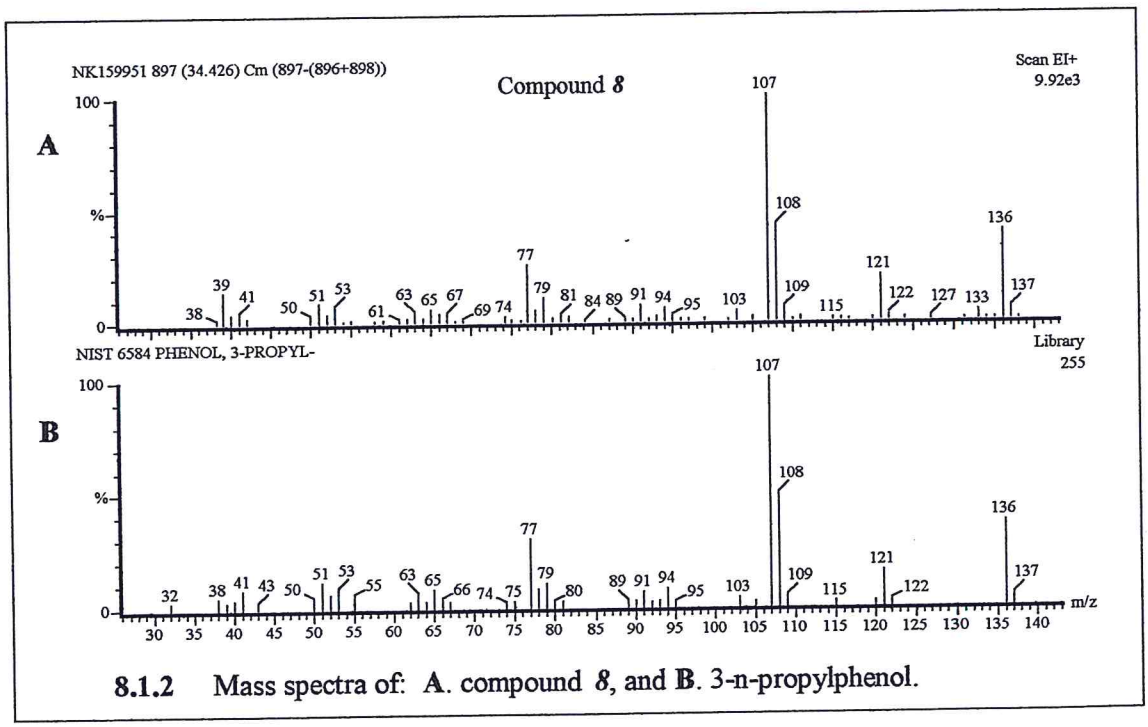
8.0 APPENDICES

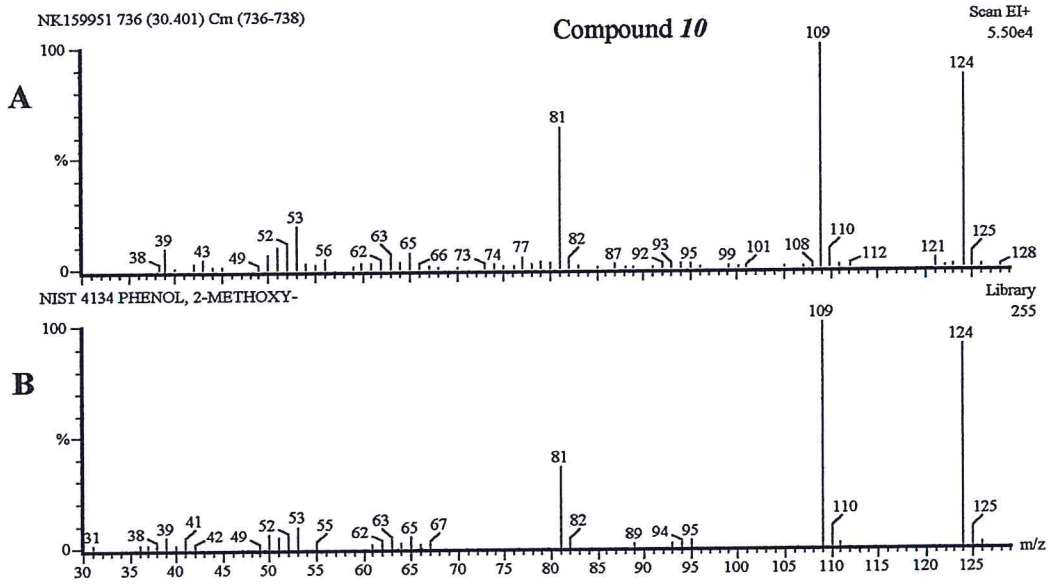
8.1 Mass spectra of EAG-active compounds on *G. m. morsitans* and *G. pallidipes* from animal body volatiles.



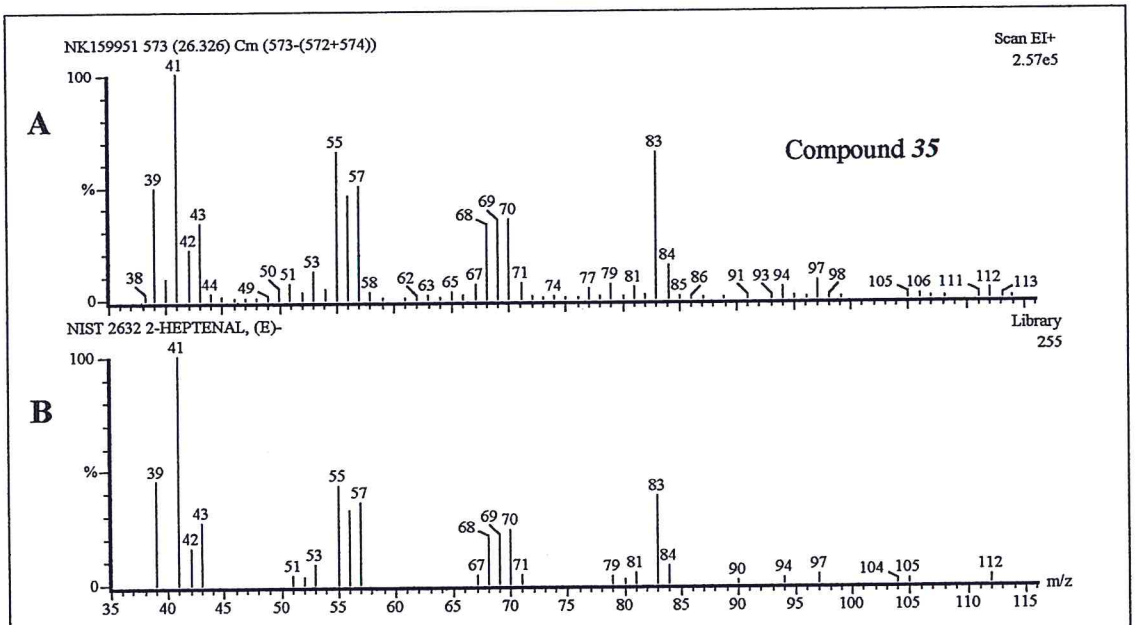
8.1.1 Mass spectra of: **A**. compound *5*, and **B**: 4-methylphenol.



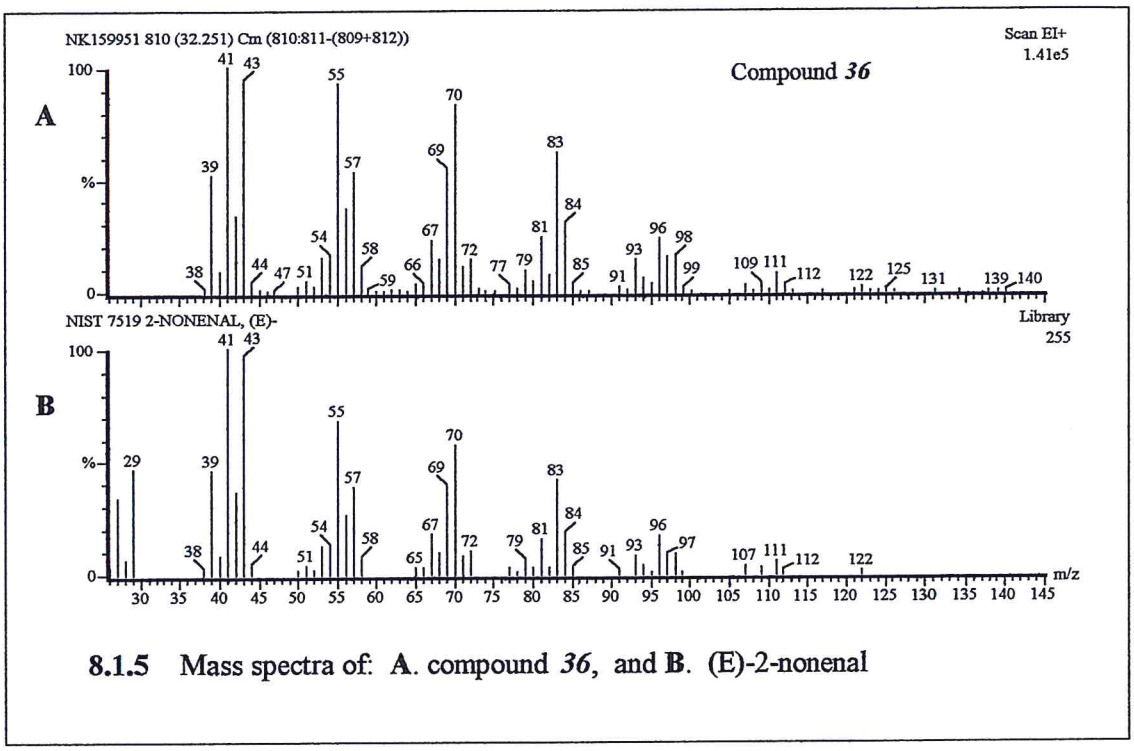
8.1.2 Mass spectra of: **A**. compound *8*, and **B**. 3-n-propylphenol.



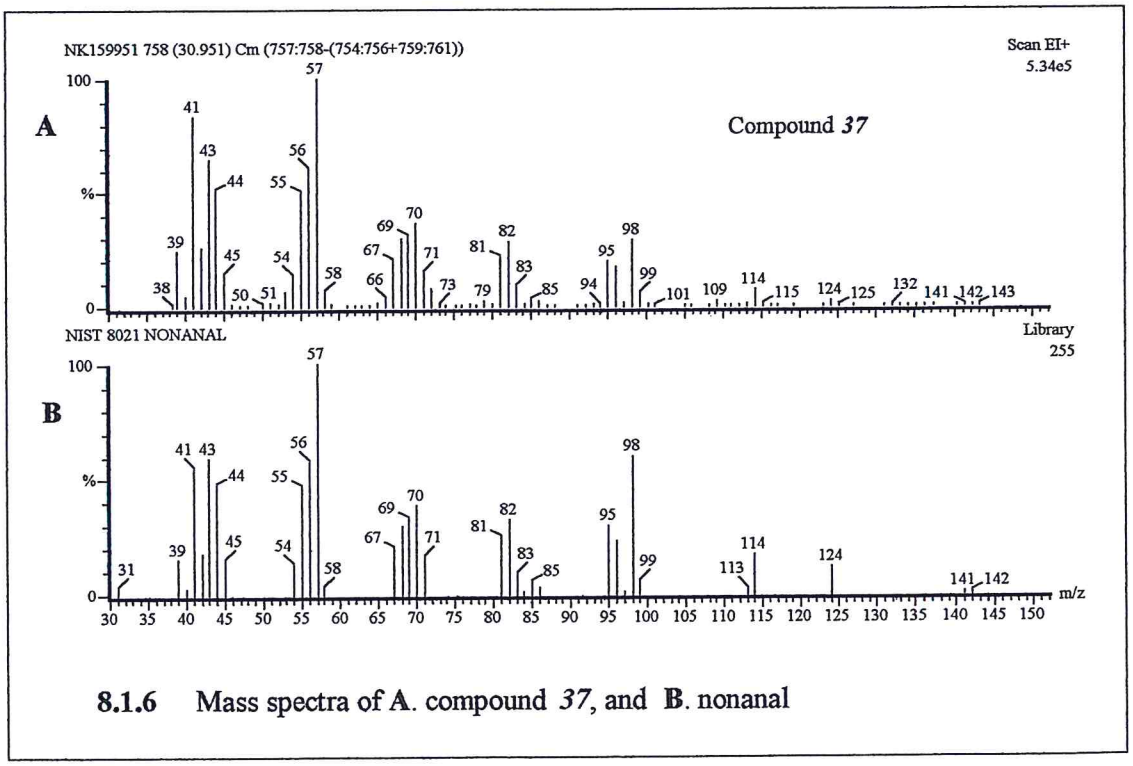
8.1.3 Mass spectra of: **A**. Compound ***10***, and **B**: 2-methoxyphenol.



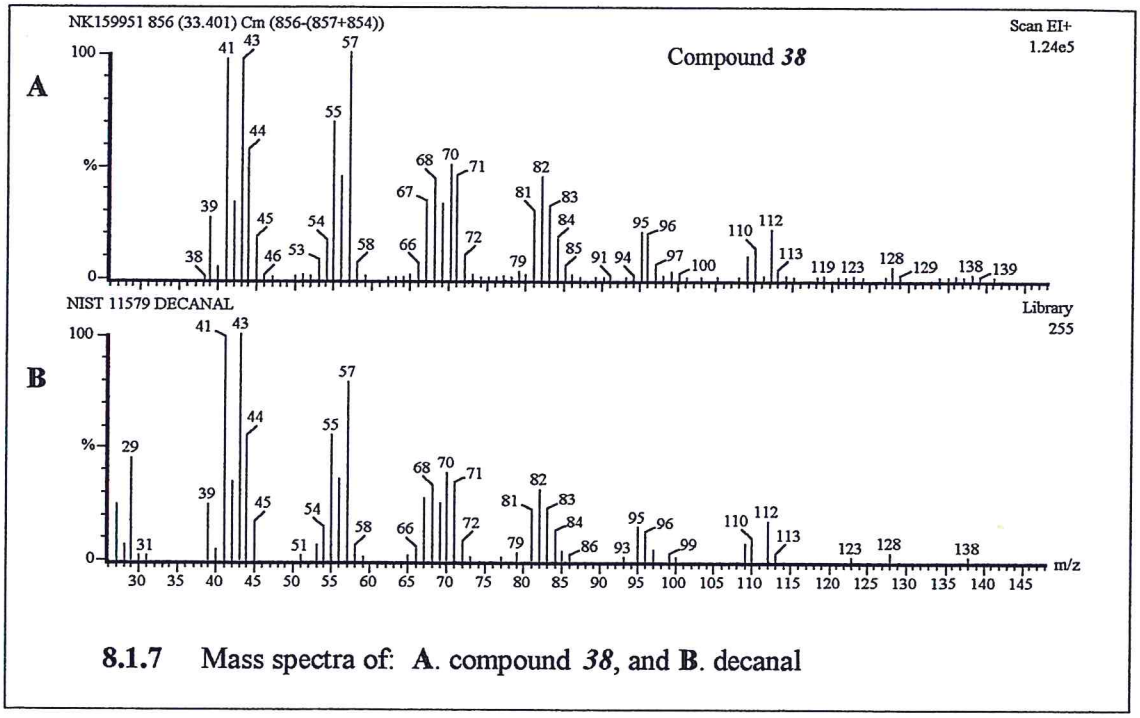
8.1.4 Mass spectra of: **A.** Compound ***35***, and **B**. (E)-2-heptenal.



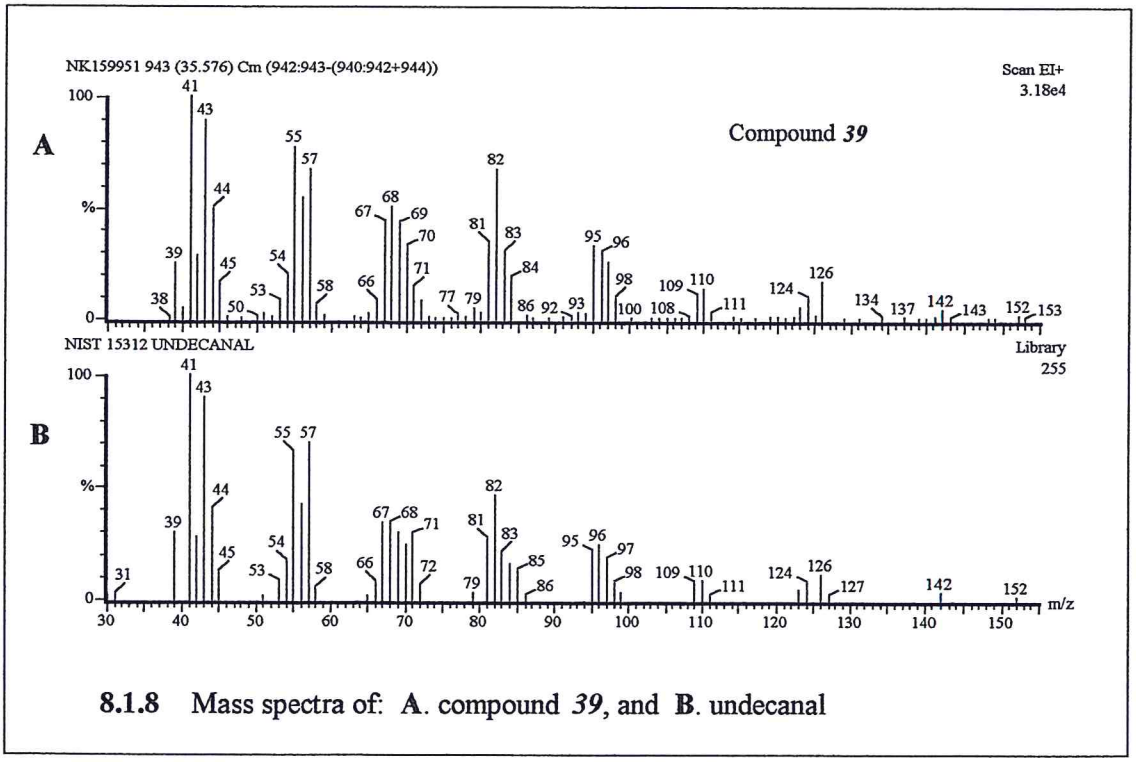
8.1.5 Mass spectra of: **A**. compound *36*, and **B**. (E)-2-nonenal



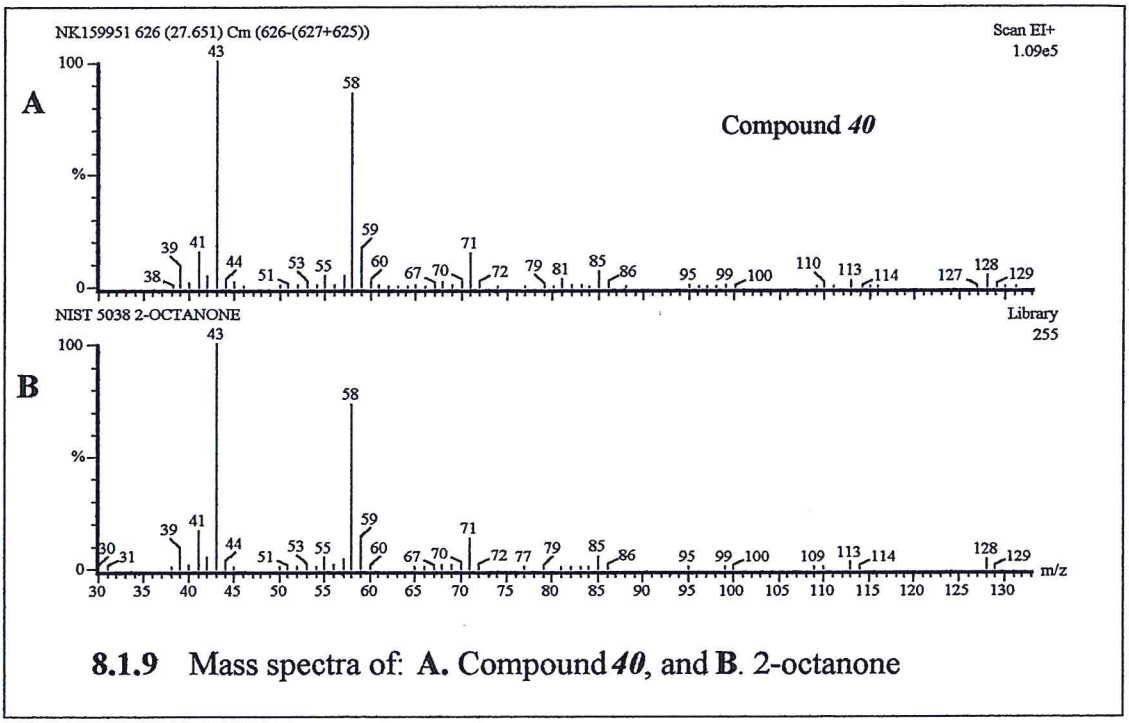
8.1.6 Mass spectra of **A**. compound *37*, and **B**. nonanal



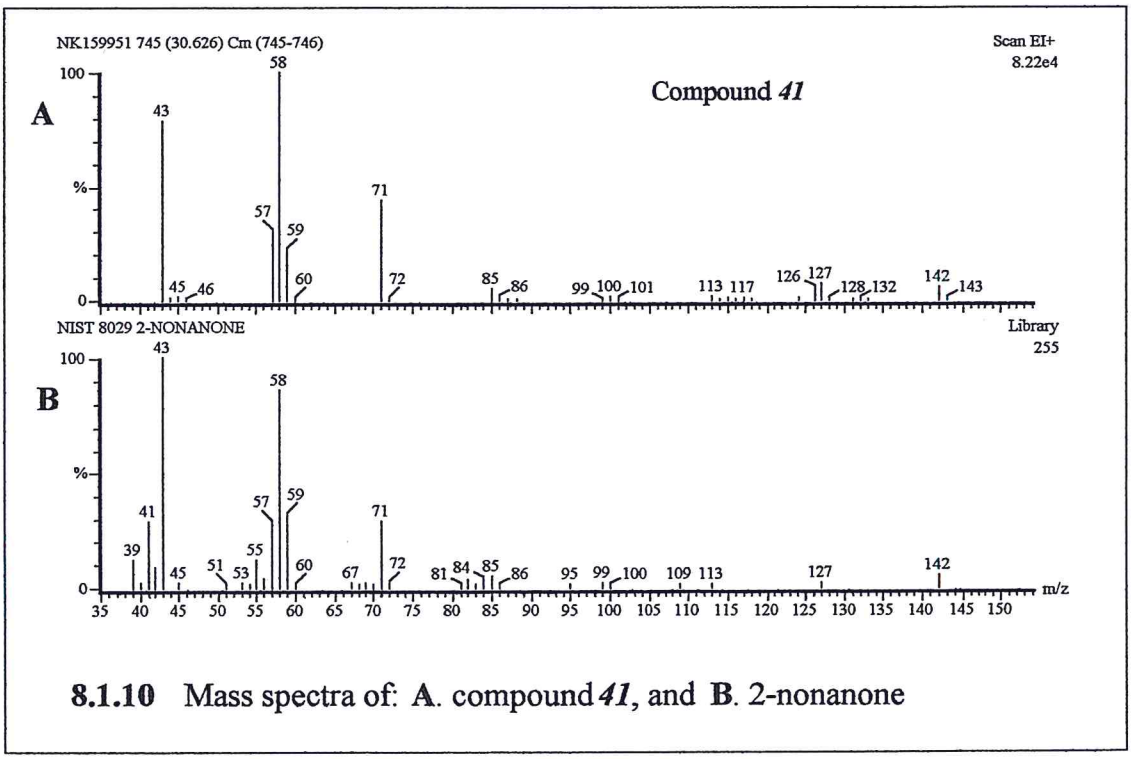
8.1.7 Mass spectra of: **A**. compound *38*, and **B**. decanal



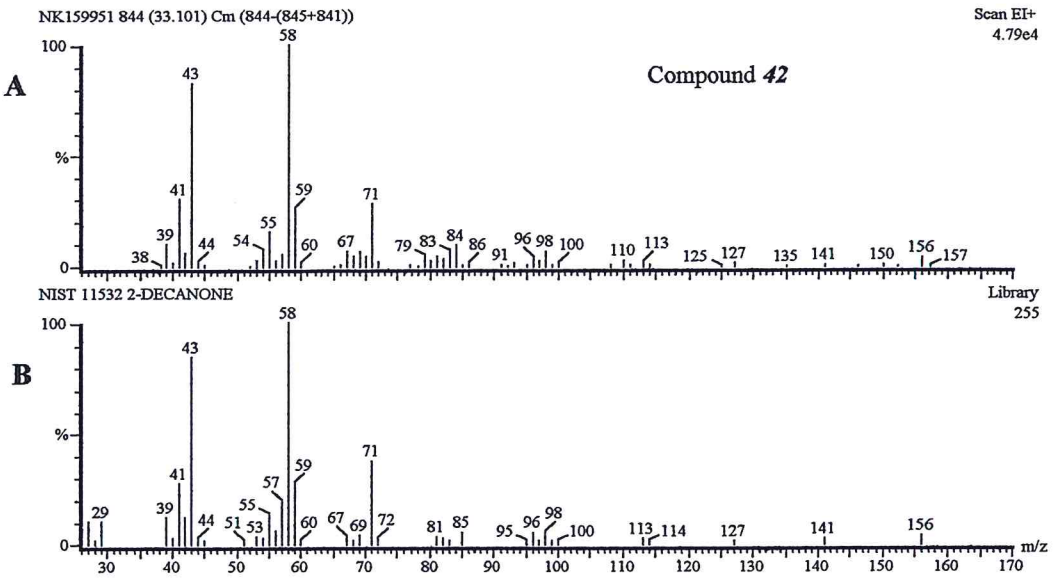
8.1.8 Mass spectra of: **A**. compound *39*, and **B**. undecanal



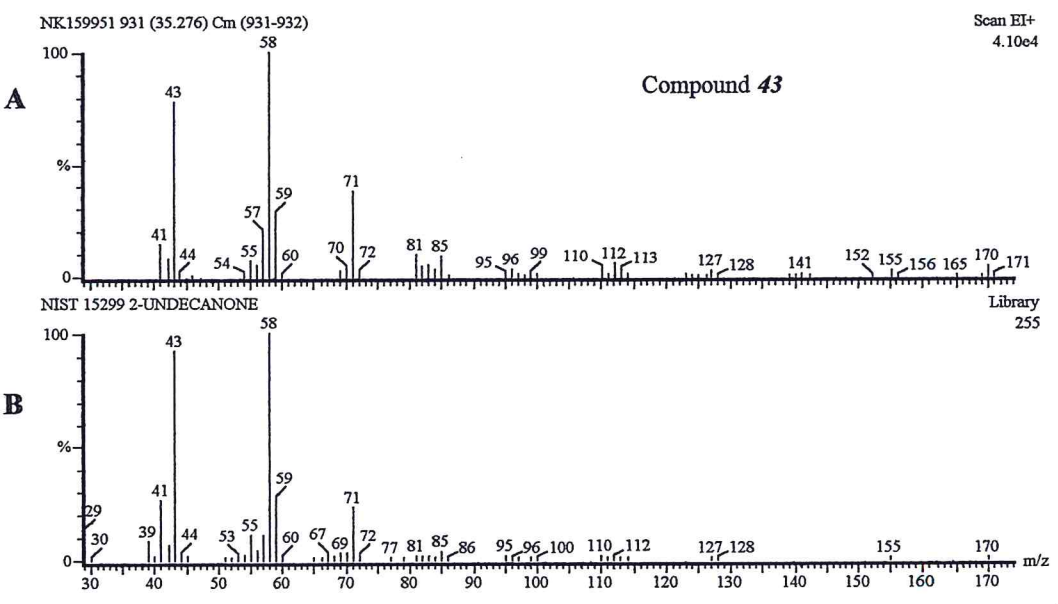
8.1.9 Mass spectra of: **A.** Compound ***40***, and **B**. 2-octanone



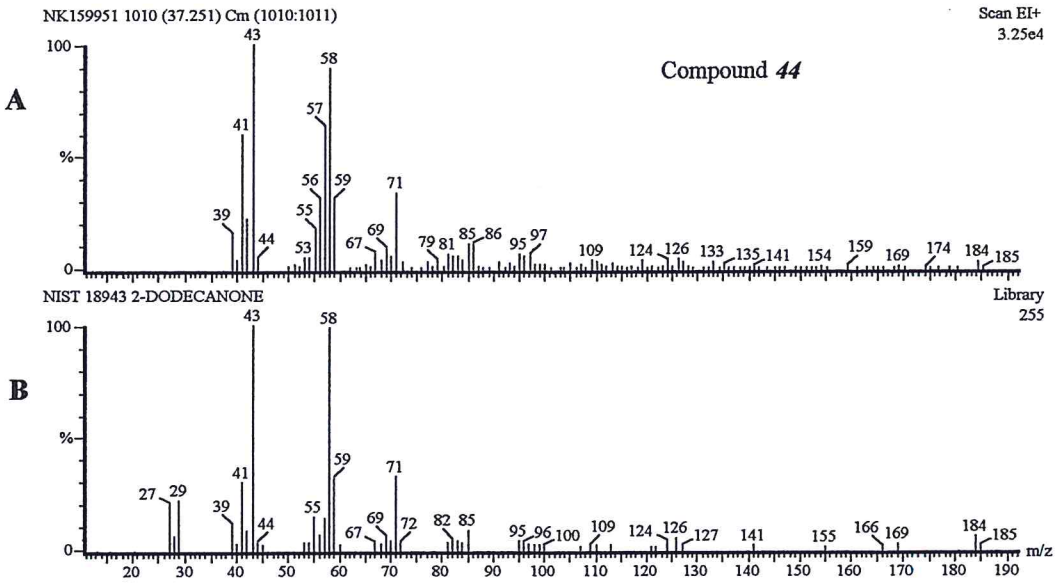
8.1.10 Mass spectra of: **A**. compound ***41***, and **B**. 2-nonanone



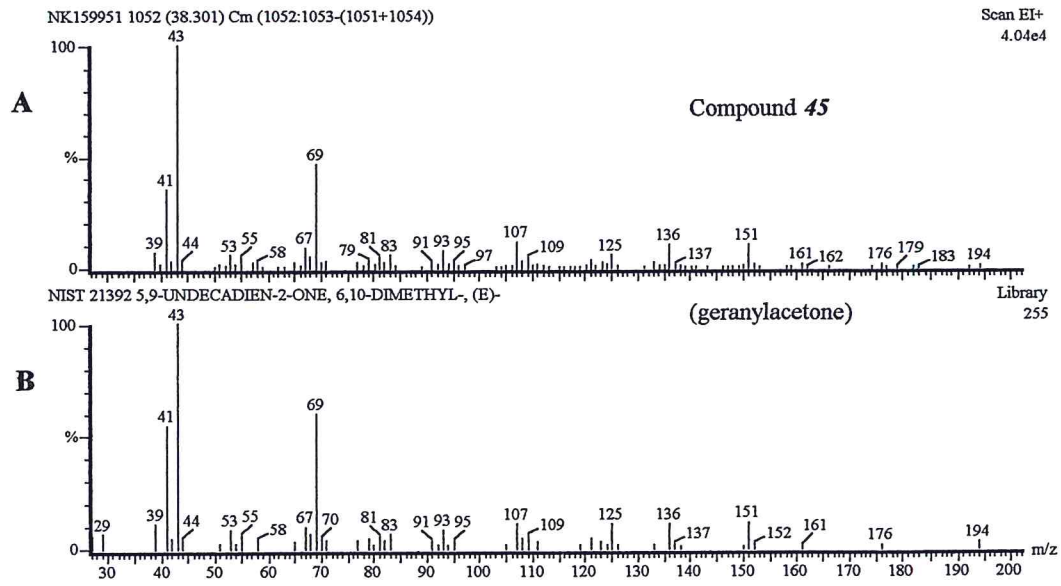
8.1.11 Mass spectra of: **A**. compound *42*, and **B**. 2-decanone



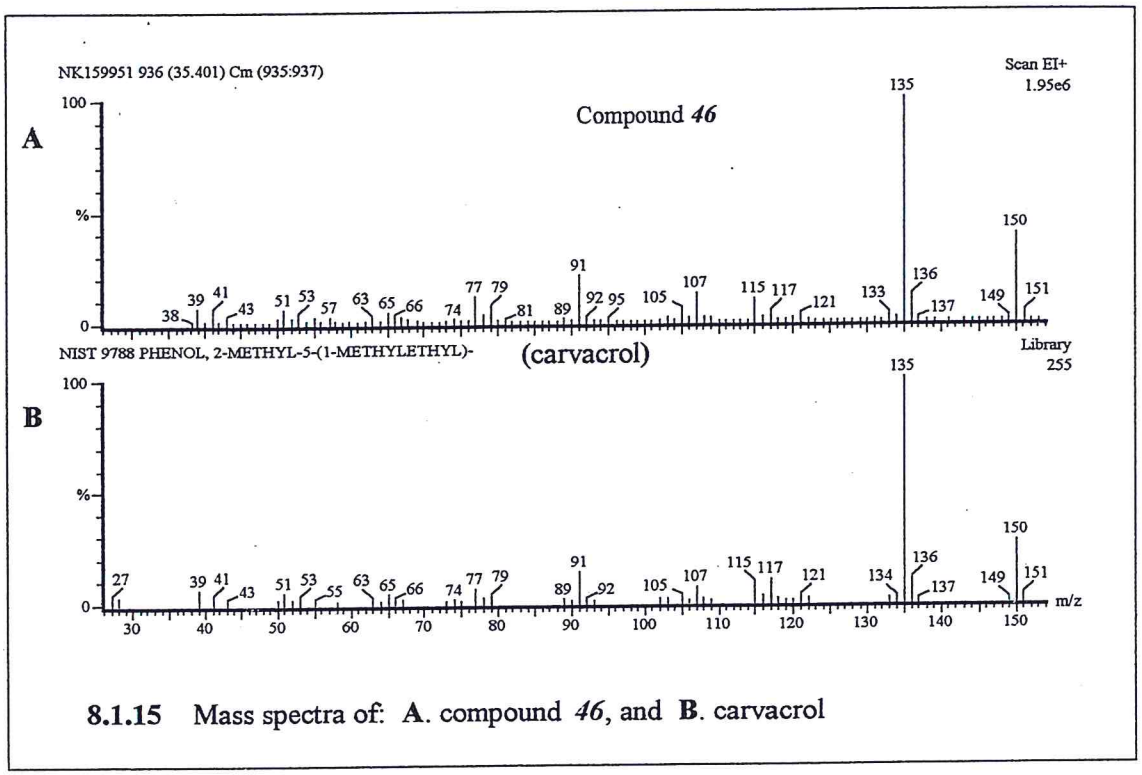
8.1.12 Mass spectra of: **A**. compound *43*, and **B**. 2-undecanone



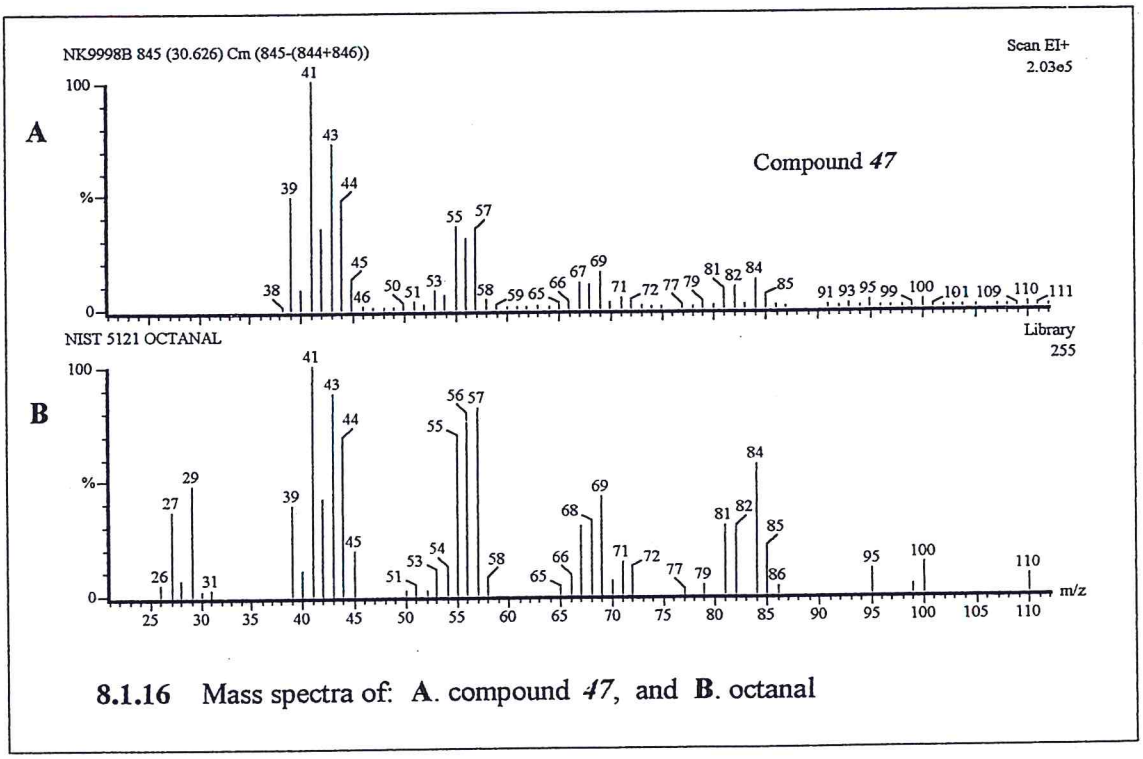
8.1.13 Mass spectra of: **A**. compound ***44***, and **B**. 2-dodecanone



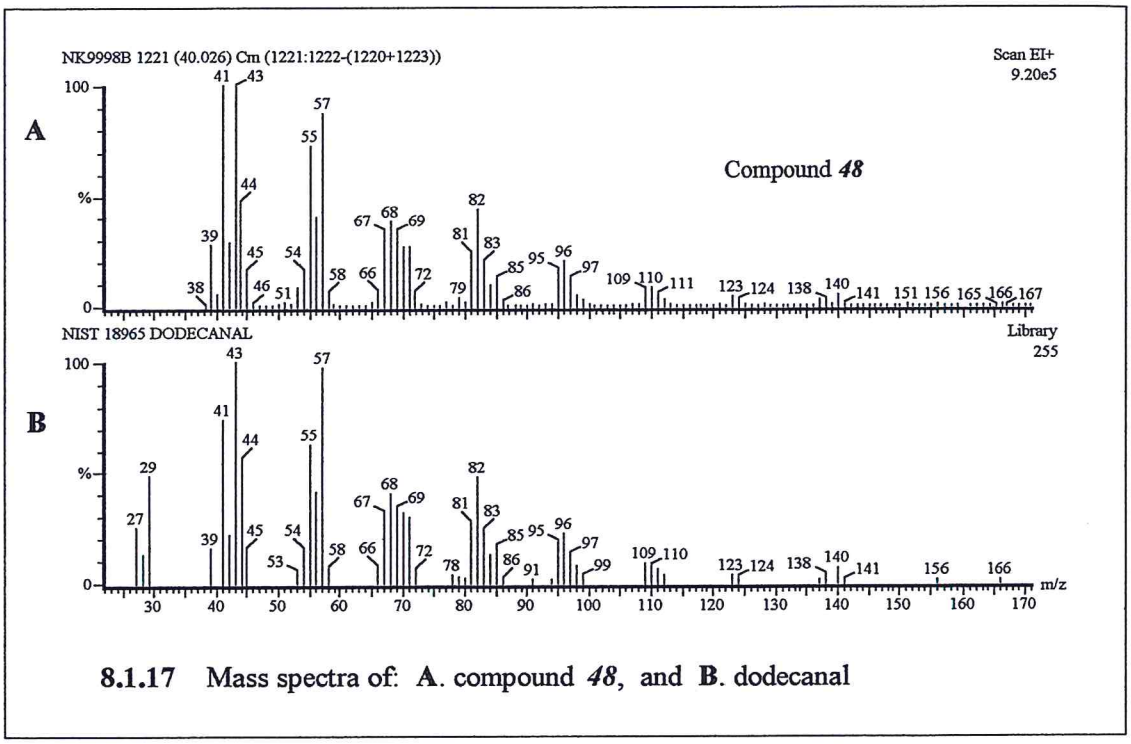
8.1.14 Mass spectra of: **A**. compound ***45***, and **B**. geranylacetone



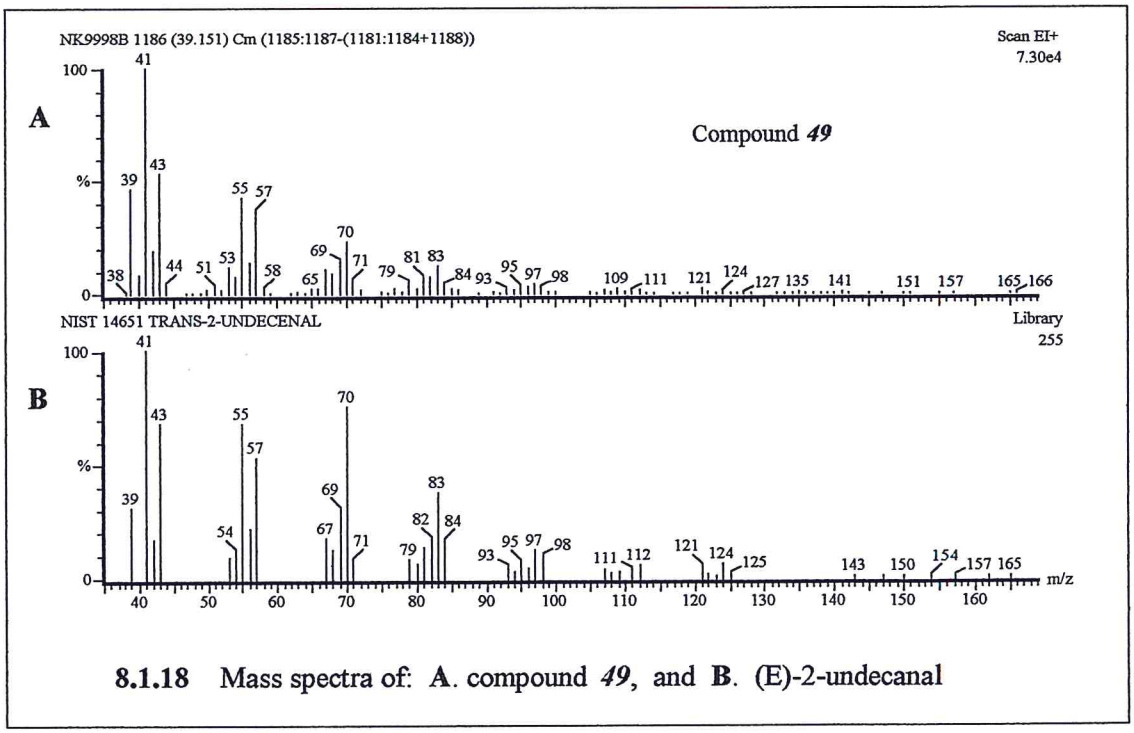
8.1.15 Mass spectra of: **A**. compound *46*, and **B**. carvacrol



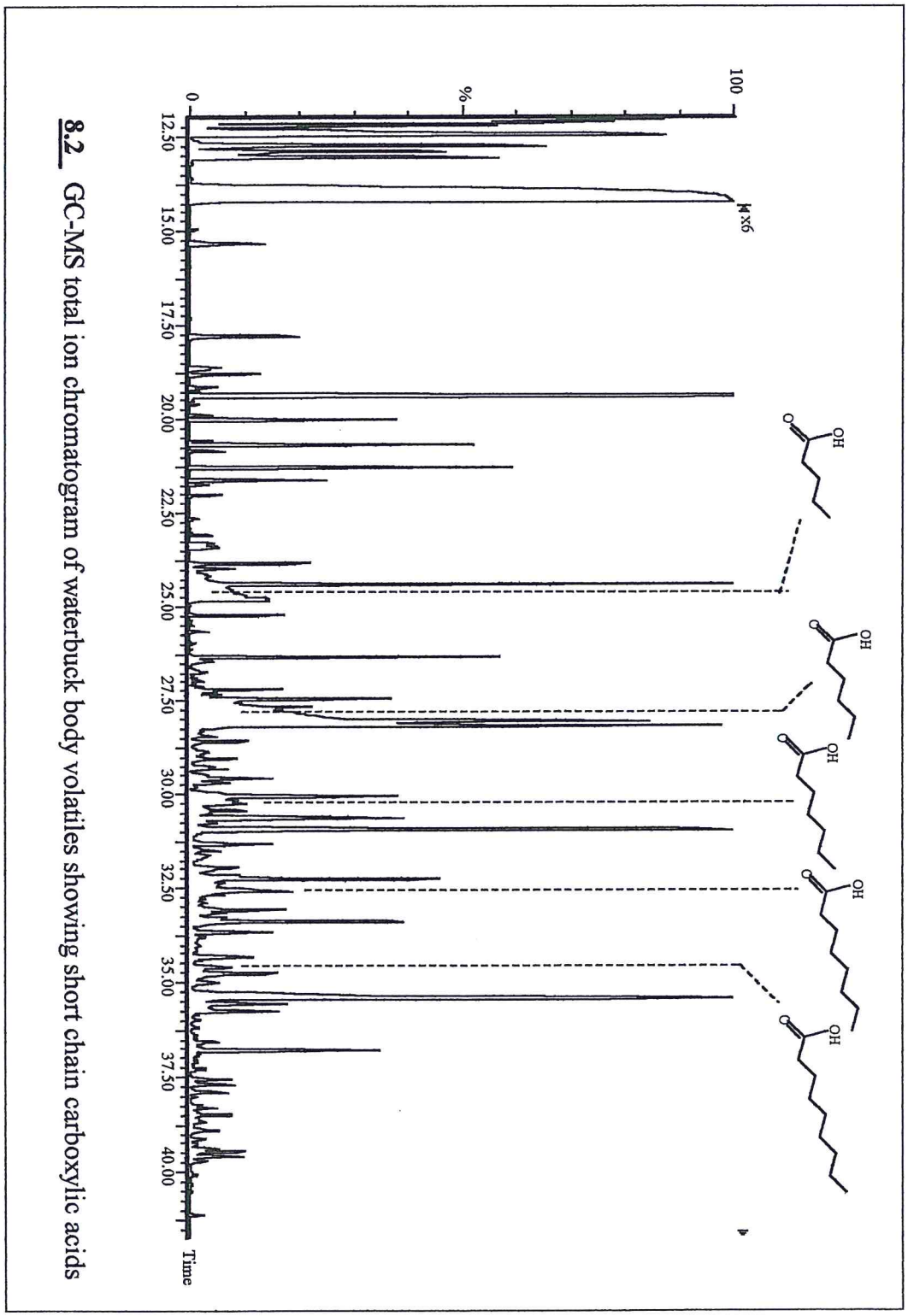
8.1.16 Mass spectra of: **A**. compound *47*, and **B**. octanal



8.1.17 Mass spectra of: **A**. compound ***48***, and **B**. dodecanal



8.1.18 Mass spectra of: **A**. compound ***49***, and **B**. (E)-2-undecanal



8.2 GC-MS total ion chromatogram of waterbuck body volatiles showing short chain carboxylic acids