1. INTRODUCTION

1.1 LITERATURE REVIEW

1.1.1 Tsetse flies and their role in disease transmission

Tsetse flies belong to the small, but highly specialized genus *Glossina* (Diptera: Glossinidae) (Pont 1980). The genus *Glossina* is limited to Africa south of the Sahara, occurring in an area of an estimated 11 million square kilometers (Nash 1969; Jordan 1995), but have also been recorded in south-western Saudi Arabia (Moloo 1993). In Africa the distribution of tsetse flies extends from latitude 14° N to 29° S in 38 African countries, with either continuous or isolated areas of infestation (Ford 1970; Kuzoe 1991, Moloo 1993). There are 23 species and eight subspecies, belonging to three species groups. These are the fusca, morsitans and palpalis groups of species, which are distinguishable on the basis of the male and female genital armatures (Newstead *et al.* 1924; Moloo 1993; Jordan 1995). Tsetse flies are remarkable for their viviparity, females producing one fully-grown larva approximately every 9-11 days (FAO 1982). Both sexes feed solely on vertebrate blood. In the course of their feeding they transmit pathogenic flagellates of the genus *Trypanosoma* (Protozoa), which occur in the blood and organs of some of the African wild ungulates. Various species of *Trypanosoma* are the cause of trypanosomosis in humans and in domestic stock where they affect cattle, horses, sheep, goats and pigs (Connor 1994). They are harmless to game animals (except when animals are stressed) which, therefore, act as reservoirs of the disease. Tsetse act as the invertebrate hosts of the parasite and are, therefore, the principal biological vectors of trypanosomosis in Africa (Bruce 1895; Newstead *et al.* 1924; Aschcroft 1959). Other flies may play a lesser role as mechanical transmitters (Leak 1999).

1.1.2 Trypanosomosis – economic importance

Tsetse-transmitted trypanosomosis is recognized widely as a major animal and human disease (Jordan 1986). The wide distribution of tsetse and

trypanosomes, and their severe impact on animal production and agricultural production systems, makes animal trypanosomosis among the most important disease constraints of the agricultural sector, restraining agricultural advancement in over 46 % of the African continent (Buxton 1955; Glasgow 1963; Ford 1971; Ford & Katondo 1977; Rogers *et al.* 1994). Bovine trypanosomosis or "nagana" can cause anaemia, production losses, abortion and mortality in domestic herds and thus depresses all aspects of production: fertility is impaired, milk yields, growth and work output (draught power for ploughing) is reduced; and the mortality rate may reduce herd size (Connor 1994; Rogers *et al.* 1994). In its chronic form the disease results in poor health to ± 50 million people (in the form of human trypanosomosis or sleeping sickness) and enormous losses in livestock and, therefore, low industrial output (Kuzoe 1991). Losses of cattle are estimated at three million deaths, mainly young stock with up to 25 % mortality in pre-weaning calves. Mortality losses are combined by lower reproduction and less milk and weight gain. It is estimated that direct losses to agricultural production amount to about 4.5 billion USD in Africa annually (Budd 1999). Approximately 7 million km^2 of the tsetse-infested areas would probably be suitable for livestock and agricultural development if trypanosomosis were controlled (Finelle, 1974).

1.1.3 Disease and vector control aspects

There are different approaches to deal with the trypanosomosis problem (Jordan 1986). They vary from parasite control by treating infected cattle with therapeutic drugs, the use of trypanotolerant livestock, and finally control of the vector.

Parasite control

Since a broadly effective vaccine is unlikely to be developed (Williams & Williams 1992), the only effective treatment is the continuous dosage of trypanocidal drugs such as the therapeutic Diminazene (Berenil®) or the prophylactic Isometamidium (Samorin® and Trypamidium®). Drug therapy

has in the past been the main control activity in many countries. This has been partially effective in some circumstances where trypanosome resistance to drugs has developed due to drug mismanagement (Fox *et al.* 1993; Budd 1999). This is of particular concern as there are few drugs available and this situation is unlikely to change in the foreseeable future (Alsop 1993).

Trypanotolerant livestock

There is an increasing interest in using cattle breeds that are naturally resistant to trypanosomosis (trypanotolerant) (Murray *et al.* 1981). These breeds, however only comprise approximately five percent of the current cattle population in Africa (ILRAD 1989, cited in McMillan & Meltzer 1996). Furthermore, trypanotolerance does not offer complete protection and the breeds can still succumb to the disease under intense tsetse challenge.

Vector control

In much of Africa trypanosomosis control focused on the large-scale control of the vector with the ultimate aim being its eradication (Jordan 1985). Vector control aims to reduce or eliminate contact between tsetse and humans or livestock. Initially the resettling of people and, therefore, the movement of domestic stock away from tsetse infested areas was undertaken (Cockbill *et al.* 1963). This approach, however, shifted to the control and eradication of tsetse populations (Mulligan 1970; Jordan 1976, 1978, 1985; Dame & Jordan 1981).

ELIMINATION OF HOSTS AND CLEARING OF HABITAT

Early approaches to tsetse control included the extermination of the preferred host species of tsetse in infested areas (du Toit 1954; Cockbill 1960; Bursell 1970) as well as random clearance of woody vegetation (Nash 1940; du Toit 1954; Jordan 1974; Davies 1981; MacLennan 1981). These methods, however, are regarded as ecologically unacceptable and would often be unsuccessful as tsetse are capable of changing their breeding habitats and also their hosts in the

absence of their favourite species (Vale & Cumming 1976), while vegetation invariably grows back.

USE OF INSECTICIDES

Since the Second World War the control of *Glossina* has become increasingly dependent upon the use of insecticides. Ground and aerial application of DDT, HCH, dieldrin and endosulfan were widely used in Africa and have proved to be very effective for the management of crisis situations (du Toit 1954; Davies 1981; Turner 1984). These methods are, however, comparatively expensive and nowadays not favoured because of logistical requirements and for environmental reasons.

Currently, two vector control methods are preferred and are more selective and environmentally friendly. Improved understanding of visual and olfactory stimuli responsible for the host-seeking behaviour of tsetse flies, led to the development of artificial bait technology. In recent years reliance for the control of tsetse flies in parts of Africa has, therefore, increasingly been placed on attracting them to stationary targets, i.e. insecticide-impregnated odour-baited targets of the right colour (Vale *et al.* 1986; Vale *et al.* 1988a; Willemse 1991; Knols *et al.* 1993; Van Den Bossche 1997).

The application of insecticide to domestic animals, primarily cattle, can be regarded as a modification of the target method whereby, instead of stationary targets, the treated animals are used as attractive, mobile, living targets (referred to as "mobile targets"). This may be applied in the form of cattle dips or pour-ons and is technically feasible and a promising technique in tsetse-infested areas where cattle occur (Thomson 1987; Thomson *et al.* 1991; Fox *et al.* 1993; Okiria & Kalunda 1994; Bauer *et al.* 1995). This system could also be used together with targets to maintain tsetse fly barriers (Warnes *et al.* 1997; Warnes *et al.* 1999). Mobile targets are, therefore, more likely to be effective on commercial ranches and communal farms where the density of wild hosts is usually low (Williams & Williams 1992). They are, however, inappropriate when it comes to tsetse fly control in game reserves or other

tsetse-infested areas where the flies cannot, for obvious reasons, come into contact with treated cattle.

The Sequential Aerosol Technique (SAT) classically involves applying a sequence (usually 5 or 6 applications at 12-20 day intervals) of extremely low dosage, non-residual, insecticide (Endosulfan or synthetic pyrethroids) (Budd 1999) as a fine aerosol applied characteristically by fixed-wing aircraft. It has proven itself to be a good method of tsetse control and eradication over the last 20 years. Its main advantage is that large areas (2,500 - 20,000 km^2) could be treated very quickly (3 months) and reduce the number of flies to the point of eradication (Budd 1999) with a minimal reliance on ground support workers (Alsop 1993). With the use of deltamethrin the active ingredient is applied at a very low dose (0,25 g per spray per 10,000 m^2) so that non-target side effects are comparatively minimal and transient (Roussel Uclaf, Glossinex® information leaflet, undated).

ALTERNATIVE CONTROL OPTIONS

The biological basis for the Sterile Insect Technique (SIT) is that tsetse female flies only mate once. Operationally the technique involves the production and release of sterilized male flies into a community where they mate with the wild females, which are then unable to produce any offspring. As the population is not replenished with new young it gradually decreases eventually leading to eradication of the whole population. Inundated release of mass-reared, radiation sterilized tsetse males of several species has been used successfully to suppress populations of tsetse flies (Politzar *et al.* 1980, cited in Langley 1999; Williamson *et al.* 1983). This SIT has also been applied and was particularly successful to eradicate *G. austeni* Newstead from Unguja Island, Zanzibar (Saleh *et al.* 1999). It is also nowadays planned in other operational control programmes (FAO/IAEA 2000). This technology is, however, costly (Williams & Williams 1992) because huge colonies of tsetse have to be reared and facilities to sterilize these flies have to be established. Therefore, it is not sustainable for implementation at the local level. It is, however, justifiable if the objective is eradication. Its advantages of species-specificity and non-

contaminating nature are almost in all circumstances outweighed by the high costs and considerable sterile insect production and release logistics (Alsop 1993). However, although not yet proven on a very large scale, its reputation in other fields suggests that it could be another effective tool in the fight against trypanosomosis (Budd 1999).

The use of Insect Growth Regulators (IGRs) does provide an alternative to the use of insecticides control (Jordan *et al.* 1979) and works on the principle of sterilization rather than killing (Langley 1999). Sterilizing tsetse in the field by treating females with juvenile hormone, pyriproxyfen, through contaminating traps or targets has proven successful in the field and could be used with fair confidence in large-scale experimental control operations (Hargrove & Langley 1990, 1993). The chitin synthesis inhibitor triflumuron has also been proven to work as an alternative (Langley 1995; Bauer *et al.* 1999). However, it was suggested that IGRs as alternatives only be applied for control with targets if resistance to pyrethroids appears in tsetse populations (Langley 1999).

Biological control with the use of entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae* has also been demonstrated as a promising alternative (Kaaya *et al.* 1991). The value of this technique, now called the Lethal Insect Technique (LIT), has also been demonstrated in the field (Mahamat & Okech 1999).

1.1.4 Control/suppression and eradication prospects

The past trypanosomosis control policies of national control organizations, significantly supported by donor agencies, have placed emphasis on the eradication of the tsetse vector (FAO 1991, cited by Alsop 1993). Although often successful in achieving their objective of controlling trypanosomosis within the project area, these actions have usually not produced sustainable results because of the inability to consolidate and protect against reinvasion in the longer term. Financial continuity and commitment have rarely been adequate so that it was recommended that the problems experienced

necessitated a change of approach and re-defining of objectives (FAO 1991, cited by Alsop 1993) with a change in emphasis from vector eradication towards vector suppression.

Whereas eradication implies a once and for all cost and solution to the problem, suppression is an ongoing process with a recurrent budgetary commitment. Suppression can only be justified if it can be achieved and maintained at a reasonable cost, and is environmentally acceptable (Alsop 1993).

A major new initiative to eradicate tsetse flies from Africa was the recent establishment of PATTEC (Pan African Tsetse and Trypanosomiasis Eradication Campaign) which demonstrated the commitment of Heads of African States. It has only been possible to clear tsetse from 5 % of its range and the PATTEC objective is to drive towards larger and larger tsetse-free and disease-free zones in Africa (OAU/IBAR 2000; PAAT 2000).

1.1.5 Tsetse and trypanosomosis situation in South Africa

The historical tsetse and trypanosomosis situation in South Africa was reviewed by Kappmeier *et al.* (1998). Presently trypanosomosis or nagana occurs only in the north-eastern areas of KwaZulu-Natal Province where the two tsetse fly species, *G. brevipalpis* Newstead and *G. austeni* Newstead are its vectors. These flies are confined to evergreen forests and thickets, often associated with water-courses and other densely or semi-forested areas. They are responsible for the transmission of various trypanosome species to livestock. These are the severely pathogenic tsetse-transmitted *Trypanosoma* species *T. brucei* Plimmer & Bradford (in horses), *T. vivax* Ziemann (in cattle), *T. congolense* Broden (in cattle) and *T. simiae* Bruce *et al.* (in pigs) (Connor 1994).

Sleeping sickness has never occurred in South Africa (Kuzoe 1991) so that the focus is on nagana. The main nagana problem areas of KwaZulu-Natal Province are in the magisterial districts of Ingwavuma, Ubombo, Hlabisa and

Nongoma in the north-east (De Waal *et al.* 1998). The area is confined to some 16,000 km^2 and contains 426,000 humans, 130,000 small ruminants (De Waal *et al.* 1998), *c.* 350,000 cattle belonging to developing farmers in communal farming areas and *c.* 9,000 cattle on commercial farms. This number of cattle amounts to *c.* 10,8 % of those in the entire KwaZulu-Natal (A. Ilemobade, unpublished report 1997).

In 1990 an outbreak of nagana contributed to severe cattle mortalities in the communal areas of the magisterial districts mentioned above, involving *T. vivax* and *T. congolense* (De Waal *et al.* 1998). Between 1990 and 1994 surveys showed cattle served by 77 of 132 diptanks to be infected with nagana. Emergency control measures, which consisted of the treatment of cattle with homidium bromide (Ethidium®) and diminazene (Berenil®) as well as the weekly to fortnightly dipping of cattle in a pyrethroid, cyhalothrin (Grenade®) brought the disease under control (Kappmeier *et al.* 1998). This dipping was maintained for only two years so that ticks would not develop resistance as they have to the chlorinated hydrocarbons.

In 1992 odour-baited targets were used in a trial to control *G. brevipalpis* in the northern parts of the Hluhluwe-Umfolozi Game Reserve (Kappmeier *et al.* 1998). Use was made of the target technology then used in Zimbabwe for the control of *G. morsitans morsitans* Westwood and *G. pallidipes* Austen (Vale *et al.* 1988a). The target consisted of a 1,5 m wide x 1 m high black cloth baited with the synthetic odours 3-*n*-propylphenol, 1-octen-3-ol, 4-methylphenol and acetone. This trial was ineffective due to a number of possibilities (Kappmeier *et al.* 1998). Control efforts have, therefore, been put on hold until further research could provide the correct tools to use target technology for these species successfully. For this purpose, in 1992, the National Directorate of Veterinary Services contracted ARC-Onderstepoort Veterinary Institute (ARC-OVI) to develop a long-term strategy for the control of nagana and the present study formed part of this effort.

1.2 JUSTIFICATION

The use of drugs for the treatment and prevention of trypanosomosis is costly. Where a significant large-scale and long-term impact is needed, emphasis is placed on the control or eradication of the tsetse vector from the infested areas. In general the cheapest approach to tsetse control is by use of pyrethroid treated cattle. Where no cattle are present then targets are likely to be the best control tools. A practical and effective coloured target and attractive odour-bait system was developed which, it is considered, could prove effective for the control of both species when used in the field. The combination of the best South African (SA) odour developed for *G. brevipalpis*, namely 1-octen-3-ol (released at *c.* 9,1 mg/h), 4-methylphenol (released at *c.* 15,5 mg/h) and acetone (released at *c.* 350 mg/h) (Kappmeier & Nevill 1999a), together with a 1,75 m black/p.blue/black target (Kappmeier & Nevill 1999b), appeared to be an effective combination to employ as a control device for both species (Kappmeier & Nevill 1999c).

However, a number of studies were still needed in order to apply target technology successfully for long-term control and/or eradication of the tsetse flies in NE KwaZulu-Natal. These were the following:

1.2.1 Odour-bait for *G. austeni*

Odours have been used to attract tsetse flies to traps and targets (Snow 1980). However, none of the odour components tested in South Africa was attractive for *G. austeni* (Kappmeier & Nevill 1999a) except for carbon dioxide (Kappmeier, unpublished), which is impractical to use. The present lack of conventional odour attractants for *G. austeni* highlights the importance of searching for attractants specific for this species and efforts should thus focus on this. Although the recommended target was still effective for *G. austeni* in the absence of any odours (Kappmeier & Nevill 1999c), finding an appropriate odour for this species would mean that more of them could be attracted to the vicinity of a target or trap and they could be attracted from further away (Hargrove & Vale 1978; Torr 1990; Hargrove *et al.* 1995) so that faster control

should then be achieved. More efficient odour attractants would also allow fewer targets to be deployed per km^2 and would reduce costs (Leak 1999).

1.2.2 Trapping devices for *G. brevipalpis* and *G. austeni*

Population monitoring using traps provides essential information before and during tsetse control and eradication operations on the distribution of tsetse flies, the degree of control achieved at any given time and also on population composition, e.g. age structure. Sticky traps have been invaluable in determining presence or absence, sexes, age structure and whether marked with a fluorescence substance or not, as in the SIT eradication campaign on Unguja Island (Vreysen 1995, Vreysen *et al.* 1998). Following the early designs (Hall 1986) of sticky panels of various shapes and colours for *G. austeni*, these needed to be tested and improved for *G. brevipalpis* and *G. austeni* in South Africa. These sticky traps would, therefore, be useful tools for monitoring purposes.

To site insecticide treated targets successfully in optimal locations and densities in the field it is, however, also necessary to use a suitable trap to obtain base-line data on the behaviour, movement, population structure, density and ecology of the two species. Sticky traps kill the flies so that they cannot be used for mark-release-recapture studies and traps that capture live flies are therefore needed. For *G. austeni* no such trap exists as its behaviour is elusive and only low numbers are caught in existing tsetse fly traps elsewhere in Africa (Takken 1984, Hall 1986, Madubunyi 1990). For *G. brevipalpis* the only trap available for this purpose was the Siamese trap but it is only partially effective for this species in Kenya (Kyorku *et al.* 1993). It was, therefore, necessary to develop a trap for the purpose of catching live flies.

1.2.3 Population density and dispersal

For targets to be utilized effectively one needs to know the tsetse flies' population density and dispersal distances and rates. For the savanna species *G. m. morsitans, G. m. centralis* and *G. pallidipes* a target density close to four

targets per km^2 (with attractive synthetic odours) is necessary and sufficient to eradicate a tsetse population in nine months to a year (Hargrove 1993). What the required densities would be for *G. brevipalpis* and *G. austeni* has not yet been established. In order to control these species successfully with the newly developed targets, it was necessary that trials be conducted to establish more or less the number of targets needed per square kilometer.

Knowledge of target density is also needed if targets are to be deployed in barriers in control operations to prevent reinvasion from uncontrolled areas (Hargrove 1993). However, unsuitable habitat between pockets of forests and thickets can also act as "natural barriers". It is, therefore, also necessary to obtain knowledge on their dispersal and movement and thereby determine the distance of "unsuitable" habitat between pockets of forests and other suitable habitat, which could act as a natural barrier between controlled and infested areas.

1.2.4 The role of livestock in the control of tsetse and nagana

Demographic pressures and increases in livestock numbers are causing overgrazing, thereby forcing cattle owners to graze their cattle in tsetse habitat in their search for pasture. This is particularly the case in rural Zululand where it could be seen that cattle graze right up to the fences of the game reserves, which act as the main sources of tsetse flies. In these situations cattle can be used as mobile targets when treated with a suitable insecticide. What proportion of *G. brevipalpis* and *G. austeni* would feed on cattle is, however, not certain. There are also high numbers (*c.* 130 000) of small ruminants, i.e. goats in the rural areas (De Waal *et al.* 1998). If tsetse also feed on goats, these could accordingly be used as mobile targets. The role/importance of livestock, therefore, needs to be established in the Zululand situation for tsetse control management. Furthermore, since treatment with insecticides will not prevent infection, disease challenge could also be high in these high-risk areas and it may be necessary to protect cattle from tsetse fly attack. Improved knowledge on the times and situations when cattle are at most risk of tsetse challenge will aid in the development of strategies for minimizing contact.

1.2.5 Tsetse fly distribution surveys

The successful planning of a control/eradication campaign depends primarily on reliable knowledge of the tsetse distribution in an area and on what species occur in which areas. The last accurate information on the distribution of *G. brevipalpis* and *G. austeni* in South Africa was that obtained through tsetse surveys in the 1950s (du Toit 1954, 1956). Therefore, it was necessary that the relative distribution of both species in KwaZulu-Natal be re-surveyed.

Furthermore, reliable tsetse distribution mapping is necessary to define tsetse distributions. Geographical Information Systems (GIS) provide the ideal means for this. GIS, together with remote sensing techniques, also provide the means for overlaying layers of spatial and temporal information including land use patterns, vegetation, climate, topography and human, livestock and disease distributions, in effect the very parameters which characterize production systems (Lillesand & Kiefer 1994, cited in Erkelens *et al.* 2000). It will, therefore, be a useful tool for integrating tsetse distribution and abundance with disease distribution and prevalence, land use and tenure, livestock distribution and abundance to facilitate with the decision-making and assist with planning interventions of the nagana problem in KwaZulu-Natal.

1.3 PROBLEM AND HYPOTHESIS

Since no control measures against tsetse and trypanosomosis in South Africa are currently applied, it is necessary to strategically plan the control and/or eradication of tsetse flies throughout the infested areas of N.E. KwaZulu-Natal. This study should provide a sound basis for planning tsetse control/eradication operations in an area-wide integrated control/eradication strategy for both tsetse species in this region.

The north-eastern region of KwaZulu-Natal where nagana and tsetse are present consists of a number of land use categories. These consist of Game Reserves and nature conservation areas, communal farms, commercial farms, indigenous forests as well as commercial plantations.

The choice of control strategy appropriate to a particular land use category is determined by many factors. For example, aerial spraying may be considered inappropriate for a conservation area, while it could be used where tsetse flies occur outside the Game Reserves. Targets, which are considered most environmentally friendly (Nagel 1995), could be a good choice in conservation areas, while in communal areas, where cattle are present, pyrethroid application to livestock could be more effective and without the danger of target theft.

Thus, *Glossina brevipalpis* and *G. austeni* can be controlled and/or eradicated by the integration of various tsetse control options in various tsetse-infested areas. A strategy will be proposed for their sustainable control and/or eradication in N.E. KwaZulu-Natal.

1.4 OBJECTIVES

- To conduct studies to find an attractive odour(s) for *G. austeni* and to obtain a better understanding on the role of odours for both species. Simultaneously these studies will give results for *G. brevipalpis*.
- To develop a trap(s) that is suitable to survey the distribution of *G. brevipalpis* and *G. austeni* and to monitor future control programmes.
- To develop a trap to capture live flies of both species in relatively large numbers for population dynamics or other ecological studies.
- To determine the density and dispersal of *G. brevipalpis* and *G. austeni* in order to establish the required target density and what constitutes a natural barrier to dispersal.
- To determine the value of livestock for *G. brevipalpis* and *G. austeni* control and determine tsetse challenge to livestock in different sites at various times of day.
- To analyze, interpret and map data of a survey of the distribution of *G. brevipalpis* and *G. austeni* and to use a Geographical Information System (GIS) to integrate tsetse distribution and abundance, disease distribution

and prevalence, land use, land designation/tenure as well as cattle distribution so as to assist in the planning of sustainable tsetse control.

- To propose a strategy for the environmentally sound and sustainable control (or eradication) of *G. brevipalpis* and *G. austeni* in N.E. KwaZulu-Natal.

1.5 EXPECTED BENEFITS ARISING FROM THIS STUDY

The proposed research will contribute to an improved understanding on the ecology, behaviour, monitoring and control aspects of *G. brevipalpis* and *G. austeni*. This could also be used by other African countries, which host these species, since very little research is conducted elsewhere on these two difficult species. Accurate information on the distribution of tsetse flies in South Africa will become available which will benefit in the planning of control operations. Appropriate intervention methods in different land use areas will be proposed and the overall research will facilitate planning for the sustainable control of tsetse flies in N.E. KwaZulu-Natal. Ultimately, the benefits to farmers will depend on the extent that a reduction in trypanosomosis challenge translates into improved health and productivity of affected livestock populations, the extent that the opportunities presented for the development of more effective and profitable systems of livestock keeping are taken up by the rural community concerned (Alsop 1993) and the extent into how it could relieve other disease challenges, e.g. tick-borne diseases, which act as major constraints for the production of cattle in N.E. KwaZulu-Natal (De Waal *et al.* 1998).

2. GENERAL MATERIALS AND METHODS

2.1 STUDY AREA

The study was conducted at Hellsgate Military Base, situated on the Ndlozi peninsula north of Charter's Creek, Lake St. Lucia (28°02'40"S 32°25'50"E), which forms part of a nature conservation area. Both *G. brevipalpis* and *G. austeni* are present in habitat consisting of evergreen sand forest, bushland and thickets occurring in a ± 0,5 - 2,0 km wide stretch along the edge of the saltwater lake-system. This forms part of the coastal forest and thornveld (Acocks 1988). The remaining vegetation consists of thorn and palmveld (*Acacia* spp., *Syzygium cordatum, Phoenix reclinata, Hyphaene natalensis*) with patches of bushed grassland, dominantly on regic sands (MacVicar *et al.* 1986).

The area has an altitude between 0 - 50 m and forms part of the coastal belt, ± 12 km from the sea. The mean annual temperature is 21 - 22 °C (Schultze 1982). Climatic records from a Stevenson Screen at Hellsgate Tsetse Research Station (1994 - 1997), showed mean maximum temperatures for the hottest months to be *c.* 29,7 °C in February and March and the mean minimum for the coldest months (June and July) *c.* 11,6 °C. The relative humidity is high with an annual mean maximum of *c.* 96,5 % and an annual mean minimum of *c.* 62 %. Summer rainfall occurs with annual precipitation of *c.* 950 mm (minimum of 0 - 5 mm in June - July and maximum of *c.* 260 mm for each month between October - February [this may vary annually]). The prevailing winds are south-westerly and north-easterly.

The animal life in the area consists *i.a.* of hippopotamus (*Hippopotamus amphibius*), warthog (*Phacochoerus aethiopicus*), bushpig (*Potamochoerus porcus*), nyala (*Tragelaphus angasii*), bushbuck (*T. scriptus*), red duiker (*Cephalophus natalensis*), grey duiker (*Cep. monticola*), reedbuck (*Redunca arundinum*), vervet monkey (*Cercopithecus pygerythrus*), samango monkey (*Cer. albogularis*) and some nocturnal small mammals like the bushbaby (*Galago crassicaudatus*), porcupine (*Hystrix africaeaustralis*), genet (*Genetta*

sp.) and serval (*Felis serval*). Kudu (*T. strepsiceros*) were introduced to the area in September 1997. Crocodiles (*Crocodylis niloticus*) and water monitors (*Varanus niloticus*) are also common and there is abundant bird-life. There is no domestic stock in the area except for two experimental cattle introduced in 1997 (until 2000). Many of the mammals that occur at Hellsgate have been shown to be natural hosts of *G. brevipalpis* and *G. austeni* (Moloo 1993).

2.2 GENERAL TECHNIQUES AND EQUIPMENT

2.2.1 Tsetse fly evaluation techniques

The use of targets and electric grids

Visual targets incorporated into electric grids (Vale 1974a) are widely used as capturing devices and were used as capturing tools of the tsetse flies throughout some of the studies following. The grids consisted of various sizes of an aluminium frame with fine copper wires spaced close together electrifying both surfaces of either a coloured cloth target or an almost invisible black gauze screen, which was inserted in the frame, so incorporating the grid into a visual or non visual target (Fig. 2.1). A high voltage (20,000 – 30,000 V) was applied between the wires (alternate wires being charged and earthed) by means of a high-tension unit (energizer unit), which operated from a 12 volt car battery. The non-visual target will henceforth be referred to as an "electric net" and was at times used together with a visual target (as in Fig. 2.1) to intercept flies flying around the visual target, or could be used on its own, as will be described later in this work. Unless stated otherwise, the grids were placed at a right angle to the wind so that tsetse flies, which flew upwind, could see the visual target. A corrugated iron tray, painted with polybutene, was placed underneath the grid. Tsetse flies colliding with the grid were electrocuted and dropped down onto the sticky surface of the tray so that flies which were stunned could be retained, counted and sexed.

Fig. 2.1 Visual (1 x 1 m pthalogen blue) and non-visual (0,5 x 1 m net) electric grids incorporated to form a flanked target (i.e. p.blue/net).

Traps

Unless stated otherwise, all trap catches were made by either XT sticky traps or the newly developed H trap. Details of all traps and their designs will be described in Chapter 4.

2.2.2 Odour baits and dispensers

Synthetic tsetse fly attractants

Initially traps (or sometimes electrified grids) were baited with a blend of acetone (500 mg/h), 1-octen-3-ol (0,4 mg/h), 4-methylphenol (0,8 mg/h) and 3-*n*-propylphenol (0,1 mg/h) at rates known to increase the catch of tsetse flies in Zimbabwe (Vale *et al.* 1988b). Henceforth this blend of odours is termed the Zimbabwe mixture (**Zim-mix**). Alternatively, following experiments on the

South African species' attractiveness to the Zim-mix (Kappmeier & Nevill 1999a), the South African blend was used, which consisted of the same components as the Zim-mix, but without 3-*n*-propylphenol and with acetone released only at 350 mg/h. This blend is henceforth referred to as the **original SA blend**. Following further experiments (Kappmeier & Nevill 1999a) a more effective odour was used, namely acetone released at *c.* 350 mg/h, 1-octen-3-ol released at *c.* 9,1 mg/h and 4-methylphenol released at *c.* 15,5 mg/h, referred to as the **best SA blend** for *G. brevipalpis*. Therefore, traps (or sometimes electrified grids) were either baited with the Zim-mix, original SA blend or best SA blend.

In some experiments, studies were made using other synthetic odour components, e.g. carboxylic acids, butanone, phenol, 3-methylphenol, 3-and 4-ethylphenol, etc. Octenol, phenols and carboxylic acids were dispensed from heat-sealed sachets of low-density polyethylene tubing (wall thickness 100 microns). These have the advantage that the release rate remains reasonably constant with age. The surface area varied between *c.* 8-75 cm^2 for the various chemicals (or mixture of chemicals) to produce the different release rates desired.

Ketones (acetone and butanone) were mostly dispensed from a glass bottle. The release rate was controlled by a hole in the lid and could be varied by varying the apertures of the hole. When minute doses of the ketones were needed these were also dispensed by means of polythene sachets, which gave a slower release.

Carbon dioxide was released from 200 kg cylinders at the required rates controlled by means of flow regulators and appropriate flow meters.

Natural attractants

In some experiments, studies were made of the numbers of tsetse attracted to a source of natural host odour. Where natural odour needed to be tested without any interference from a visual effect (i.e. without the visibility of the animal

itself), odour was obtained by placing an animal in an underground, ventilated pit (chamber) (see Fig. 3.1). The details will be given in Chapter 3.

Otherwise, e.g. in feeding response studies (Chapter 6), the animals were tethered to a post at a particular site.

Experiments with animals

Approval was given by the ARC-OVI animal ethics committee regarding the use of all animals involved in this study.

2.3 EXPERIMENTAL DESIGN AND ANALYSIS

All field experiments were carried out during the hours between early morning until dark, when both *G. brevipalpis* and *G. austeni* were found active (Kappmeier 2000). Treatments were incorporated into a series of replicated Latin Squares described by Fisher (1935, cited in Perry *et al.* 1980) consisting of days x sites x treatments or randomized block designs were used. In the **Latin square designs** the number of sites and days equaled the number of treatments. Since each treatment occured once at each site and on each day the treatment means were independent of any differences due to sites and days. Only one treatment occupied a site on any one day, so treatment interaction was avoided (Perry *et al.* 1980). Sites were far enough apart to ensure that interactions between treatments could not occur, but near enough to experience similar weather on any given occasion. The design was randomized to prevent systematic errors. Where only one site was available, treatments were compared using a **randomized block design**; groups of adjacent days were regarded as different blocks and treatments were randomly allocated to days within these blocks.

Unless stated otherwise, the daily catches (n) were normalized using a $\log_{10}(n)$ or, where zero catches were obtained, a $\log_{10}(n+1)$ transformation and subjected to analysis of variance (ANOVA). Differences between more than two means were assessed by a least significant difference test. The general test level was P

= 0,05. Use was made of either a programme designed specifically for the analysis of series of Latin Squares or using GLIM4 (Francis *et al.* 1993, cited in Torr *et al.* 1996).

Male and female catches were usually analyzed separately. The detransformed means are generally reported as a catch index, which was estimated by expressing the detransformed mean catch with the test treatment as a proportion of the detransformed mean control (standard) catch. Thus catch indices significantly greater than the control indicated attraction/ superiority.

Further details on the methodology and analysis of the various types of studies will be explained in the following chapters.

3. STUDIES TO FIND AN ATTRACTIVE ODOUR BAIT

3.1 ABSTRACT

Studies were conducted on the attractiveness of carbon dioxide, synthetic and natural host odours to find an attractive odour for *Glossina austeni* and, less importantly, to improve attraction of *G. brevipalpis*. Carbon dioxide was the most attractive component for *G. austeni* at release rates between 2 - 20 l/min. Natural cow odour was also very attractive for both species. An attempt was made to evaluate the effect of natural odour components other than carbon dioxide on the attraction of especially *G. austeni*. For this species cow odour was found to be equally effective to CO_2 released at the same rate as that expired by the cow. Also there was no difference in attraction when adding supernormal doses of octenol, 4-methylphenol and acetone to cow odour or to carbon dioxide. This addition rather had a repellent effect, suggesting that the remaining components of cow odour were not attractive. For *G. brevipalpis* the presence of these high doses of octenol and 4-methylphenol played a major role in its attraction. In an attempt to simulate cow odour, chemical absorbent filters were used to absorb the main components of cow odour. When synthetically simulated cow and natural cow odours were compared there was no significant difference in their attractiveness for *G. austeni* but natural cow odour significantly increased catches of *G. brevipalpis* as compared to its synthetic concoct. For *G. austeni* it was assumed that the attractiveness of natural cow odour as well as other host odours could be attributed to the presence of carbon dioxide in their breath and that this is probably the single most important component in host odour. Vision was also found to play a very important role in the attraction of *G. austeni*. For *G. brevipalpis* there seemed to be additional unknown attractive components of cow odour. Compared to odour vision played a minor role in its attraction.

3.2 INTRODUCTION

Tsetse flies recognise potential hosts by olfactory and visual cues. They approach a stationary host or target baited with odour by upwind flights (Torr

1989; Gibson *et al.* 1991; Brady & Griffiths 1993; Willemse & Takken 1994; Groenendijk 1996) modulated by olfactory stimuli, with visual responses operating only at short range. Odours have, therefore, been used to enhance the effects of shape and colour in the attraction of tsetse flies to traps and targets (Snow 1980).

Studies in Zimbabwe (Vale 1974b, 1977a) demonstrated that ox-breath is an important odour bait for *G. morsitans morsitans* Westwood and *G. pallidipes* Austen. One of the effective components of ox-breath is carbon dioxide (Vale 1980; Owaga 1984), but it is too expensive and inconvenient to use. Acetone and a number of aldehydes and other ketones are attractive to tsetse (Vale 1980) and are cheaper and more convenient to use. The most attractive element in ox-breath was identified as 1-octen-3-ol (henceforth referred to as octenol) (Hall *et al.* 1984). This attractant enhances the effects of ketones and CO_2 (Vale & Hall 1985a). Butanone was identified as a substitute for acetone and can be used at a lower dosage rate (Vale & Hall 1985b). A further breakthrough occurred when Owaga (1985) demonstrated in Kenya that the urine of the African buffalo (*Syncerus caffer*) could be used for the attraction of *G. pallidipes*. Much of the efficacy of the urine is due to phenols (Hassanali *et al.* 1986; Bursell *et al.* 1988; Owaga *et al.* 1988; Vale *et al.* 1988b) comprising the parent phenol, 3- and 4-methylphenol (3- and 4-cresol), 3- and 4-ethylphenol as well as 3- and 4-*n*-propylphenol (Hassanali *et al.* 1986). Of these, 4-methylphenol and 3-*n*-propylphenol have been found to be the most compelling for tsetse flies (Saini 1990; Saini & Hassanali 1992) and act synergistically as the crucial components of the phenolic mix (Owaga *et al.* 1988; Vale *et al.* 1988b).

While workers in Kenya used acetone and cow urine (Okech & Hassanali 1990), the Zimbabwean workers used blends of synthetic octenol, 4-methylphenol and 3-*n*-propylphenol. Owaga (1992) tested African buffalo (*S. caffer)* and cow urine, acetone, 3-*n*-propylphenol, 4-methylphenol and carbon dioxide, in the form of dry-ice, for *G. austeni* in Kenya. Only dry ice attracted this species significantly. Kyorku (personal communication, 1994) also conducted studies on attractive odours (octenol and acetone) for *G. brevipalpis* in Kenya and found acetone and octenol to be the main attractive components.

In South Africa studies were conducted on the use of these conventional odour attractants for *G. brevipalpis* and *G. austeni* (Kappmeier & Nevill 1999a) and it was found that a very effective attractant combination with optimal release rates could be recommended for *G. brevipalpis*. This consisted of octenol released at *c.* 2,3 - 9,1 mg/h, 4-methylphenol released at *c.*15,5 mg/h and acetone released at *c.* 350 mg/h. This combination increased the catches of this species by 2,3 - 2,8 times when compared to the Zimbabwe mixture (Zim-mix) and by 10,1 - 12,3 times compared to 'no odour'. Unfortunately, none of the components or combinations attracted *G. austeni*. Further phenols, namely 4-*n*-propylphenol, 3-methylphenol, 3- and 4-ethylphenol as well as butanone were tested at low, medium and high release rates together with their synergistic effect to the Zim-mix. None of these components were, however, attractive to either species (Kappmeier 1999).

Considerably greater attraction of *G. pallidipes* and *G. m. morsitans* in Zimbabwe was obtained when Hargrove & Vale (1978) utilized live animals in pits and used the extracted odour as bait. It was suggested that other factors were also probably involved. Further detailed studies in Zimbabwe showed the attraction of these species to be greater to natural ox odour than to the synthetic ox odours (Torr *et al.* 1995; Hargrove *et al.* 1995) suggesting the presence of unidentified attractant(s) in ox odour.

The present lack of odour attractants for *G. austeni*, highlighted the importance of searching for attractants specific for this species and efforts during this study thus focused on this. It was suggested (G.A. Vale, personal communication, 1997) that since *G. austeni* did not react positively to any of the conventional odours, it might be that other more volatile components could be effective and that it could be the link to finding the unidentified odour components of ox odour. If present, the attractive odour component could be isolated and synthesized and so be used as part of an attractive odour bait.

In the present chapter, five types of study were undertaken to determine the significance of olfactory attraction for specifically *G. austeni*, as well as to find attractants in natural host odours. Firstly, studies were made on the effect of

carbon dioxide (CO_2) at various dosage rates on tsetse fly catches. Secondly, studies were made to determine the relative importance of vision vs. olfaction for their attraction as was done by Vale (1974b). Thirdly, studies were made to assess the responses of *G. brevipalpis* and *G. austeni* to natural cow odour. This included studies to determine if there is an animal odour besides CO_2, which is attractive for *G. austeni*. Comparisons were further made on the effect of adding large doses of known attractants to the natural cow odours and to CO_2, based on the concept that large doses of these components would eliminate the effect of the small quantities released by the cow. Following from this, comparisons were made of the numbers of tsetse attracted to natural cow odour and a synthetic cow odour simulate (henceforth referred to as synthetic cow) containing all the known attractants at their natural concentrations. Fourthly, studies were made to determine the attractiveness of other host odours in the search for an attractive odour for *G. austeni*. Lastly, studies were made on the effect of the presence of human odour for the attraction of both species.

3.3 MATERIALS AND METHODS

3.3.1 Evaluation methods

Tsetse flies orientate imprecisely to an odour source unless it is marked by a visual stimulus (Vale 1974b). To gauge the numbers of tsetse flies attracted to various odours, an electric grid consisting of a visual 1 x 1 m phthalogen blue part and an adjacent non-visual 0,5 x 1 m net (Fig. 2.1), was placed ± 30 - 50 cm downwind of the odour source to act as a focal point. This was at times replaced by a similar grid, but flanked with 2 x 1 m electric nets at each side of the visual target. The grids were operated from noon until dark, during which time both species could be attracted (Kappmeier 2000).

3.3.2 Odours

All odours (natural and synthetic), except CO_2 (released from cylinders), were extracted from a ventilated underground chamber (pit) (Fig. 3.1). The pit was constructed according to Vale (1974b). The pit dimensions are 3,5 x 2 x 2 m

with a roof of corrugated iron (at ground level) and an entrance door. Part of the roof contained a corrugated fibreglass sheet for light. A 5 m ramp led from ground level to the entrance of the chamber. The opposite side was fitted with a 3 m long (150 mm diam.) ventilation shaft of PVC piping, fitted in the top third of the pit wall. Air was exhausted from the pit at 2000 l/min via the ventilation shaft fitted with a 12 V (120 mm dia.) co-axial fan.

During these pit experiments, the electrified grid was placed 50 cm downwind of the extractor outlet (Fig. 3.2). Carbon dioxide cylinders were hidden away from the direct scene with CO_2 released through extended tubing placed at the extractor outlet at the release rates indicated in the experiments below.

Natural host odours

Natural host odours were obtained by placing the animals in the roofed pit and exhausting the air at 2000 l/min. The pit was cleaned daily to minimize the accumulation of phenolic materials present in animal excreta. The animals tested during the course of the study consisted of:

- a bull calf (*c.* 75 kg)
- a cow (*c.* 350 kg)
- three goats (total weight *c.* 68 kg)
- a bushpig (*c.* 100 kg)
- three men (total weight *c.* 270 kg)

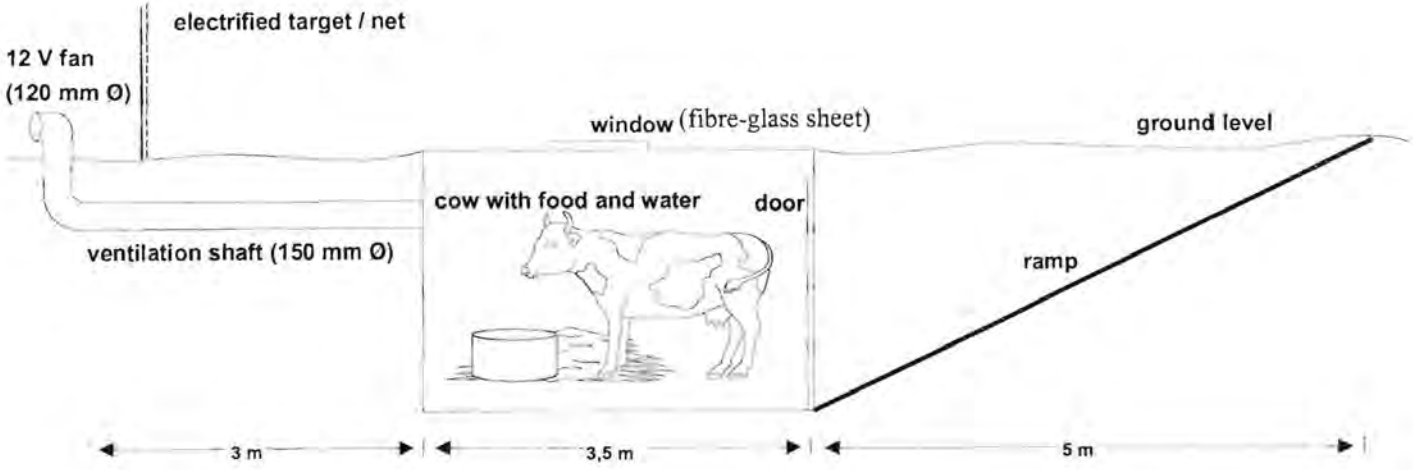

Fig. 3.1 Schematic representation of cow in underground ventilated pit (design after Vale 1974b)

Fig. 3.2 Extractor fan outlet placed *c.* 50 cm downwind of a flanked p/blue electric target

Synthetic odours

Blends of carbon dioxide and other synthetic odour components were dispensed at various rates as explained in Chapter 2.2.

3.3.3 Air sampling of attractants

Carbon dioxide

Measures of the natural CO_2 levels (ppm) released by the experimental cow were obtained. With this information, the release rate of the cow's CO_2 could be simulated at the correct rate for comparison purposes. Air was drawn at 300 ml/min via a tube inserted through a port in the pit ventilation shaft. The concentration of CO_2 was measured using an infrared gas analyzer (EGA, ADC Ltd., Hoddesdon, UK). The

output from the analyzer was recorded continuously by a data logger and the data were subsequently downloaded onto a personal computer for analysis. The logger recorded the mean levels of CO_2 at 1-minute intervals.

Natural cow

During October 1997 odour samples were taken of the 350 kg cow used in the experiments for the identification of its components and to obtain their release rates. This study was done in collaboration with researchers of the Natural Resources Institute[1] (NRI) where samples were analysed by the chemists, to be able to compare natural cow odour with synthesized cow odour.

During sampling at Hellsgate (initially by Dr. S.J. Torr (NRI)), the animal was housed in the underground pit (ventilated at *c.* 2000 l/min). Air was extracted via tubing with air pumps and filters were inserted through the sampling ports in the ventilation shaft of the pit (Fig. 3.3 a & b). Ketones and aldehydes (carbonyl compounds) were trapped with silica SepPak® (Waters Corporation, Milford, MA 01757, USA) cartridges containing DNPH (2,4-dinitrophenyl-hydrazine; 360 mg) at a rate of 0,5 l/min for approximately 2 h. Volatiles (i.e. phenols and octenol) were collected on Porapak® filters (200 mg; 50 – 80 mesh) at a rate of 2,0 l/min for 2 h. Collections of carboxylic acids were made by sampling at 2,0 l/min for 2 h on filters containing Chromasorb P AW impregnated with 2,5 % tetrabutyl-ammonium hydroxide (200 mg). Samples were taken of both the experimental cow as well as an empty pit (to be used as the background odour).

Since the samples taken in October 1997 showed contamination, which obscured the analyses of the ketones and octenol, these odours were resampled in December 1998 in order to get a proper analysis of the experimental cow odour for synthesis. Immediately after collection the filters were stored in a deep freeze and thereafter sent

[1] Natural Resources Institute/ University of Greenwich, Central Avenue, Chatham Maritime, Kent ME4 4TB, United Kingdom

by courier to the NRI for analyses where they were analysed within seven days of collection. Four samples (replicates) were taken of the cow and three of the empty pit with each of the three filter types during the first trial and five samples of each treatment during the second trial.

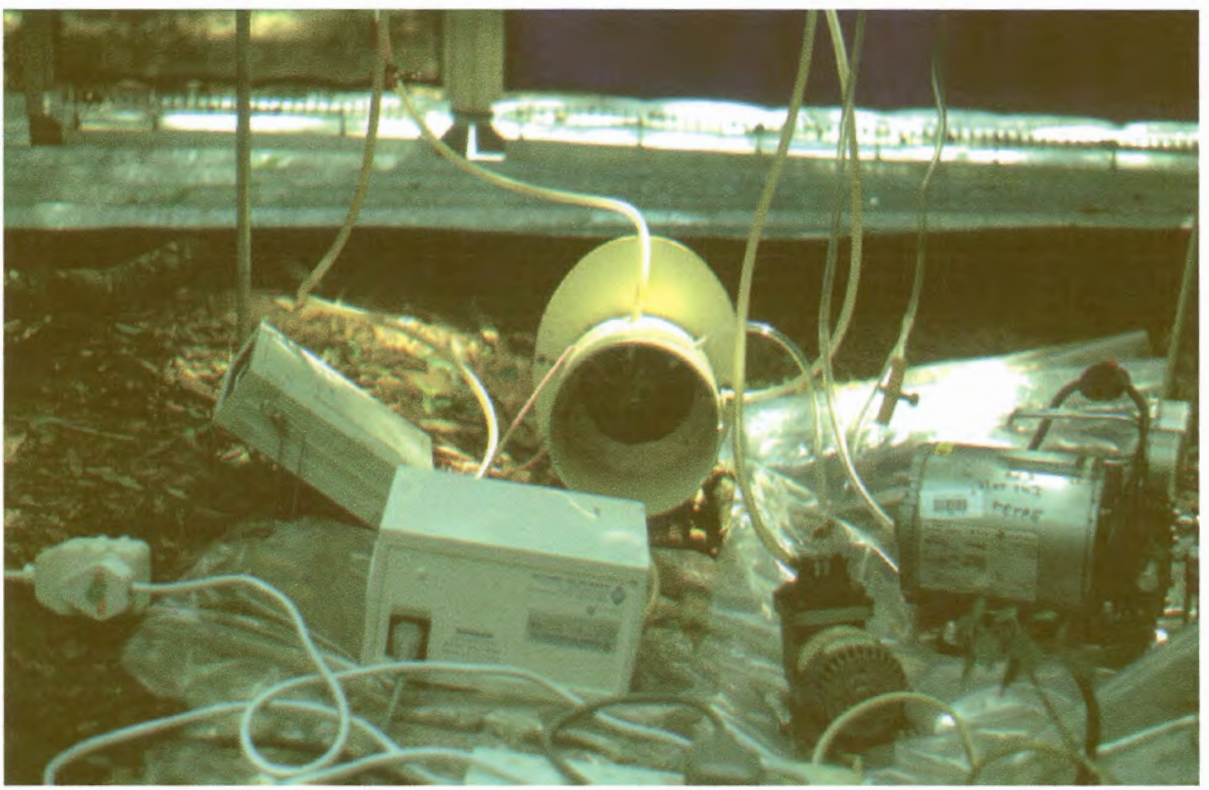

Fig. 3.3 a Odour extraction and sampling setup. Extracted air from the pit (housing an animal) was sampled via tubing with air pumps (shown right). Filters were inserted through the sampling ports in the ventilation shaft of the pit (centre). Carbon dioxide was measured similarly by means of an infrared gas analyzer (shown left).

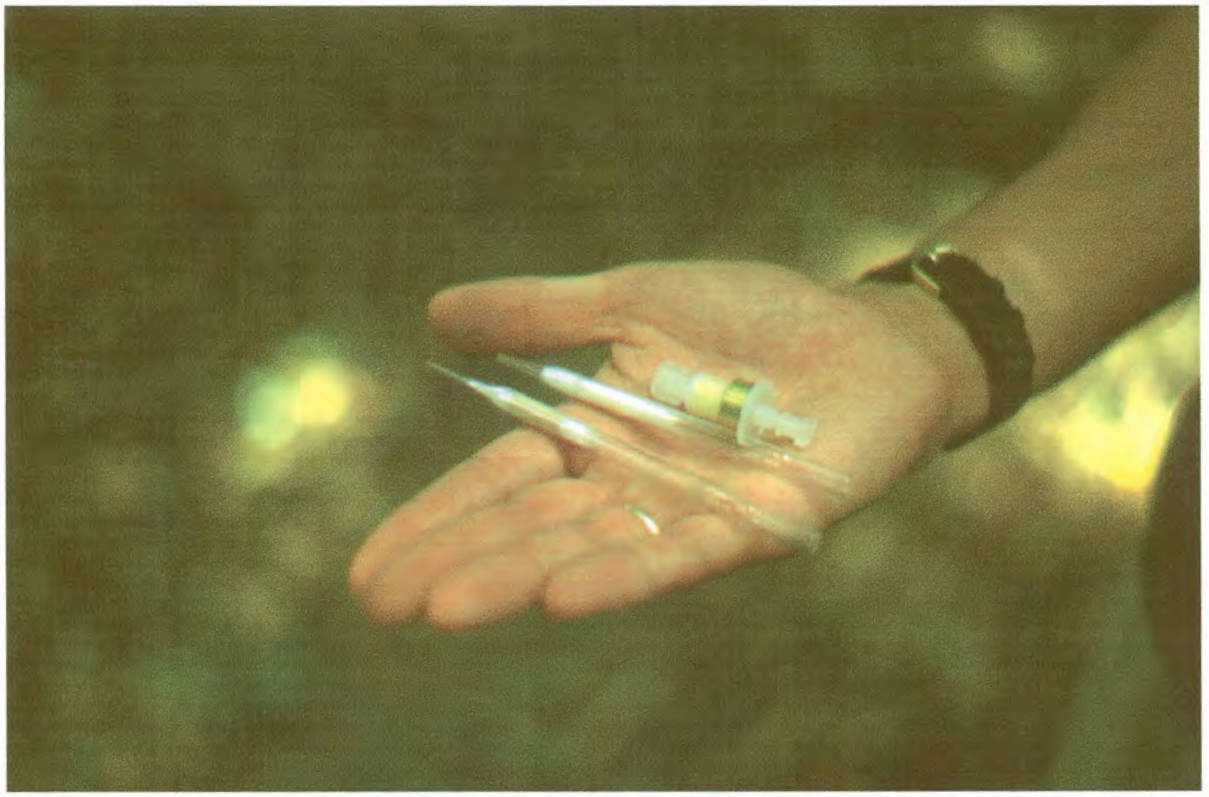

Fig. 3.3 b Collections of carboxylic acids were made by sampling through filters containing Chromasorb P AW filters (left), volatiles (i.e. phenols and octenol) were collected on Porapak® filters (centre) and ketones and aldehydes (carbonyl compounds) were trapped with silica SepPak® cartridges (right)

3.3.4. Details of chemical analyses by NRI

NRI chemists analyzed the filters following the procedures described below (D.R. Hall, unpublished report, 1998):

- *Carbonyl compounds:* At NRI, the trapped 2,4-dinitrophenylhydrazones (DNPHs) were eluted with 3 ml of HPLC grade acetonitrile and the eluate made up to 5 ml. Analyses used a Spherisorb5 ODS2 column (25 cm x 4,6 mm; HPLC Technologies) eluted with a 60:40 acetonitrile/water mixture at 1 ml/min. The eluate was monitored by an UV detector at 350 nm. DNPHs of acetone, butanone,

formaldehyde and acetaldehyde were synthesized at NRI and amounts in the test samples were quantified by external standard.

- Collections on Porapak: The Porapak was pre-purified by soxhlet extraction with dichloromethane and further washing with dichloromethane after making up the filters. At NRI trapped volatiles were eluted with dichloromethane (3 x 0,5 ml) and decyl acetate (2 μg) added as internal standard. The solution was analyzed by gas chromatography (GC) coupled directly to mass spectrometry (MS) using a Finnigan ITD 700 ion trap detector operated in electron impact mode. The GC column was fused silica (25 m x 0,25 mm i.d.) coated with polar CPWax52CB (Chrompack) with helium carrier gas (0,5 kg/cm^2) and oven temperature held at 50 °C for 2 min then programmed to 240 °C at 6 °C/min. Components were identified by their GC retention times and mass spectra and comparison with synthetic standards where possible. Components were quantified against the internal standard and rates of production calculated from the flow rates used.

- Collections of carboxylic acids: At NRI the tetrabutylammonium salts were eluted with acetone (3 x 0,5 ml) and benzyl bromide (2 μl) added to convert the salts to the benzyl esters. After standing for 2 hr, an internal standard, e.g. decyl acetate (2 μg), was added and the solution analyzed by GC-MS as above. Benzyl esters were detected by single ion scanning at m/z 91 and 108. Synthetic standard benzyl esters were prepared *via* the tetrabutylammonium salts on a preparative scale. The internal standard was calibrated against these and amounts of benzyl esters converted to amount of acid trapped.

3.3.5 Experimental design and analysis

In comparisons of different odour treatments, the various treatments were incorporated into a series of replicated Latin squares or, where only one site was available, treatments were compared using a randomized block design, as explained in Chapter 2. Unless stated otherwise, 'no odour' acted as the control treatment in all experiments. Details of each of the experiments will be given in the sections following. Detailed analyses of the results are described in section 2.3.

Where comparisons were made between the same treatments in more than one experiment and various indices (of detransformed mean catches) were obtained (e.g. comparisons with cow and 'no odour'), the overall mean index was calculated. Indices were transformed to log (n) and then the averages were calculated and detransformed. For more precise estimates the indices of the treatments were weighted by the reciprocal of the scale parameter (variance) for each experiment. This deals with problems of differences in the variance between different experiments when there are varying numbers of catches. The weighting procedure thus gives greater weight to the values with smaller variance.

3.4 EXPERIMENTS AND RESULTS

The experiments are described in detail in the following sections. The results will be summarized in Tables. Detransformed mean catches will be expressed as indices of increase relative to the control treatment. The detransformed mean catch of the control treatment (i.e. 'no odour') will be given in brackets. Indices followed by the same symbols (a, b or c) indicate no significant differences. Table summaries will incorporate the number of replicates (n) for each treatment, the degrees of freedom (df) for error, the transformed standard errors (s.e.) as well as the levels of probability (P) that the means are different at $P < 0,05$ (*), $P < 0,01$ (**), $P < 0,001$ (***), or not significantly different (n.s.).

3.4.1 Evaluation of carbon dioxide for the attraction of *G. brevipalpis* and *G. austeni*

Attraction with carbon dioxide at various release rates

Carbon dioxide was released from cylinders through appropriate flow regulators and evaluated for its attractiveness for the two species. Three treatments of CO_2 with release rates of 0,2 l/min, 2,0 l/min and 20,0 l/min were compared to 'no odour' as the control and to the best SA blend. Fifteen replicates were carried out during May - October 1997.

The results are given in Table 3.1 for both species. For *G. brevipalpis* males it was shown that only the best SA blend and none of the CO_2 treatments increased catches significantly compared to 'no odour'. For females CO_2 increased the catches significantly only at a rate of 2,0 l/min compared to 'no odour'. For *G. austeni* males no significant increase in the catches occurred when baited with any of the CO_2 treatments (or the best SA blend) and when released at 20,0 l/min only a 1,7 x increase in the catches was obtained when compared to 'no odour'. For *G. austeni* females, however, CO_2 increased the catches significantly at all three release rates by *c.* 2,3, 3,2 and 4,0 times, respectively, compared to those attracted to 'no odour'. The differences between the catches with these three CO_2 treatments were not significant, although catches increased as the release rates of CO_2 increased. CO_2 released at 20,0 l/min also increased the catches significantly (2,2 - 2,3 x) compared to the best SA blend, while the lower release rates were also better but not significant.

Table 3.1 Attractiveness of targets baited with various release rates of CO_2 and the best SA blend [Results are expressed as indices of increase relative to the control treatment (index = 1) with detransformed mean catches of the control given in brackets. The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*), $P < 0,01$ (**) levels of probability, or not significantly different (n.s.) are indicated]

	No odour	CO_2 (0,2 l/min)	CO_2 (2,0 l/min)	CO_2 (20 l/min)	Best SA blend	*n*	df	*P*	± s.e.
G.brevipalpis									
Males	1 (3,766)a	1,817a	2,020a	1,742a	4,506b	15	52	**	0,236
Females	1 (3,072)a	1,550ab	2,906bc	2,245abc	4,605c	15	52	*	0,231
G.austeni									
Males	1 (4,484)a	1,457a	1,361a	1,722a	1,048a	15	52	n.s.	0,331
Females	1 (4,711)a	2,252bc	3,172bc	3,971c	1,714ab	15	52	*	0,269

abc Treatments followed by the same symbol are not significantly different

Synergistic effect of carbon dioxide on components of the SA blend

The synergistic effect of CO_2 when added to other synthetic odours was determined in two experiments with treatments as set out in Tables 3.2 and 3.3. In these experiments CO_2 was always released at 2,0 l/min, since the increase in catches with CO_2 at a high dose of 20,0 l/min was not significant compared to the catches at 2,0 l/min and would not have warranted the expense. Fifteen replicates of each experiment were carried out from June - December 1997.

The results of the first experiment for both species are given in Table 3.2. For *G. brevipalpis* males and females it was shown that all treatments were significantly better than 'no odour'. The synergistic effect of CO_2 when added to the original SA blend is apparent but only significant for females.

For *G. austeni* males and females CO_2 also increased catches when added to the original SA blend, however, the original SA blend appears to have a repellent effect on the CO_2 catches.

The results for the second experiment are given in Table 3.3 for both species. For *G. brevipalpis* males and females all treatments were significantly better than 'no odour'. The addition of CO_2 to the original SA blend or the best SA blend increased the catches of both males and females (not significantly) compared to the respective blends. The best SA blend together with CO_2 increased the catches of males by 7,7 x and females by 6,9 x compared to 'no odour'.

For *G. austeni* males and females treatments were not significantly different to 'no odour' and the addition of CO_2 to the original and the best SA blends did not significantly increase the catches. It is possible that the components of the SA blends have a repellent effect on CO_2 (as was also indicated in the results from Table 3.2), since the blends were also less attractive than 'no odour'. Thus CO_2 is found attractive

Table 3.2 Indices of catches of targets baited with CO_2, phenols and acetone relative to the control treatment [Control index = 1 and detransformed mean catches of the control are given in brackets. The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*) and $P < 0,01$ (**) levels of probability are indicated]

	No odour	CO_2 (2,0 l/min)	Oct, 4mp + CO_2	Original SA blend[1]	Original SA blend + CO_2	*n*	df	*P*	± s.e.
G.brevipalpis									
Males	1 (2,647)a	3,297b	6,161bc	3,566bc	7,557c	15	52	**	0,277
Females	1 (2,428)a	3,785b	5,930bc	2,652b	8,787c	15	52	*	0,293
G.austeni									
Males	1 (4,059)ab	2,047b	1,741b	0,677a	1,679b	15	52	*	0,254
Females	1 (11,135)ab	1,450ab	1,508b	0,750a	1,232ab	15	52	*	0,225

abc Indices followed by the same symbol are not significantly different

[1] Original SA blend: octenol (0,4 mg/h); 4-methylphenol (0,8 mg/h); acetone (350 mg/h)

Table 3.3 Indices of catches of targets baited with CO_2, the SA blend and a combination of CO_2 added to the blends relative to the control treatment [Control index = 1 with detransformed mean catches of the control given in brackets. The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,01$ (**), $P < 0,001$ (***) levels of probability, or not significantly different (n.s.) are given]

	No odour	Original SA blend[1]	Original SA blend + CO_2	Best SA blend[2]	Best SA blend + CO_2	*n*	df	*P*	± s.e.
G.brevipalpis									
Males	1 (3,876)a	3,772b	4,064b	5,750b	7,714c	15	52	***	0,191
Females	1 (5,029)a	2,555b	3,622bc	3,900bc	6,858c	15	52	**	0,245
G.austeni									
Males	1 (3,475)a	0,786a	1,097a	0,987a	1,105a	15	52	n.s.	0,252
Females	1 (11,662)a	0,631a	1,288a	1,020a	1,319a	15	52	n.s.	0,290

abc Indices followed by the same symbols are not significantly different

[1] Original SA blend: octenol (0,4 mg/h); 4-methylphenol (0,8 mg/h); acetone (350 mg/h)

[2] Best SA blend: octenol (9,1 mg/h); 4-methylphenol (15,5 mg/h); acetone (350 mg/h)

for *G. austeni*, but it was suggested by the latter two experiments that the components of the SA blends might diminish its attractiveness.

3.4.2 Relation of visual vs. olfactory attraction (with natural odour)

In previous studies (Kappmeier & Nevill 1999a; 1999c) it was shown that vision could play a significant role for *G. austeni* since targets not baited with odour were still highly effective , while for *G. brevipalpis* odour clearly played a major role in its attraction. The importance of vision vs. olfaction for *G. austeni* and *G. brevipalpis* were evaluated before commencement to search for an odour, other than CO_2, for *G. austeni*. The numbers of tsetse attracted to a visual vs. non-visual target, each baited with and without natural cow odour (extracted from the ventilated pit), were compared. The treatments were, therefore, as follows:

- Non-visual target, not baited with odour (control)
- Visual target, not baited with odour
- Non-visual target, baited with cow odour
- Visual target, baited with cow odour

The non-visual target consisted of a 1,5 x 1 m electric net (Fig. 3.4) and the visual target incorporated a 1 x 1 m phthalogen blue target (flanked by 0,5 x 1 m net) as in Fig. 2.1. In one experiment (Exp. 1) a young bull calf of approximately 75 kg was used. A larger animal later replaced the small animal, producing more odour. The second experiment (Exp. 2) was, therefore, conducted with the full-grown cow weighing ± 350 kg. Six replicates were carried out for each treatment.

The results are summarized in Table 3.4. The control treatment was 'no odour' and no visual target (-/-).

Fig. 3.4 Front view of ramp of pit. Setup shows 1,5 x 1 m electric net at far side of pit where ventilation shaft exits. Note fibre-glass sheet in roof of pit allowing light into pit

Table 3. 4 Summary of the results to evaluate the importance of natural cow odour vs. visual stimuli [Results are expressed as the indices of increase relative to the control treatment (with (+) and without (-) odour, with (+) and without (-) visual) (index = 1) with detransformed mean catches of the control given in brackets. The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*) and $P < 0,01$ (**) levels of probability are given]

		Odour* / Visual**							
		- / -	- / +	+ / -	+ / +	*n*	df	*P*	± s.e
Exp.	*G.brevipalpis*								
1	Males	1 (3.036) a	3.945 b	6.291 bc	7.859 c	6	15	*	0,084
	Females	1 (2.772) a	3.260 b	7.534 c	10.370 c	6	15	**	0,073
Exp.	*G.brevipalpis*								
2	Males	1 (2.595) a	5.063 b	8.956 bc	11.625 c	6	15	*	0,109
	Females	1 (2,554) a	2.599 ab	5.501 bc	9.029 c	6	15	*	0,126
Exp.	*G.austeni*								
1	Males	1 (0.944) a	5.695 bc	2.082 ab	14.327 c	6	15	**	0.165
	Females	1 (1.836) a	7.142 b	2.708 ab	9.036 b	6	15	*	0.170
Exp.	*G.austeni*								
2	Males	1 (0.648) a	7.793 b	7.863 b	19.185 b	6	15	*	0,156
	Females	1 (2.573) a	10.382 b	4.010 c	12.082 b	6	15	*	0,105

+/- with/without (odour/visual)
* Odour : Exp. 1= 75 kg bull calf; Exp. 2 = 350 kg cow
** Visual target = 1x1 m p.blue plus 0,5x1 m net; Non-visual target = 1,5x1 m net
abc Means of treatments with the same symbols are not significantly different

For *G. brevipalpis* males it was shown that the presence of a visual stimulus with no odour (- / +) increased the catches significantly by *c.* 3,9 and 5,1 x in Exp. 1 and 2 respectively and *c.* 2,6 (not significant) to 3,3 x for females. The importance of odour for this species was confirmed when the presence of the 75 kg bull calf odour only (Exp. 1) and no visual target (+/ -) increased catches significantly by 6,3 x for males and 7,5 x for females and also significantly with the 350 kg cow (Exp. 2), i.e. *c.* 9,0 and 5,5 x for males and females, respectively. It also seems that odour plays by far a more significant role in the attraction of this species than does vision especially for females where the odour attraction (with the 75 kg calf) was even significantly greater than the visual attraction. For both sexes the addition of odour to the visual target (+/+) also increased the catches significantly compared to the visual target only (- /+), i.e. with the 75 kg calf an increase of 2,0 and 3,2 x (for males and females), and with the 350 kg cow an increase of 2,3 and 3,5 x for males and females, respectively.

For *G. austeni* the presence of a visual stimulus without any odour (- /+) increased catches significantly by 5,7 - 7,8 x for males and 7,1 - 10,4 x for females. The presence of the 75 kg calf odour (no visual), however, did not increase the catches of either sex significantly (2,1 x for males and 2,7 x for females) and vision played a more important role. However, with the 350 kg cow this addition of natural odour (no visual) (+/ -) increased the catches of both males and females significantly by 7,9 and 4,0 x respectively. Vision was, however, still significantly more important than odour for females. Also the addition of the natural odour to the visual target (+/+) did not increase the catches of both sexes significantly, although there was still a large increase, namely 2,5 x in males and 1,2 - 1,3 x in females in both experiments. It seems, therefore, that vision plays a greater role in the attraction of this species than does odour. There is, however, an indication, especially with the larger animal, that odour could play an important role in this species' attraction. Whether this odour attraction is due to the attractiveness of CO_2 given off by the animals will be determined in the experiments described below.

3.4.3 Studies to evaluate natural cow odour to find an attractive odour for *G. austeni*

Measurement of CO_2 levels of the experimental cow

The results of the CO_2 concentrations (ppm) as measured with the logger are given in Table 3.5 for various treatments together with their estimated actual rates in l/min. CO_2 measures (ppm) of the experimental cow were taken in the early morning (when metabolism is still low) and during the afternoon (when experiments were conducted). Measures from an empty pit and the background (natural levels in the air) were also taken.

Furthermore, the concentration of CO_2 was measured while releasing CO_2 from cylinders with flow-meters in the pit at various known (nominal) rates (0,2; 2,0; 4,0 and 6,0 l/min). With this information a regression (Fig. 3.5) was plotted of the known rates (l/min) against the known concentrations (ppm minus background of 356 ppm). The cow's morning and afternoon rates are also indicated on this graph. With the

Table 3.5 Summary of CO_2 measurements taken during October 1997

Nominal rate (l/min)	Concentration (ppm)	Estimated actual rate (l/min)
Background	356	0,0
Pit	371	0,1
Cow	993	2,9
0,2	427	0,3
2,0	654	1,4
4,0	1180	3,8
6,0	1703	6,2
3,4	954 – 969,75	2,8

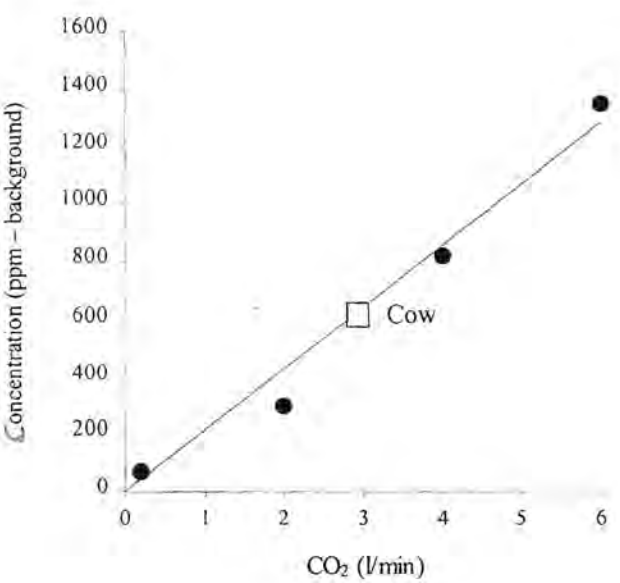

Fig. 3.5 CO_2 release rates of cow during the mornings and afternoons as determined by means of the regression between measured concentration (ppm minus background) against nominal CO_2 rates (l/min) obtained in Table 3.5

estimated actual rate of the experimental cow measured at *c.* 993 ppm (taken as an average over a number of afternoons' readings), it was on two occasions aimed to release CO_2 with the flow-meters at between 950 – 1000 ppm, in order to approximately simulate the CO_2 dose given off by the cow. The nominal rate of 3,4 l/min as indicated in Table 3.5 showed the obtained concentrations to be *c.* 954 – 970 ppm which could be estimated at an actual rate of *c.* 2,8 l/min. This rate was, therefore, used in future experiments to simulate the rate of CO_2 released by the cow.

Effect of CO_2 vs. natural cow odour

For *G. austeni* CO_2 was very attractive and is so far the only proven effective odour attractant (see section 3.4.1). To evaluate the importance of the natural cow odours, other than CO_2, the catches obtained with natural cow odour were compared with those obtained with CO_2 released at the dose of the natural cow, i.e. 2,8 l/min (as established in the previous section - see Table 3.5). The difference in attraction between the two treatments should indicate the attractiveness of the remaining odour components (other than CO_2). 'No odour' was included as the control treatment. Twelve replicates of each treatment were carried out. The results are summarized in Table 3.6.

Table 3.6 Mean catches of targets baited with natural cow odour and with CO_2 released at the same rate as produced by the cow [Expressed as the indices of increase relative to the control treatment (index = 1) with detransformed mean catches of the control given in brackets. The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*) and $P < 0,01$ (**) levels of probability are shown]

	No odour	Cow (350 kg)	CO_2 (2,8 l/min)	*n*	df	*P*	± s.e.
G.brevipalpis							
males	1 (14,15)a	4,298b	1,904c	12	22	*	0,086
females	1 (12,27)a	4,290b	2,580b	12	22	**	0,086
G.austeni							
males	1 (14,20)a	1,996b	2,137b	12	22	*	0,083
females	1 (29.29)a	2,140b	1,979b	12	22	*	0,074

abc Means of treatments followed by the same symbols are not significantly different at $P < 0,05$

The results showed that natural cow odour increased the catches of male and female *G. brevipalpis* significantly by *c.* 4,3 times compared to 'no odour'. CO_2 (released at 2,8 l/min) also increased catches significantly compared to 'no odour', namely a *c.*1,9 x increase for males and a *c.* 2,6 x increase for females. The comparison between CO_2 and cow odour showed significant differences between their attractiveness for *G. brevipalpis* males but not for females. This suggests that the remaining components of cow odour, other than CO_2, are very important, especially for males.

For *G. austeni* there was a significant increase in the catches of both males (*c.* 2,0 times) and females (*c.* 2,1 times) obtained by the cow odour compared to 'no odour'. There was also a significant increase with CO_2 compared to 'no odour', i.e. a *c.* 2,1 x increase for males and a *c.* 2,0 x increase for females. The comparison between CO_2 and cow odour showed no significant differences between their attractiveness. This suggests that CO_2 is the main attractive part of cow odour and no further important attractant is present in cow odour for *G. austeni*.

Effect of natural odour components other than carbon dioxide, acetone, octenol and 4-methylphenol

The following experiment was aimed to evaluate the importance of the natural aldehydes and ketones produced by the cow, other than octenol, 4-methylphenol, acetone as well as carbon dioxide. The experimental design included the synthetic odour chemicals, which are already in use to attract *G. brevipalpis*, namely 1-octen-3-ol, 4-methylphenol and acetone. This synthetic odour was included as the best SA blend. The best SA blend was added to CO_2 released at the equivalent rate produced by the cow, i.e. 2,8 l/min, and also to the cow. Note that the cow's natural levels of the components of the best SA blend are *c.* 0,1 mg/h (for 4-methylphenol), *c.* 5,0 – 9,0 mg/h (for acetone) and *c.* < 0,5 mg/h (for octenol). The additional large doses of the best SA blend, added to the natural cow odour, means that the small amount of phenols produced by the cow should not have any significant effect (i.e. the large doses of the SA blend will overwhelm the effect of these chemicals produced by the cow). A significant difference between the natural and synthetic odours will, therefore, indicate the presence of another attractive odour component(s).

In this experiment the visual focal point consisted once again of a 1 x 1 m phthalogen blue electric target, but instead of being flanked by a 0,5 x 1 m electric net at one side, it was flanked by two 1 x 1 m electric nets (non-visual) on each side of the visual part of the grid.

The results are summarized in Table 3.7. For *G. brevipalpis* the results indicated a significant increase in the catches with the Cow + best SA blend and the CO_2 + best SA blend for both sexes compared to 'no odour'. The comparison of catches obtained with the Cow + best SA blend and the CO_2 + best SA blend for males and females indicated no significance. This suggests that the increase in catches obtained by the remaining odour components will not make a significant difference and that CO_2, octenol, 4-methylphenol and acetone are the main attractive components of cow odour for this species.

Table 3.7 Summary of the results showing the importance of the remaining odour components other than octenol, 4-methylphenol, acetone and carbon dioxide [Expressed as indices relative to the control treatment (index = 1) with detransformed mean catches of the control given in brackets. The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*) level of probability, or not significantly different (n.s.) are given]

	No odour	Cow (350kg) + best SA blend	CO_2 (2,8 l/min) + best SA blend	*n*	df	*P*	± s.e.
G.brevipalpis							
Males	1 (26,49)a	2,826b	1,824b	9	16	*	0,074
Females	1 (16,99)a	3,713b	2,216b	9	16	*	0,098
G.austeni							
Males	1 (20,48)a	1,195a	1,370a	9	16	n.s.	0,133
Females	1 (25,84)a	1,816a	1,595a	9	16	n.s.	0,108

ab Means of treatments followed by the same symbols are not significantly different

For *G. austeni* there was no significant difference between the treatments. Thus there is no significant indication that any of the remaining cow odour components would be attractive. It seems that the high doses of the best SA blend rather had a repellent effect and clearly suppressed the effect of CO_2. This repellent effect of the SA blend was also indicated in previous experiments.

Results of chemical analyses of experimental cow odour used in pit experiments

A summary of the results of the chemical analysis (by the NRI) of the odours absorbed from the 350 kg cow sampled at Hellsgate during 1997 and 1998 are given in Tables 3.8 a-d below (D.R. Hall, unpublished results, 1998). These analyses revealed the components and doses of the main carbonyl compounds (ketones and aldehydes), volatiles (octenol and phenols) and carboxylic acids present in the experimental cow. This will enable the simulation of the known components at the same doses as produced by the cow.

Table 3.8a Rates of production of acetone and butanone from cow and synthetic source (AOP) [AOP is a synthetic source of tsetse attractants consisting of a "mini-sachet" containing acetone and releasing at 6 mg/hr and a 5 cm x 5 cm x 150µm sachet containing a 100:3:1 mixture of 4-methylphenol + 3-*n*-propylphenol + octenol releasing at 1,0 mg/hr]

	RATE OF PRODUCTION[1] (mg/hr)			
	ACETONE	BUTANONE	MEAN ACETONE	MEAN BUTANONE
Cow	0,80	9,05	-2,79	7,39
Cow	-5,70	-1,72		
Cow	-7,25	9,42		
Cow	0,99	12,82		
AOP	1,01	12,36	-2,16	12,26
AOP	-9,57	5,91		
AOP	2,067	18,50		
Empty pit	-0,90	17,04	-0,99	12,86
Empty pit	-2,65	13,39		
Empty pit	0,58	8,15		

[1] corrected for blank

Table 3.8 b Analyses of volatiles collected on Porapak (ratio relative to 4-methylphenol = 100)

	RATE OF PRODUCTION (mg/hr)							
	OCTENOL	2-METHOXY PHENOL	PHENOL	4-METHYL PHENOL	3-METHYL PHENOL	4-ETHYL PHENOL	3-ETHYL PHENOL	3/4-PROPYL PHENOL
Empty pit	0,953	0,000	0,000	0,000	0,000	0,000	0,000	0,000
SE (n=3)	0,374	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Cow	0,500	0,000	0,008	0,107	0,000	0,001	0,001	0,001
SE (n=4)	0,102	0,000	0,008	0,107	0,000	0,001	0,001	0,001
Ratio	467,300	0,000	7,513	100,000	0,000	1,027	0,513	1,283
AOP	0,806	0,000	0,018	0,434	0,000	0,008	0,004	0,008
SE n=3	0,268	0,000	0,010	0,208	0,000	0,008	0,004	0,008
Ratio	185,956	0,000	4,143	100,000	0,000	1,779	0,889	1,779

AOP is a synthetic source of tsetse attractants consisting of a "mini-sachet" containing acetone and releasing at 6 mg/hr and a 5 cm x 5 cm x 150µ sachet containing a 100:3:1 mixture of 4-methylphenol + 3-*n*-propylphenol + octenol releasing at 1,0 mg/hr.

Table 3.8 c Rates of production of carboxylic acids in cattle odour

	RATE OF PRODUCTION[1] (mg/hr)													
SOURCE/REPS	FORM	ACET	i-BUTYR	PROPION	BUTYR	?	PENTAN	HEXAN	HEPTAN	LACTIC	OCTAN	4MP	NONAN	BENZ
Scan[2]	1115	1165	1235	1250	1340	1435	1454	1565	1670	1735	1776	1800	1881	2107
ECL[3]	10,10	10,52	11,10	11,23	11,98	12,77	12,93	13,87	14,86	15,48	15,87	16,11	16,95	19,28
Unused(3)	12,584	13,373	0,050	1,070	0,112	0,208	0,245	1,781	0,282	0,383	0,408	0,000	0,329	0,149
Empty pit (3)	13,475	13,849	0,069	1,030	0,114	0,211	0,262	1,781	0,191	0,561	0,444	0,064	0,358	0,176
Cow(4)	11,189	11,385	0,052	0,883	0,085	0,187	0,208	1,400	0,161	0,578	0,324	0,009	0,284	0,136

[1] Form = formic; acet = acetic; i-butyr = iso butyric; propion = propionic; butyr = butyric; pentan = pentanoic; hexan = hexanoic; heptan = heptanoic; octan = octanoic; nonan = nonanoic; 4MP = 4-methylphenol; benz = benzoic; ? = unknown.

[2] scan in GC-MS analysis (= seconds)

[3] ECL = GC retention time in equivalent chain length relative to retention times of straight-chain acetates.

Table 3.8 d Estimates of the mean rates of production of various tsetse attractants as obtained for the second run of chemical absorbtion (1998) and analyses [S.J. Torr & Sara Phythian, unpublished report, 1999. The mean rate for a cow is estimated from the difference between the blank filter and the cow+pit]

Odour	Treatment	Mean (mg/h)	s.e.	*n*
Octenol				
	Blank filter	0,027	0,02	5
	Empty pit	0,040	0,02	5
	Cow + pit	0,059	0,02	5
Mean rate (mg/cow/h)		0,03		
4-methylphenol				
	Blank filter	0	0,00	5
	Empty pit	0	0,00	5
	Cow + pit	0,06	0,06	5
Mean rate (mg/cow/h)		0,06		
Acetone				
	Blank filter	0,97	0	5
	Empty pit[1]	0,86	0,85	4
	Cow + pit	4,7	1,2	6
Mean rate (mg/cow/h)		3,7		
Butanone				
	Blank filter	0	0,00	5
	Empty pit	0,17	0,10	5
	Cow + pit	0,33	0,02	6
Mean rate (mg/cow/h)		0,33		

[1] Excludes the result for one (contaminated) filter which indicated a release rate of 25,4 mg/h compared to 0,4-1,1 mg/h for the remaining four filters

Analyses of carbonyl compounds showed samples were contaminated with UV-absorbing impurities and in some samples amounts of the acetone DNPH were lower than that in unused filters, such that no useful results could be obtained (Table 3.8 a). These collections and analyses were repeated in 1998 (Table 3.8 d). Presumably the impurities came from the pit set-up used, but the reason for the low acetone DNPH in some samples is less obvious. Possibly large amounts of moisture were a factor (D.R. Hall, unpublished report, 1998).

Rates of production of the volatiles by the cow were lower than from the synthetic source (AOP). Contamination of filters with octenol made it impossible to give a reliable figure for this component (Table 3.8 b) as rates of production are typically very low (D.R. Hall, personal communication, 1998). These measurements were repeated during 1998 with completely clean filters (Table 3.8 d).

Collections of carboxylic acids showed no significant amounts (< 1 mg/hr) of any of the carboxylic acids analyzed attributable to the cow.

Evaluation of natural vs. synthetic cow

The previous experiments showed that both natural cow odour and CO_2 increased the catches of *G. brevipalpis* and *G. austeni* males and females significantly compared to 'no odour'. The remaining cow odour components, other than carbon dioxide, were significant for the attraction of *G. brevipalpis*, especially for males, but not for *G. austeni*. It was also indicated that the very volatile components of natural cow odour might be important for the attraction of *G. brevipalpis* (although not significant), but not for *G. austeni*. From the previous results it could be concluded that no further attractant in cow odour, other than carbon dioxide, is present for *G. austeni*.

The final and ultimate test to prove this, or which could assist to find anything else that might attract *G. austeni*, will be to test the natural cow odour and compare it against synthetically simulated cow odour (henceforth synthetic cow (SC)). Any significant increase in the catches with the natural cow,

compared to SC, will suggest the presence of a very volatile and unidentified attractant.

SC consisted of a number of chemicals based on the analysis of chemists at the NRI (see previous section) as summarized in Table 3.9. SC was released more or less at the indicated dosages through the corresponding sachet sizes as set out in Table 3.9.

Table 3.9 List of synthetic cow (SC) components used to simulate the natural cow and the recommended release rates. The sachet sizes, which gave more or less the correct dosages, are indicated

Synthetic cow odour components	Release rate (mg/hr)	Sachet size (measurements in cm)
Octenol	0,03	
4-methylphenol	0,107	
4-ethylphenol	0,001 } 0,14	} 2x2
3-ethylphenol	0,001	
3-propylphenol	0,001	
2-methoxyphenol	zero	-
phenol	0,008	2,5 x 2
3-methylphenol	zero	-
acetone	3,7	2x2
butanone	0,33	2x2 in 3x3
acid mix*	0,002	2x2
carbon dioxide	2,8 l/min	released from cylinder

* Acids: Formic, acetic, iso butyric, propionic, butyric, pentanoic, hexanoic, heptanoic, octanoic, nonanoic, benzoic

Two weeks of experimentation was done so as to obtain the correct release rates for the components, by weighing chemicals released from different sized sachets. The acids were dispensed as a mixture of equal amounts of acids and combined with sunflower oil[2] to make a 30:1 solution (i.e. 1 part acids) and dispensed from the sachets to aim for a release rate of 0,002 mg/h. Carbon dioxide was released from a cylinder at 2,8 l/min as was measured to be the release rate of the cow during times of experimentation.

During the course of the experiment, sachets of the SC were weighed before and after each day's use in the pit, to keep record of the release rates.

Natural cow and SC odours were compared with each other and to 'no odour'. Eight replicates were carried out. A summary of the results is given in Table 3.10.

Table 3.10 Indices of mean catches of flies attracted to natural and synthetic cow (SC) odour relative to the control ['No odour' index = 1 with detransformed mean catches of the control given in brackets. The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*) and $P < 0,01$ (**) level of probability are indicated]

	No odour	Cow (± 350 kg)	Synthetic Cow (SC)	*n*	df	*P*	± s.e.
G. brevipalpis							
Males	1 (8,38)a	2,700b	1,143a	8	14	*	0,079
Females	1 (3,39)a	8,167b	2,997c	8	14	**	0,093
G. austeni							
Males	1 (10,05)a	4,293b	2,032ab	8	14	*	0,109
Females	1 (79,41)a	2,051b	1,528ab	8	14	*	0,073

abc Means of treatments followed by the same symbols are not significantly different at $P < 0,05$

[2] Acids are normally dissolved in dioctyl phthalate, however, this chemical was not available at the time and NRI chemists suggested the use of sunflower oil since this would be sufficiently polar and a good mimic for the former

The results showed SC to be significantly less attractive for both *G. brevipalpis* males and females, i.e. cow odour increased catches significantly by 2,4 and 2,7 times, respectively, for males and females compared to SC. For *G. austeni* males and females there were no significant differences between the SC and natural cow, although the cow still increased catches by 2,1 and 1,3 times, respectively, for males and females compared to SC.

3.4.4 Testing of other host odours

The attractiveness of other host odours was tested by extracting their odours from the ventilated pit, to see whether their odours could be more attractive and, therefore, hold something else than cow odour. The animals tested were a bushpig and goats, which were compared to cow and 'no odour'. The same target/net combination was used as shown in Fig. 3.4. Six replicates of each treatment were carried out. The results are given in Table 3.11.

Table 3.11 Indices of mean catches attracted to cow, bushpig and goat odours relative to 'no odour' ['No odour' index = 1 with detransformed mean catches of the control given in brackets. The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*) and $P < 0,01$ (**) level of probability are given]

	No odour	Cow (± 350 kg)	Bushpig (± 100 kg)	Goats (±68 kg)	*n*	df	*P*	± s.e.
G. brevipalpis								
Males	1 (15,11)a	3,778b	1,870ab	1,535a	6	15	*	0,125
Females	1 (6,29)a	7,460c	4,298cb	2,183ab	6	15	*	0,142
G. austeni								
Males	1 (10,57)a	3,979b	4,470b	2,984b	6	15	**	0,114
Females	1 (29,81)a	3,360b	2,937b	2,133ab	6	15	*	0,122

abc Means of treatments followed by the same symbols are not significantly different at $P < 0,05$

The results indicated for *G. brevipalpis* males and females showed that cow odour was still the most attractive, increasing catches by *c.* 3,8 and 7,5 x respectively, compared to 'no odour'. For *G. brevipalpis* males, bushpig and goat odours did not increase the catches significantly compared to 'no odour'. For females, bushpig odour increased catches significantly by *c.* 4,3 x compared to 'no odour'. Goat odour was significantly inferior to cow odour for both sexes.

For *G. austeni* males, the cow, bushpig and goat odours were all significantly more attractive than 'no odour'. For females, cow and bushpig odours were significantly more attractive than 'no odour', but the goat odour was not significantly better. Bushpig odour was even more attractive than cow odour for males (although not significantly) but not for females. Considering the combined male and female catches, cow and bushpig odours were about equally effective with an increase of *c.* 3,6 with cow odour and *c.* 3,4 with bushpig odour, as compared to 'no odour'.

This suggests that no better attractants may be found in bushpigs and goats for either tsetse species.

3.4.5 Effect of human odour

Human smell was found to have a repellent effect when added to cow odour for *G. m. morsitans* and *G. pallidipes* in Zimbabwe (Vale 1979). The attractiveness of human odour for *G. brevipalpis* and *G. austeni* was determined together with its effect on the attractiveness of cow odour. Six replicates of the following treatments were compared in one experiment, the results of which are summarized in Table 3.12:

- No odour (control)
- Cow
- 3 Men
- Cow + 3 Men

Table 3.12 Indices of mean catches obtained with odours released from cow, man and a combination of cow and man relative to the control [Control/'no odour' index = 1 with detransformed mean catches of the control given in brackets. The number of replicates (n), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*) or $P < 0,01$ (**) level of probability, or not significantly different (n.s.) are shown]

	No odour	Cow (± 350 kg)	3 Men (± 270 kg)	Cow + 3 Men	n	df	P	± s.e.
G. brevipalpis								
Males	1 (10,96) a	6,199 b	2,073 ac	3,461 bc	6	15	**	0,102
Females	1 (8,916) a	8,926 b	1,791 a	3,913 c	6	15	*	0,090
G. austeni								
Males	1 (6,583) a	3,609 a	2,289 a	2,091 a	6	15	n.s.	0,144
Females	1 (22,46) a	2,111 a	1,795 a	1,811 a	6	15	n.s.	0,100

abc Means of treatments with the same symbols indicate no significant difference at $p < 0,05$
The overall treatment F for *G. austeni* is not significant

For *G. brevipalpis* it was shown that the natural cow odour increased the catches of males and females significantly by 6,2 and 8,9 x respectively. Human odour, on the other hand, also increased the catches slightly, but not significantly (i.e. 2,1 and 1,8 x for males and females). The presence of human odour with cow reduced the attractiveness of the cow significantly for females but not for males, i.e. a 0,5 and 0,4 x reduction for females and males respectively.

For *G. austeni* the catches with the natural cow odour were also better than with no odour, i.e. 3,6 and 2,1 x for males and females respectively, but this was not significant. Further the same basic trend held for *G. austeni* as for *G. brevipalpis* in that human odour alone also increased the catches (not significantly). The presence of human odour with cow odour also seemed to have a repellent effect, however, this is not significant.

3.4.6 Overall mean index of cow odour

The indices of increase of natural cow odour relative to 'no odour' as obtained in the various experiments above are summarized in Table 3.13 for each

species and sex. To summarize these indices, the overall mean index of cow odour was obtained. Indices were transformed to log and then the averages were calculated and detransformed. The detransformed mean indices are calculated (Table 3.13) as 3,622 and 6,053 for *G. brevipalpis* males and females, respectively, and 3,133 and 2,051 for *G. austeni* males and females, respectively.

For more precise estimates these indices were weighted according to the reciprocal of the scale perimeter (variance) for each experiment as summarized in the Table. This deals with problems of differences in the variance between different experiments when there are different numbers of catches. The weighting procedure thus gives greater weight to the values with smaller variance. Weightings are indicated as 1 (low) - 5 (high). This procedure showed, for weightings of 5, indices of cow odour as 2,700 and 8,926 for *G. brevipalpis* males and females, respectively, and 3,979 and 2,051 for *G. austeni* males and females, respectively.

Table 3.13 Summary of indices of the attractiveness of natural cow odour vs. 'no odour' for five experiments (A-E) [The overall mean index is indicated in bold. Weights are allocated to the indices obtained in the various experiments]

	G. brevipalpis				*G. austeni*			
	Males		Females		Males		Females	
Exp.	Index	Weight[2]	Index	Weight	Index	Weight	Index	Weight
A	2,296	3	3,474	2	2,462	1	1,164	2
B	4,298	2	4,290	3	1,966	4	2,140	3
C	2,700	5	8,167	4	4,293	3	2,051	5
D	3,778	1	7,460	1	3,979	5	3,360	1
E	6,199	4	8,926	5	3,609	2	2,111	4
Mean[1]	**3,622**		**6,053**		**3,133**		**2,051**	

[1] Detransformed mean of log transformed indices
[2] Weighting index allocated to reciprocal of scaled parameters between 1 (low) – 5 (high)

3.5 DISCUSSION

CO_2 and other synthetic components

Carbon dioxide is an attractive component for *G. brevipalpis* and *G. austeni* in South Africa. For both these species the rate of CO_2 released from cylinders was comparatively suitable at 2-20 l/min and significantly increased the numbers of these flies attracted to a target. These rates are, however, regarded to be not worth the expense, since it is costly, impractical to use in the field and, furthermore, impossible to use on a large scale. Since studies started on testing conventional odour components for these species (Kappmeier & Nevill 1999a), CO_2 was the only single component found to attract significant numbers of *G. austeni*. It had also been found attractive, in the form of dry ice, for the Kenyan *G. austeni* population (Owaga 1992).

Carbon dioxide, acetone, octenol and 4-methylphenol all added synergistically to the attraction of *G. brevipalpis* males and females. Of these components it seemed that acetone and CO_2 act as the most important synergists, even at high doses of octenol and 4-methylphenol, in that the increase in catches were only significant when acetone and/or CO_2 were present. Torr (1990) also has found that acetone and CO_2 acted as valuable synergists. For *G. austeni* it seemed that the addition of octenol, 4-methylphenol and acetone had a repellent effect and diminished the attractiveness of CO_2. Kappmeier & Nevill (1999a) have noted a similar repellent effect of these components when targets baited with some combinations of these chemicals caught even less than those baited with no odour. Whether this repellency was the combined effect of all three components is not certain. Vale & Hall (1985a) have observed for *G. m. morsitans* and *G. pallidipes* that, even in the presence of CO_2, attraction can be depressed by high doses of octenol.

Natural cow odour

Natural cow odour also increased catches of *G. austeni* males and females with indices weighted at *c.* 4,0 and 2,0 times, respectively, compared to 'no

odour'. The emphasis of the study, therefore, focused on finding an attractive odour component(s) for *G. austeni*, other than CO_2, which may be present in natural cow odour and which could perhaps also further enhance the attraction of *G. brevipalpis*. The natural cow odours (other than CO_2) were confirmed to be valuable for the attraction of *G. brevipalpis*. However, the components of cow odour (other than CO_2) had no significant effect on the catches of *G. austeni*. These results were expected since some of the main synthetic components of ox odour were known to be very attractive for *G. brevipalpis* but not for *G. austeni* (Kappmeier & Nevill 1999a).

The components of natural cow odour excluding CO_2, acetone, 4-methylphenol and octenol looked promising for the attraction of *G. brevipalpis*, even when supernormal doses of the latter three components were added to cow odour, although the remaining components' contribution to the increase of catches were not significant. This was not the case for *G. austeni*, thus the results generally indicated that, other than carbon dioxide, no further attractant for *G. austeni* was present in cow odour. The implication that acetone, octenol and 4-methylphenol had a repellent effect for this species, was verified when the attractiveness of cow odour and CO_2 were both reduced so that their increases in catches compared to 'no odour' were unexpectedly not significant with the presence of supernormal doses of these components.

Natural vs. synthetic cow odour

When all identified attractants of the cow odour were dispensed synthetically at seemingly appropriate levels (this blend referred to as synthetic cow [SC]), no significant difference was found between catches of *G. austeni* obtained with the natural cow and its synthetic concoction. For *G. austeni* males, cow odour still doubled the catches compared to SC, although this was not significant. Therefore, despite the indication that there might be some attraction by a volatile component(s) present in the cow odour, this attraction is certainly not significant, so that most, and probably all of its attraction, especially for females, seems to be due to CO_2. For *G. brevipalpis* cow odour increased catches of males and females significantly by *c.* 2,4 and 2,7 times,

respectively, compared to SC. This supports the findings of Torr *et al.* (1995) and Hargrove *et al.* (1995) that there are possibly attractive components in cow odour, other than the acids, ketones and not so volatiles (octenol and phenols), which are probably relatively volatile and still remain to be identified, as was suggested in their respective studies on *G. pallidipes* and *G. m. morsitans* in Zimbabwe.

Other host odours

The subsequent testing of other host odours, which gave an idea of their relative attractiveness as compared to cow odour and 'no odour', showed that cow odour was still the most attractive for *G. brevipalpis* and bushpig odour to a lesser extent (not significant for males). Goat odour was relatively unattractive and did not increase the catches of this species significantly compared to 'no odour'. For *G. austeni* cow, bushpig and goat odours were all better than 'no odour', with cow and bushpig odours as the most attractive (significant) and goats to a much lesser extent (not significant). For *G. pallidipes* and *G. m. morsitans* it has been shown that increases in the body mass resulted in an increase in the catch of the tsetse flies (Hargrove *et al.* 1995). Therefore, it is possible that this is the reason that cow odour is still the best source of attraction for the two Zululand species. It could, therefore, be presumed that no better attractant might be found in either bushpigs or goats and that their effects on the increases in catches, specifically for *G. austeni*, were most probably due to CO_2. Thus the fact that the numbers of this species increased according to increased body mass can probably be attributed to increased carbon dioxide release.

Human odour

While human odour on its own was somewhat attractive (not significantly) for *G. brevipalpis* and *G. austeni*, its presence with cow odour clearly had a repellent effect on the attractiveness of cow odour for both species. This suggests that cow odour is attractive but the odour of man has a mixture of repellents and attractants. Similar results were obtained for *G. m. morsitans*

and *G. pallidipes* (Vale 1974b, 1977b, 1979) where it was shown that chemicals present in human odour reduce the numbers of tsetse attracted to a host and also the proportion of flies that feed. The identities of the repellents present in human odour remain unknown, however, work was underway to identify these repellents (Torr *et al.* 1996).

Vision vs. olfaction

In an experiment, separating the cues that might be responsible for attracting tsetse, the numbers of tsetse attracted to a visual and non-visual target with and without the smell of a cow were compared. Thus, when measuring the role of vision vs. olfaction the results for *G. brevipalpis* indicated that natural cow odour played a significantly more important role in its attraction than did vision. For *G. austeni*, on the contrary, vision seemed to play a major significant role in its attraction. However, there is a good indication that natural cow odour still played a role in its attraction, which is, as previously suggested, most likely exclusively due to CO_2.

This notable importance of vision and CO_2 is fascinating. Carbon dioxide normally occurs in large concentrations in nature, especially in woody vegetation where daytime concentrations of CO_2 exceed the concentration in the open (Gillies 1980). Carbon dioxide is naturally present at ≈ 300-400 ppm during the day (see Table 3.5), rising to as much as ≈ 1000 ppm at night (Gibson & Torr 1999). As *G. austeni* is most active during day-time (Kappmeier 2000), it would suggest that it would not easily detect host CO_2 which is released into a potentially competing background. However, the CO_2 released by the cow or via cylinders were measured at ≈ 993 ppm and ≈ 954-970 ppm respectively (see Table 3.5) (at the pit outlet). These CO_2 exhalations or releases could, therefore, be fairly dispersed and diluted before falling to background levels.

In a study on CO_2 odour plumes by G.E. Zollner (pers. comm., 2000), the background concentration of CO_2 at Hellsgate was very "noisy" in the middle

of the day in that the atmospheric concentrations of CO_2 fluctuated highly. Following release of natural or synthetic CO_2, it was difficult to detect CO_2 with gas analyzers at 16-32 m downwind from the source. Taking the foregoing into account, the background CO_2 will therefore probably not prevent detection of hosts by *G. austeni* at close range, but from further away host CO_2 should be lost in atmospheric "noise". It might, therefore, be difficult for tsetse in general to detect host-derived CO_2 from far away. This was also demonstrated in Zimbabwe, where it was shown that CO_2 stimulated close-range responses in tsetse, i.e. the alighting responses on targets (Vale 1983) as well as entering responses into traps (Vale & Hall 1985b).

G.E. Zollner (pers. comm., 2000) also showed during her study at Hellsgate that the catches of *G. austeni* decreased as they were measured from further away downwind of a CO_2 source. On the other hand, the catches improved as they were measured closer downwind of the CO_2 source (4-8 m). However, this range of attraction also probably coincides with the visual range of attraction of this species, which was clearly demonstrated earlier to be an important cue in their host-detecting behaviour. Their reliance on vision may on the other hand be linked to their smaller size and strength which may mean that they are unable to pursue odour trails for as far as *G. brevipalpis* and thus depend more on the visual detection of hosts (walking past its resting sites) than on the location of essentially stationary hosts using olfactory cues. However, Hargrove *et al.* (1995) indicated that one should be wary of any oversimplified explanations of differences based on differential flight ability.

The fact remains, however, that *G. austeni* is still attracted significantly more by a visual target baited with CO_2 than to an unbaited target. It may, therefore, be that this species (and other tsetse as well) probably have a highly sophisticated sense of smell and a higher sensitivity to CO_2. Zollner *et al.* (1998) suggested that tsetse may well be able to detect CO_2 from large distances (at least 64 m) downwind of an animal host in riverine habitats.

Implications

Consequently, it is concluded that *G. austeni* probably makes use of a combination of carbon dioxide and visual cues of a host. It could also be confidently deduced that the likely existence of other volatile components in cow odour would have no significant implications for this species. Due to its practical unsuitability, carbon dioxide can best be utilized with monitoring tools, such as electric grids, to confirm tsetse presence or absence. This can particularly be applied in such situations where *G. austeni* density is low, for example at the completion of control or eradication operations or at the limits of distribution so as to confirm their presence or absence. However, for practical application of odour-baits during monitoring and control operations, no odour bait is necessary to attract this species and reliance on the flies' visual capabilities should be sufficient to attract them to targets and traps.

For *G. brevipalpis*, on the other hand, the existence of an additional effective odour component is a possibility. It would, however, be worth doing an economic assessment to determine how much of an improvement such an odour would have over the present odours which have been found to be suitable for this species.