The role of host cues in the transmission of sleeping sickness

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ABSTRACT

Tsetse (*Glossina* spp.) transmit species of *Trypanosoma* which cause trypanosomiases in livestock and humans. To improve the cost-effectiveness of baits used to control tsetse, studies were made of the host-oriented behaviour of the following Palpalis-group species: *Glossina tachinoides* and *G. palpalis gambiensis* in Burkina Faso, *G. p. palpalis* in Côte d'Ivoire, *G. fuscipes quanzensis* in the Democratic Republic of the Congo, and *G. f. fuscipes* in Kenya. In each country, electrocuting grids and traps were used to quantify the responses of tsetse to natural and artificial host stimuli.

The results showed that riverine tsetse respond to certain natural host odours. For example, studies of the numbers of tsetse attracted to traps or grid baited with natural host odours showed that cattle odour doubled the catches of *G. p. gambiensis* and increased the numbers of *G. tachinoides* by five-fold; pig odour increased the catch of *G. p. palpalis* five-fold and doubled the numbers of *G. f. quanzensis*; and lizard odour doubled the catch of *G. f. fuscipes*. Responses of *G. tachinoides* and *G. p. gambiensis* to natural host odours were due largely to kairomones identified for savannah-tsetse (carbon dioxide, 1-octen-3-ol, acetone and 4-methylphenol). For instance, blends of 3-*n*-propylphenol, octenol, 4-methylphenol and acetone increased catches of *G. tachinoides* about five-fold, it doubled the catches of *G. p. gambiensis* and increased the catches of *G. p. palpalis* about 1.5-fold. Comparable catch ratios were obtained when acetone was removed from the blend; both *G. tachinoides* and *G. palpalis* were attracted by CO_2. None of these chemicals was effective for *G. f. fuscipes*, suggesting that unidentified semiochemicals are present in lizard odour. For *G. f. fuscipes*, the response of female flies increased from 18% to 24% with lizard odour, but mammalian odours did not have any affect. For *G. tachinoides* the landing response increased significantly with cattle odour in one experiment only, and none of the odours had any effect in the landing responses for other species. The use of odours in control operations is discussed.

Studies of visual stimuli showed that large targets ($1m^2$) doubled the catches of *G. p. palpalis* and *G. f. fuscipes* compared to $0.25m^2$ targets, the smallest being eight times more cost-efficient. Horizontal oblongs were more attractive than vertical ones for *G. f. quanzensis* and *vice versa* for *G. p. palpalis*. For all species, square targets were as effective as the most attractive oblong. Landing responses were generally about 30%, and although consistently higher for larger targets, differences were not statistically significant. The addition of flanking nets increased the catches about four-fold.

In conclusion, results suggest that cost-effective control of Palpalis-group tsetse could be achieved by using *tiny targets* (0.25×0.25m) flanked by nets of the same size.

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ABBREVIATIONS

AAT	Animal African Trypanosomiasis (*nagana*)
AIDS	Acquired immunodeficiency syndrome
BTV	Bluetongue virus
C.E.Q.	cost-effectiveness quotient
CAR	Central African Republic
CDC	Centers for Disease Control and Prevention
CHF	Crimean-Congo haemorrhagic fever
CNS	central nervous system
CO_2	carbon dioxide
CSF	cerebrospinal fluid
CTV	Citrus tristeza virus
DDT	dichloro-diphenyl-trichloroethane
DRC	Democratic Republic of Congo
ECDC	European Centre for Disease Prevention and Control
Eflornithine	DL-alpha-difluoromethylornithin
e-grids	electric grids
e-net	electric net
ERG	electroantennography
e-target	electric target
GC	gas chromatography
GC-EAG	gas chromatography linked with electroantennography
GMCS	Global Malaria Control Strategy
GMEC	Global Malaria Eradication Campaign
HAT	Human African trypanosomiasis (sleeping sickness)
HBI	human biting index
HIV	Human immunodeficiency virus
IRS	indoor residual spraying
ITC	Insecticide-treated cattle
ITMN	Insecticide-treated mosquito nets
MDG	Millennium Development Goals (WHO)
MS	mass spectrometry
NGOD	non-governmental organizations for the development
Octenol	1-octen-3-ol
p.p.m.	parts per million
PAAT	Programme Against African Trypanosomiasis
PATTEC	Pan-African Tsetse and Trypanosomosis Eradication Campaign
PCR	polymerase chain reaction
PVC	polyvinyl chloride

RC	Republic of Congo
RVF	Rift valley fever
S.E.	standard error
S.E.D.	standard error of the difference
SAT	sequential aerosol technique
SIT	sterile insect technique
UK	United Kingdom
UN	United Nations
USA	United States of America
UV	Ultraviolet
VAT	variant antigen type
VSG	variant surface glycoprotein
WHO	World Health Organisation
WNF	West-Nile fever

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CHAPTER ONE

INTRODUCTION

1.1. Vector-borne diseases

Since Sir Patrick Manson discovered the transmission of *Wuchereria bancrofti* by *Culex* (Service, 1978), almost all groups of haematophagous arthropods have been associated with the spread of pathogens (Gubler, 1991). Historically, malaria, dengue, yellow fever, plague, filariasis, louse-borne typhus, trypanosomiasis, leishmaniasis, and other vector-borne diseases were responsible for more human disease and death from the 17th century through the early 20th than all other causes combined (Gubler, 1991).

Control programmes of vector-borne diseases were based on a variety of interventions and/or prevention strategies, where the control of the arthropod vectors played a major role. Early in the twentieth century, yellow fever in Cuba was the first vector-borne disease to be effectively controlled by means of vector control (Table 1-1). Soon thereafter, yellow fever and malaria were controlled in Panama (Gubler, 1998) (Table 1-1). Over the next 50 years, other campaigns against vectors achieved a widespread reduction in the incidence of diseases. Thus, urban yellow fever and dengue, transmitted by *Aedes aegypti*, was effectively controlled in Central and South America and eliminated from North America; similarly, malaria was nearly eliminated in the Americas, the Pacific Islands, and Asia (Gubler, 1998) (Table 1-1). The discovery and effective use of residual insecticides in the 1940s onwards contributed greatly to these successes. However, since the 1960s there has been a resurgence of previously controlled vector-borne infectious diseases.

Disease	Location	Year
Yellow fever	Cuba	1900-1901
Yellow fever	Panama	1904
Yellow fever	Brazil	1932
Anopheles gambiae infestation	Brazil	1938
An. Gambiae infestation	Egypt	1942
Louse-bornetyphus	Italy	1942
Malaria	Sardinia	1946
Yellow fever (*Aedes aegypti*)	Americas	1947-1970
Malaria	Americas	1954-1975
Malaria	Global	1955-1975
Yellow fever	West Africa	1950-1970
Onchocerciasis	West Africa	1974-present
Bancroftian filariasis	South Pacific	1970s
Chagas disease	South America	1991-present

Table 1-1: Examples of vector-borne disease control/elimination programmes (Brunhes *et al.*, 1994; Simarro *et al.*, 2008; CDC, 2009)

Vector-borne infections remain as major causes of mortality and morbidity, particularly in the poorest regions of the world, affecting children with particular virulence. For example, today malaria alone is responsible for approximately 11% of the total disease burden in Africa, while all vector-borne diseases combined are responsible for less than 0.1% in Europe (Campbell-Lendrum *et al.*, 2005). Vector-borne diseases are not just an effect of poverty, but also a contributory cause to it. This association is illustrated by the per capita incomes, which in countries with hyperendemic malaria are only about 33% of those without malaria (Gallup & Sachs, 2001).

Nevertheless, concerns in western countries about vector-borne diseases are increasing. Among other reports, a risk assessment of vector-borne diseases in Europe prepared by the European Centre for Disease Prevention and Control (ECDC) listed Crimean-Congo haemorrhagic fever (CHF), chikungunya, tick-borne encephalitis, West-Nile fever (WNF) and leishmaniasis, among the vector-borne diseases that have the greatest potential to affect European citizens (Vesenjak-Hirjan *et al.*, 1991; Dedet & Pratlong, 2000; Karti *et al.*, 2004; ECDC, 2006; ECDC/WHO, 2007; Pugliese *et al.*, 2007; Papa *et al.*, 2008; Senior, 2008).

The factors responsible for the emergence/resurgence of vector-borne diseases are complex. They include insecticide and drug resistance, changes in public health policy, emphasis on emergency response as prevention programmes are disregarded, and

demographic and social changes – due to population growth, social development, resettlements or social unrest (Lederberg *et al.*, 1992).

Urbanization, deforestation and agricultural practices are among the main reasons for the re-emergence of vector-borne diseases (Table 1-2). Unplanned and uncontrolled urbanization in poor countries has led to inadequate housing and sanitation, and had an impact in the transmission of mosquito-, rodent-, and water-borne diseases (Gubler, 1998). Irrigation systems and dams built since the 1950s have provided suitable breeding sites for vectors. Similarly, large areas of the tropical forests are being cleared, and replaced by agricultural practices such as rice cultivation, providing plentiful mosquito breeding sites (Gubler, 1998).

Urbanization	Deforestation	Agricultural Practices
Dengue fever	Loaiasis	Malaria
Malaria	Onchocerciasis	Japanese encephalitis
Yellow fever	Malaria	St. Louis encephalitis
Chickungunya	Leishmaniasis	West Nile fever
Epidemic polyarthritis	Yellow fever	Oropouche
West Nile fever	Kyasanur Forest disease	Western equine encephalitis
St. Louis encephalitis	La Crosse encephalitis	Venezuelan equine encephalitis
Lyme disease	Eastern equine encephalitis	
Ehrlichiosis	Lyme disease	
Plague		

Table 1-2: Influences on emergent/resurgent vector-borne diseases (Gubler, 1998)

Improved air-, sea- and land-transport networks also play a role in the dissemination of these diseases into the Western countries. Pathogens and their vectors can now move further, faster and in greater numbers than ever before (Tatem *et al.*, 2006). Thus, movements of passengers, animals and goods have been incriminated in the spreading of several arboviruses – *e.g.* chikungunya, dengue and WNF – across Europe and the Americas (Gould *et al.*, 2003; Tatem *et al.*, 2006; Gould & Higgs, 2009).

In addition to human health, new and emerging animal and plant vector-borne diseases have also greatly affected regional ecologies and economies. For instance, bluetongue virus (BTV) – a virus transmitted to ruminants by the midges *Culicoides* spp. – costs the United States cattle and sheep industry an estimated $125 million annually in lost trade and monitoring. From 1998 to 2005, multiple incursions of different strains and serotypes of

the same virus have moved northwards into the European continent with a frequency never before recorded, causing substantial disease-related costs through mortality and morbidity, and socioeconomic costs through implementation of control measures (Simon, 2007).

Vector-borne diseases will continue to represent a significant threat, not just because of their direct effect on human health, but also for their negative economic impact on families, communities and countries.

The transmission of vector-borne diseases is governed by complex interactions between parasites, vectors and hosts. Insights into each of these interactions have practical implications: (i) they provide opportunities for the rational development of control campaigns, aiming to break the transmission, and (ii) interactions between parasites, vectors and hosts contribute to an understanding off the epidemiology of the diseases. Elucidating the behavioural mechanisms by which vectors locate their hosts can help to develop efficient sampling and control systems. For example, monitoring and control devices can be designed to attract biting insects by mimicking their hosts (Muirhead-Thomson, 1991). In addition, cues used by the vectors to select and approach suitable hosts, together with the relative availability of human and non-human hosts, govern host choice, and thereby the transmission of human diseases.

Host-orientated behaviour has been extensively studied and reviewed for tsetse, *Glossina* spp., vectors of Human African Trypanosomiasis (HAT), also known as sleeping sickness, and Animal African Trypanosomiasis (AAT), known as *nagana* (Colvin & Gibson, 1992; Vale, 1993a; Green, 1994; Torr, 1994b; Willemse & Takken, 1994; Gibson & Torr, 1999). However, although most of the studies have investigated the host-orientated responses of the main AAT vectors, much less is known about the main HAT vectors. The subsequent sections in this chapter review the importance of sleeping sickness as a public health problem, the host-orientated behaviour of tsetse, with an emphasis on the main vectors of the HAT, and the contribution of this knowledge towards the control of the vectors.

1.2. Sleeping sickness: a nightmare

1.2.1. Generalities

HAT is caused by the parasitic flagellate protozoa *Trypanosoma brucei gambiense* and *T. b. rhodesiense*, transmitted to humans by the bite of tsetse (*Glossina* spp.). *Trypanosoma* are motile, with a kinetoplast associated with the basal body of the flagellum, and range in length between 15 and 35 μm.

The disease is only found in sub-Saharan Africa, between 14°N and 29°S, within the limits of the geographical distribution of tsetse (Figure 1-1).

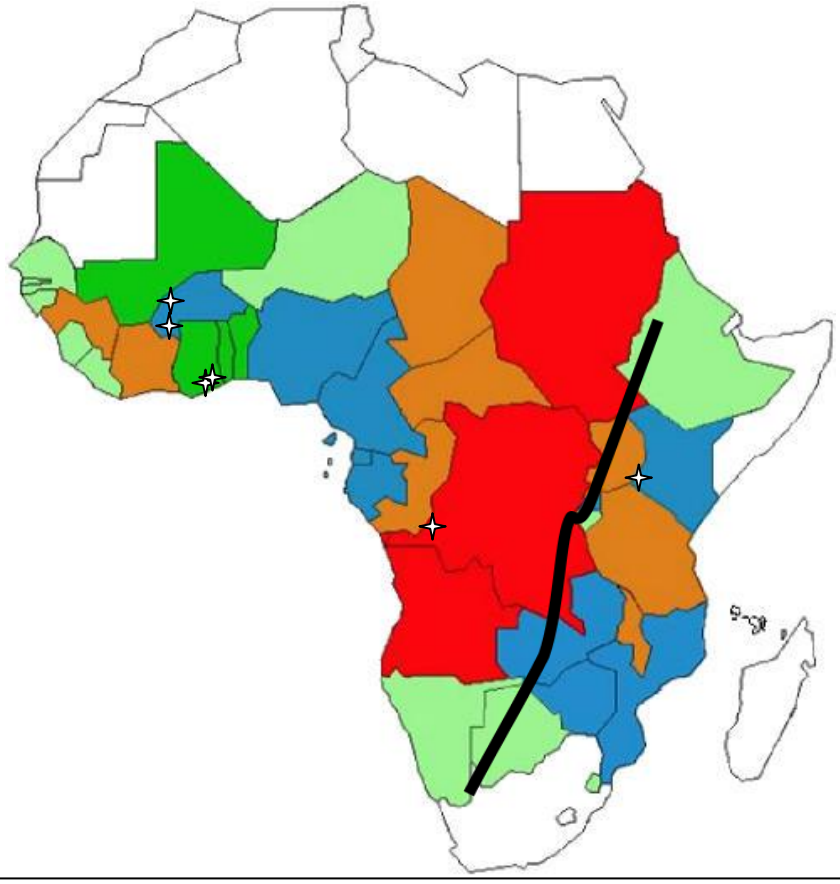


Figure 1-1: Distribution of HAT. Those countries coloured in red are currently reporting in excess of 1000 cases per year. Those in brown currently report between 50 and 1000 cases per year. Those in blue report fewer than 50 cases per year, while those in green currently report no cases of HAT. (Barrett *et al.*, 2007). No HAT cases in white areas. Field sites in the study are marked with a start. The black line delimit the Rift Valley, and the distribution of *T. b. rhodesiense* in the East, and *T. b. gambiense* in the West

The Rift valley, separating East and West Africa, defines the distribution of the two subspecies of *Trypanosoma* (Welburn *et al.*, 2001): *T. b. gambiense* being present in central and western Africa, and *T. b. rhodesiense* in east and southern Africa.

The reason for the distinctive distribution of the two subspecies of *T. brucei* remains unclear. Some authors suspect that the current distribution of the two subspecies of *T. brucei* may be, at least in part, a consequence of the co-evolution of parasites and hosts in different environments (Welburn *et al.*, 2001; Welburn *et al.*, 2011). The fact that the disease advances or contracts within a given focus but ancient foci tend to exist to this day is an indication of importance of this theory (Welburn *et al.*, 2001; Welburn *et al.*, 2011). Evolution of hominids has been intimately connected with the Rift (Haile-Selassie *et al.*, 2010; Reed *et al.*, 2013). Climate changes of the earth about five million years ago caused a reduction of the forest and an increased in the savannah areas in East Africa. This change in the habitat resulted in the evolution of bipedalism and, ultimately, the evolution of hominids in the Rift Valley (Johanson & Edey, 1990; Stringer & McKie, 1998). The colonisation of new habitats brought hominids into contact with parasites different from those found in the forest, including trypanosomes circulating in the reservoirs of savannah-adapted game animals. *T. b. rhodesiense* is known to be zoonotic, and is transmitted from wild (Heisch *et al.*, 1958) and domestic (Onyango *et al.*, 1966) animals to humans. Apes, like humans, are partially adapted to *T. b. gamginese,* and Welburn *et al.* (2001; 2011) speculated that this adaptation was achieved over long periods of exposure in the forested areas to the west of the Rift Valley.

There are over 250 discrete endemic foci of HAT, distributed in some 36 countries. The World Health Organization (WHO) estimates that 70 million people are at risk, with about 7,000 new cases each year (Simarro *et al.*, 2012). The severity of the disease, the complexity of diagnosis in rural areas, the toxicity of the drug treatments and the potential of HAT to develop into epidemics makes the disease a major public health problem (Hide *et al.*, 1996; Kigotho, 1997; Smith *et al.*, 1998; Hide, 1999; Moore *et al.*, 1999; WHO, 2000; Louis *et al.*, 2002; Fèvre *et al.*, 2004; WHO, 2004).

Depending on the causative subspecies of the parasite implicated, *i.e. T. b. gambiense* or *T. b. rhodesiense*, HAT develops into two forms of the disease: *gambiense*- and *rhodesiense*-sleeping sickness, with their distinctive epidemiology and pathology. In both cases, symptoms begin with fever, headaches, and joint pains. If untreated, the disease slowly

overcomes the defences of the infected person, and symptoms include anaemia, pruritus and skin rash, oedema, disruption of the endocrine rhythms, thrombocytopaenia, splenomegaly, and cardiac and renal dysfunction. Then, the parasite passes through the blood-brain barrier, initiating the meningo-encephalic phase (Enanga *et al.*, 2002). The symptoms during the neurological phase include confusion and reduced coordination, accompanied by fatigue and disrupted sleep patterns. Severe mental disorders are common in the second stage of the disease, and patients frequently show memory loss, depression, agitation, and symptoms evolving into mania, irritability, dementia, and lethargy. Without treatment, the disease is invariably fatal, with progressive mental deterioration leading to coma and death. The distinctive features of the two forms of the disease include:

- ***gambiense*-sleeping sickness** (Table 1-3) represents more than 90% of reported cases of sleeping sickness and causes a chronic infection. A person can be infected for months, or even years, without major signs or symptoms of the disease. When symptoms are apparent, the disease has often developed into the neurological phase (WHO, 2006a). *Gambiense*-sleeping sickness is generally confined to a human-fly-human cycle (Malvy & Chappuis, 2011).

Tryp. sp	Host	Disease	Disease course	Distribution	Main Vectors	Transm.
T. b. gambiense	Humans Pigs	Sleeping sickness	Chronic	Western Africa	*G. palpalis* *G. fuscipes*	Biological
T. b. rhodesiense	Humans Cattle Wild ruminants Monkeys	Sleeping sickness	Acute in humans Mild infection in animals	Eastern Africa	*G. morsitans* *G. swynnertoni* *G. pallidipes* *G. fuscipes*	Biological
T. b. brucei	Antelope Cattle Camels Horses Sheep Goats	Nagana	Acute	Africa	*G. morsitans* *G. swynnertoni* *G. pallidipes* *G. palpalis* *G. tachinoides* *G. fuscipes*	Biological
T. vivax	Cattle Camels Horses Sheep Goats	Nagana	Acute	Africa	*G. morsitans* *G. palpalis* *G. tachinoides* *G. swynnertoni* *G. pallidipes* *G. austeni* *G. vanhoofi* *G. longipalpis*	Biological
T. congolense	Cattle Camels Horses Sheep Goats Pigs	Nagana	Chronic	Africa	*G. palpalis* *G. morsitans* *G. austeni* *G. swynnertoni* *G. pallidipes* *G. longipalpis* *G. tachinoides* *G. brevipalpis*	Biological
T. simiae	Domestic pigs Cattle Camels Horses	Nagana	Acute	Africa	*G. palpalis* *G. fuscipes* *G. morsitans* *G. tachinoides* *G. longipalpis* *G. fusca* *G. tabaniformis* *G. brevipalpis* *G. vanhoofi* *G. austeni*	Biological
T. uniforms	Cattle Camels Horses Sheep Goats	Nagana	Acute	Africa	*G. morsitans* *G. palpalis* *G. tachinoides* *G. swynnertoni* *G. pallidipes* *G. austeni* *G. vanhoofi* *G. longipalpis*	Biological
T. suis	Pigs Warthogs	Surra	Chronic	Africa	*G. palpalis* *G. fuscipes* *G. morsitans* *G. tachinoides* *G. longipalpis* *G. fusca* *G. tabaniformis* *G. brevipalpis* *G. vanhoofi* *G. austeni*	Biological
T. evansi	Horses Donkeys Camels Deer Llamas Cattle Buffalo Dogs Cats	Surra	Chronic	North Africa Middle East Asia South America	*G. palpalis* *G. fuscipes* *G. morsitans* *G. tachinoides* *G. longipalpis* *G. fusca* *G. tabaniformis* *G. brevipalpis* *G. vanhoofi* *G. austeni* *Stomoxys* *Lyperosia* Tabanidae	Mechanical
T. equiperdum	Equines	Dourine	Chronic	Africa Asia	*N/S*	Venereal disease
T. cruzi	Human Domestic rodents Wild mammals Dogs Cats	Chagas (American trypanosomiasis)	Chronic	America	Reduvidae bugs (*Triatoma*, *Rhodnius*, *Panstrogylus*)	Biological

Table 1-3: List of the main *Trypanosoma* species of medical or veterinary importance. In pink, causative agents of Human African Trypanosomiasis (also known as sleeping sickness). In mauve, main pathogens of African Animal Trypanosomiasis (also known as *nagana*). In green, other causative agents of *nagana*. In yellow, *Trypanosoma* species causing other diseases in livestock (i.e. *surra* and *dourine*) and humans (i.e. Chagas disease)

- ***rhodesiense*-sleeping sickness** (Table 1-3) represents less than 10% of reported cases and causes an acute infection. First signs and symptoms are observed in days or weeks after the infection. The disease develops rapidly and invades the central nervous system (WHO, 2006a). This form of sleeping sickness is a zoonotic disease, and requires animal reservoirs (Malvy & Chappuis, 2011).

Trypanosoma species also infect vertebrate animals other than humans, causing AAT. AAT is a disease complex transmitted by tsetse, and caused by several protozoan species of the genus *Trypanosoma*, whereof *T. b. brucei*, *T. vivax* and *T. congolense* are responsible for most of the cases in livestock (Table 1-3). AAT affects primarily cattle, but it also causes serious losses in pigs, camels, goats and sheep. The parasite infects the blood of the vertebrate host causing fever, weakness, immunosuppression and lethargy, which leads to weight loss and anaemia. The disease is an important cause of abortion in cattle, and is fatal in some animals unless treated. The impact of AAT in the African economy is severe: US$ 1-1.2 billion are lost each year in attempts to control the disease and in direct losses in meat and milk production (FAO, 2002). Affected animals are less suitable for ploughing, leading to further impoverishment of farmers. In order to limit the effects of AAT, African farmers have traditionally made efforts to prevent livestock from having contact with tsetse by avoiding tsetse-infested areas. In this way, out of 165 million cattle in sub-Saharan Africa, only 10 million are located in tsetse-infested areas, while the remainder are distributed in the highlands or the semi-arid Sahel zone (Cecchi & Mattioli, 2009). The uneven cattle distribution has two negative implications: (i) it leads to land overuse in the areas where livestock concentrate; and (ii) access to fertile and cultivable areas, where trypanosomiasis is present, is restricted (Jordan, 1986; Swallow, 1999). Thus, the overall agricultural production loss is estimated as US$ 5 billion (Budd, 1999; FAO, 2002).

Both human and animal Trypanosomiasis are implicated in the underdevelopment of the African continent. They are considered to be major obstacles in the establishment of a flourishing agriculture to provide food security, and therefore represent causes of poverty and disease (Simarro *et al.*, 2006).

1.2.2. Life cycle of the human parasites Trypanosoma brucei s.l.

While feeding on mammalian hosts, infected tsetse inject metacyclic trypomastigotes into the blood stream. The parasites enter the lymphatic system and pass into the bloodstream. Inside the host, they transform into bloodstream trypanomastigotes, where they are carried to other sites throughout the body. Eventually, the parasites cross the blood-brain barrier, establishing in the central nervous system (CNS), which determines the beginning of the the neurological phase of the disease. Although trypomastigotes multiply by binary fission in the bloodstream, the evidence suggests that they do not proliferate in the cerebrospinal fluid (CSF) (Pentreath *et al.*, 1992; Pentreath, 1999). Tsetse become infected with bloodstream trypomastigotes while taking a bloodmeal from an infected mammalian host. In the fly's midgut, parasites transform into procyclic trypomastigotes, multiply by binary fission, leave the midgut, and transform into epimastigotes. The epimastigotes reach the fly's salivary glands and continue multiplication. The cycle in the fly takes approximately 3 weeks. Humans are the main host for *T. b. gambiense*, but this species can also be found in other mammals. Wild game animals are the main reservoir of *T. b. rhodesiense* (Figure 1-2) (CDC, 2009).

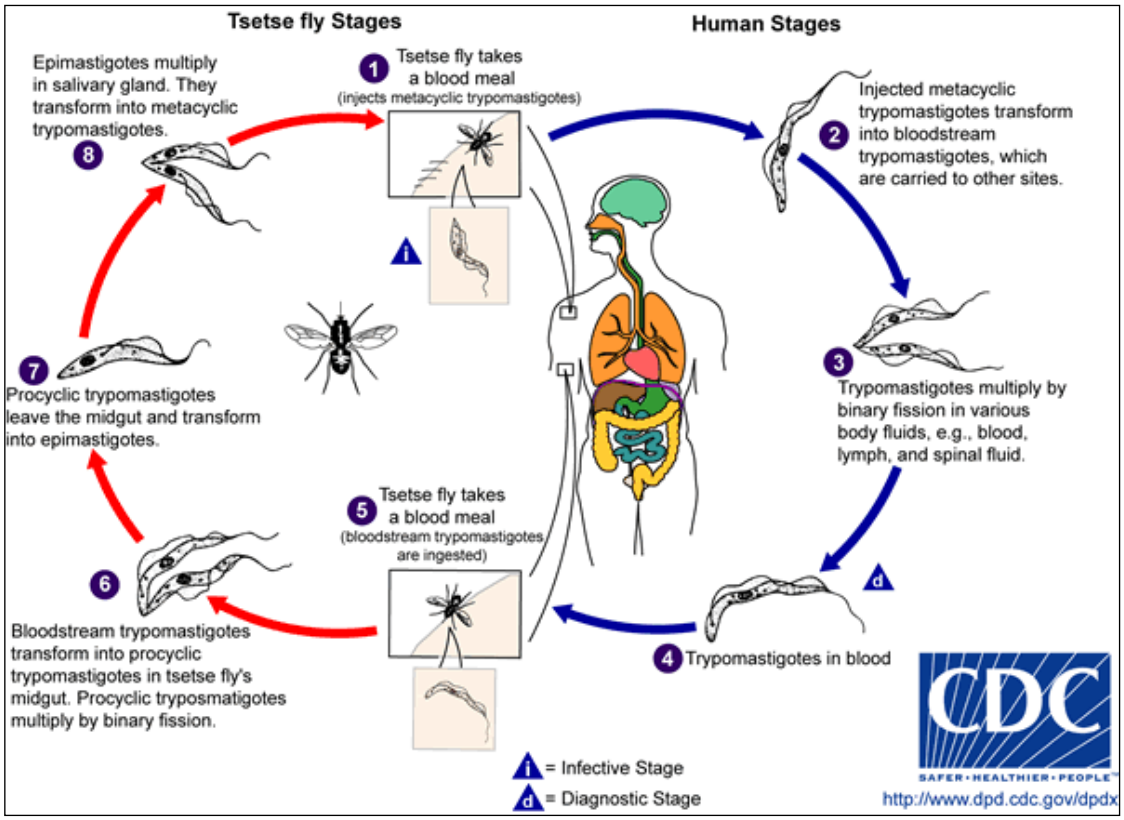


Figure 1-2: Life cycle of *Trypanosoma bruce s.l.* (CDC, 2009)

How the parasites penetrate the CNS is not fully understood. Schultzberg *et al.* (1988) suggested that in an early stage of the infection the parasites enter the CNS through areas where the blood-brain or blood-nerve barrier are absent, *i.e.* the sensory ganglia and circumventricular organs. Although the hypothesis has not been refuted, more recent studies suggest that *Trypanosoma* can invade the CNS crossing the blood-brain barrier (Enanga *et al.*, 2002). This barrier shows a selective permeability, given by the presence of the tight junctions that restrict paracellular passive diffusion between endothelial cells of the cerebral vessels. However, the selective permeability of the blood-brain barrier is compromised during the inflammation process (Enanga *et al.*, 2002; Masocha *et al.*, 2004). *T. brucei* are extracellular pathogens, and as such, they are continuously exposed to the host's immune system. Cytokines are released in response to the presence of the pathogen antigens, which is followed by the migration of neutrophils, and subsequently, the antigen-specific B and T lymphocytes, and the monocytes (Osborn, 1990). The migration of the mononuclear cell into the CNS increases the permeability of the blood-brain barrier, which can influence the invasion of the CNS by the parasites (Enanga *et al.*, 2002). Pro-inflammatory cytokines released during the infection by cells of the blood-brain barrier induce the synthesis of nitric oxide, which also increases the permeability of the barrier (Enanga *et al.*, 2002).

The host's immune response plays an important role in the pathogenesis of HAT. The manifestations of the disease in the meningo-encephalitic phase are triggered by a self-propagating autoimmune response (Enanga *et al.*, 2002). *Trypanosoma* evade the immune system, primarily through antigenic variation (Figure 1-3): the appearance of successive parasitic waves correlates with changes in the specific glycoproteins, responsible of each variant antigen type (VAT) of *Trypanosoma* (Vickerman, 1985). The variant surface glycoprotein (VSG) is the predominant surface antigen of African trypanosomes, and covers nearly the whole membrane of the bloodstream trypomastigotes. The continuous stimulation of the immune system due to variant antigens leads to a dysfunction in the cytokine balance and the production of autoantibodies. Autoantibodies trigger the demyelination and atrophy of the CNS, leading to the death of the patient (Vincendeau *et al.*, 1996; Vincendeau & Bouteille, 2006).

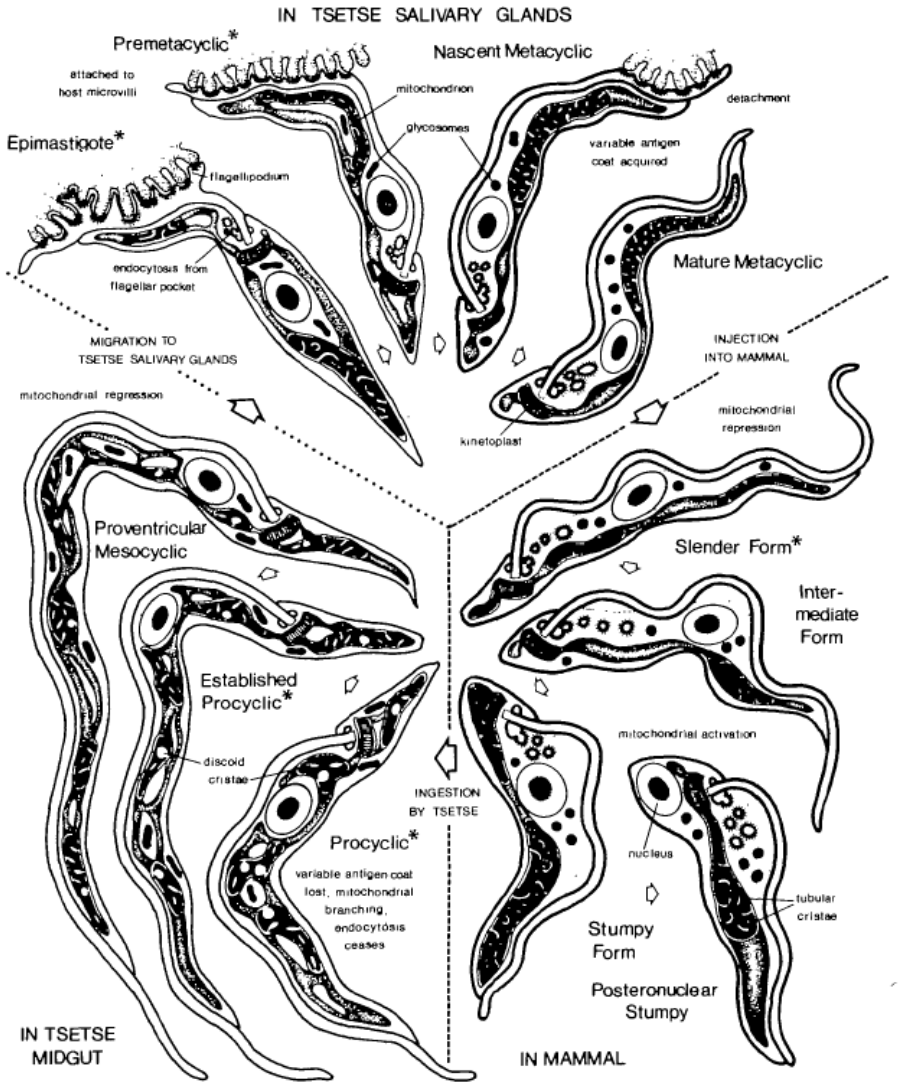


Figure 1-3: ***Developmental cycle and biology of pathogenic trypanosomes:*** Schematic diagram of *T. brucei* developmental cycle in mammal and tsetse, showing changes in cell surface, mitochondrion, glycosomes and receptor mediated endocytosis, also in relative size of different stages. Stages possessing the variable antigen coat lie to the right uncoated stages to the left. * Cellular division (Vickerman, 1985)

While antigenic variation constitutes a major obstacle to the development of effective vaccines (WHO, 1978; Pays, 1995), the migration of the parasite into the CNS requires the use of highly toxic drugs to treat patients in the meningo-encephalitic phase (Fairlamb, 2003; Kumar *et al.*, 2006).

1.2.3. History of HAT

HAT in early African civilisations (<XVth century)

The lack of documentation makes it difficult to assess the prevalence of sleeping sickness in early African civilisations. However, a number of reasons suggest that it was relatively low (De Raadt, 2005):

- in general, Africa was sparsely populated, hindering parasite transmission
- large areas around the villages were maintained clear of vegetation, protecting people against wild predators, enemy tribes and slave raids; as a side effect, clear areas served as barriers against tsetse
- tribes and kingdoms were isolated from each other, preventing the dissemination of the disease from one community to another
- villages devastated by diseases were abandoned, and the locations avoided for generations
- wild hosts were abundant, reducing the likelihood of tsetse turning to humans

The existence of terms in local languages to describe the disease (*e.g. marree*, *'nluoi*, *naganloe*, *kadeera*, *kee kollee kondee*, *seenoyuncaree* in West Africa, and *meki abe*, *meze'e*, *matsegue* in Central Africa) suggests that people knew about sleeping sickness and differentiated it from other diseases, although the first unequivocal documentation was provided by the Arabian writers.

By 700 AD, the Arabian powers had invaded most of North Africa. However, the impact of animal trypanosomiasis influenced their movements, restricting the occupation to the Sahel limits (McKelvey, 1973). Instead, trans-Saharan trade routes linked the Arab word with some of the kingdoms in West Africa, such as Benin, Ghana, Mali and Songhai. As a result of this contact, Arabs provided the first known reference to HAT, when the historians Ibn Khaldum and Alqalaqshandiy reported in 1401 the death of King Diata II in 1373, sultan of Mali, from a lethargy (Louis *et al.*, 2002). The historians stated that the

disease was common in the kingdom, although large-scale outbreaks were not described (De Raadt, 2005).

HAT described by Europeans: early contacts (XVth-XIXth centuries)

Trade routes between Africa and Europe were established from the 15th century onwards, first by the Portuguese, and then by the French, British and Dutch. European traders, including slave drivers, were supplied on the coast, and their incursions into the continent's interior were rare before the XIX century. During the 15th-19th centuries, slavery took millions of Africans overseas, mainly to the Americas. John Atkins, a British navy surgeon serving on slave ships, described in 1734 the cerebral oedema produced by sleeping sickness on the coast of Guinea (McKelvey, 1973). At that point, adenopathies were identified as a sign of poor health condition, and slaves with such symptoms were discarded. Thereafter there is no record until 1803, when Winterbottom reported some cases of 'lethargy' among the inhabitants of Sierra Leone (Scott, 1939). References to the disease became more frequent later in the same century, mostly in West Africa, *i.e.* in Sierra Leone, Senegal, Angola and Congo, and among slaves exported into the Americas (Scott, 1939; Duggan, 1970).

At the end of the 19th century, Europeans had explored and colonised the interior of Africa. At this time, devastating epidemics of sleeping sickness occurred in Kenya, Tanzania, Uganda, Nigeria, and the Democratic Republic of Congo (DRC). These epidemics were associated with social and environmental disruptions during colonial administration (Ford, 1971; Lyons, 1992). In addition, the devastating panzootic of rinderpest between 1889 and 1892 has been associated with the spread of HAT in Uganda in the 1900s, as it killed over 90% of the livestock, and the greater part of wildlife. Consequently, tsetse fed more on humans and hence increased the incidence of disease (Fèvre *et al.*, 2004).

Before the discovery of the aetiology of sleeping sickness, the role of vectors in the transmission, and effective therapies, control campaigns were based upon the isolation of the patients and the transfer of exposed populations. Scientific and technological advances during the twentieth century permitted the implementation of new and more efficient ways of controlling the disease.

Outcomes of early scientific missions (1900s)

Between 1900 and 1905, sleeping sickness killed over a quarter of a million Africans in the British Protectorate of Uganda (Lyons, 1992). Consequently, colonial administrations sponsored scientific expeditions to study the disease. As a result, *Trypanosoma* protozoa were identified from a blood sample in 1902 (Dutton, 1902; Forde, 1902a, b), allowing a chain of discoveries during the subsequent sixty years (Ford, 1971). Thus, Castellani (1902-1903) proposed the trypanosomes as the causative organism of sleeping sickness; Bruce (1895) discovered the role of tsetse in the transmission of *nagana*, and in 1903, in collaboration with Nabarro, demonstrated that the same vectors also transmit sleeping sickness to humans (Bruce & Nabarro, 1903); and over a five-year period, the cycle of the parasite in the *Glossina* was described (Kleine, 1909).

Advances in pharmacology and vector control (1900s-1940s)

Pharmacology also saw rapid advances in the early part of the twentieth century. In particular, Thomas (1905) demonstrated the trypanocidal properties of atoxyl. Atoxyl was followed by the discovery of suramin (Bayer 205) in 1917, pentamidine in 1939, and melarsoprol in 1949 (Lourie & Yorke, 1939; Friedheim, 1949; Jonchère *et al.*, 1953; Cross, 2001). In spite of these initial advances, after the launch of melarsoprol, the pharmacology research suffered an impasse of over 40 years, before the market saw a new trypanocide drug for medical use: Eflornithine.

Alongside the development of trypanocides, new tools for vector control were implemented before 1950. For example, traps for tsetse control were developed, and the insecticidal properties of Dichloro-Diphenyl-Trichloroethane (DDT) discovered in the 1940s. These technologies, *i.e.* traps and insecticide, were applied individually or in combination by 1949 (Hargrove, 2003a).

Technological advances provided the tools used during the oncoming campaigns conducted in affected countries.

Control campaigns during the colonial era (1910s-1960s)

Trypanosomiasis control in West and East Africa

In general, different control strategies were followed in west and east Africa. During the colonial regimes, Francophone western Africa pursued technologies suited for the control of *gambiense*-HAT, while in Anglophone Africa in the east and south of the continent, *nagana* was the main concern, followed in importance by *rhodesiense*-HAT. The reasons for this difference lie in (i) the distinct epidemiology of the two forms of sleeping sickness, *i.e. rhodesiense* in west Africa, and *gambiense* in east Africa; and (ii) the distinctive ecology of the tsetse species involved in the transmission of *Trypanosma* parasites.

Most western and central Africa was heavily forested (*e.g.* the Congo River Basin), and inhabited by riverine tsetse species. *Gambiense*-HAT occurs mainly in this region, where reducing the parasitaemia in the human reservoir decreases the chances of further generations of tsetse becoming infected and passing on the infection (Welburn *et al.*, 2001).

By contrast, vast areas of savannah extend over east Africa, providing a suitable environment for rearing cattle. Savannah-tsetse are predominant in this habitat, and *nagana* was an important economic burden for the colonial authorities. Campaigns against *rhodesiense*-HAT were mainly reduced to controlling large, but rare and usually self-limiting, epidemics (Langlands, 1967). Detection and treatment of human cases, infected with *T. b. rhodesiense*, have little impact in the transmission of the parasite (Fèvre *et al.*, 2007). Consequently, control activities in East Africa were based largely upon reducing transmission by game destruction and vector control.

Jamot's postulates; control campaigns in colonial Cameroon

During the 1920-1930s, Eugène Jamot established the protocols for HAT control, used commonly in West Africa during the colonial times, known as "Jamot's postulates". Jamot employed mobile teams to screen actively entire populations in affected areas, carried out the diagnosis *in situ*, and treated the cases; if the prevalence was high, mass prophylaxis would be provided. In his postulates, among the possible means of controlling the disease, *i.e.*, through reservoir sterilizations, vector eradication or protection of healthy individuals,

Jamot proposed to operate those that were most suitable for achieving a large scale intervention (De Raadt, 1999). As a result of Jamot's methods, the prevalence in Cameroon declined approximately 300-fold (Lapeyssonnie, 1992), and by 1930 HAT was no longer considered a major cause of mortality in the country (Milleliri, 2004).

Other examples of control campaigns during the colonial administrations

Following the results in Cameroon, Jamot's postulates were implemented in the 1940s onwards throughout West and Central Africa, by the French (French West Africa), Belgians (DRC), British (Ghana and Nigeria), Portuguese (Angola) and Spanish (Equatorial Guinea) colonial authorities with similar effects.

In the former Belgian Congo, an all-time peak of 33,562 new sleeping sickness cases was reported in 1930, but the annual number of cases decreased progressively over the next three decades to about 1000 cases in 1959 (Ekwanzala *et al.*, 1996). The country became independent in 1960.

The colonial Portuguese government in Angola created a national programme in 1949. Mobile teams crossed the country, visiting each village at least once a year. In the 1950s, 5000 cases were reported and treated each year, while in 1974, two years before independence, only three new cases were recorded countrywide (Smith *et al.*, 1998).

The focus of Luba (Bioko island, Equatorial Guinea) was described in 1910 (Pittaluga, 1910). Twenty years later, a control programme was implemented, based upon 'case detection and treatment'. The intervention resulted in a reduction in the number of new cases, from 2785 cases in 1927 to 748 cases in 1934 (González-Vicente, 1947), and finally to four cases in 1967 (González-Vicente *et al.*, 1968). The country became independent one year later.

Different strategies were used during the colonial era in Uganda. Uganda is the only country known to be affected by both *T. b. rhodesiense* and *T. b. gambiense*. Both forms of the parasite are located in different regions of the country. Thus, *T. b. gambiense* affects populations in the West Nile region in the north-west, whereas *T. b. rhodesiense* occurs

traditionally, with far fewer cases per annum, in the south-east's Busoga region, in the Lake Victoria Basin (Odiit *et al.*, 2004).

The 'Tsetse Control Department', under the Ministry of Animal Industry and Fisheries, was created in 1947 to control outbreaks of sleeping sickness and *nagana*. The East African Trypanosomiasis Research Organization (EATRO) was established in 1956 to carry out research, and advise the Government on control strategies. Active and passive surveillance was carried out by sleeping sickness assistants, whereas the 'Veterinary Department' was responsible for removing the parasites from animal reservoirs.

Anti-trypanosomiasis campaigns in Africa implemented in the 1910s onwards led almost to a halt in transmission, before the responsibilities for controlling trypanosomiases were transferred to the local authorities (Simarro *et al.*, 2008). Thus, by the late 1960s the overall percentage of new *T.b. gambiense* cases had fallen below 0.01% (Figure 1-4). The campaigns during the colonial administrations involved the mobilisation of large resources, human and material, the backup of the colonial armies, and strict policies to guarantee the participation of the population in the screening. With independence, this support was no longer sustained, and disease resurgence rapidly took place.

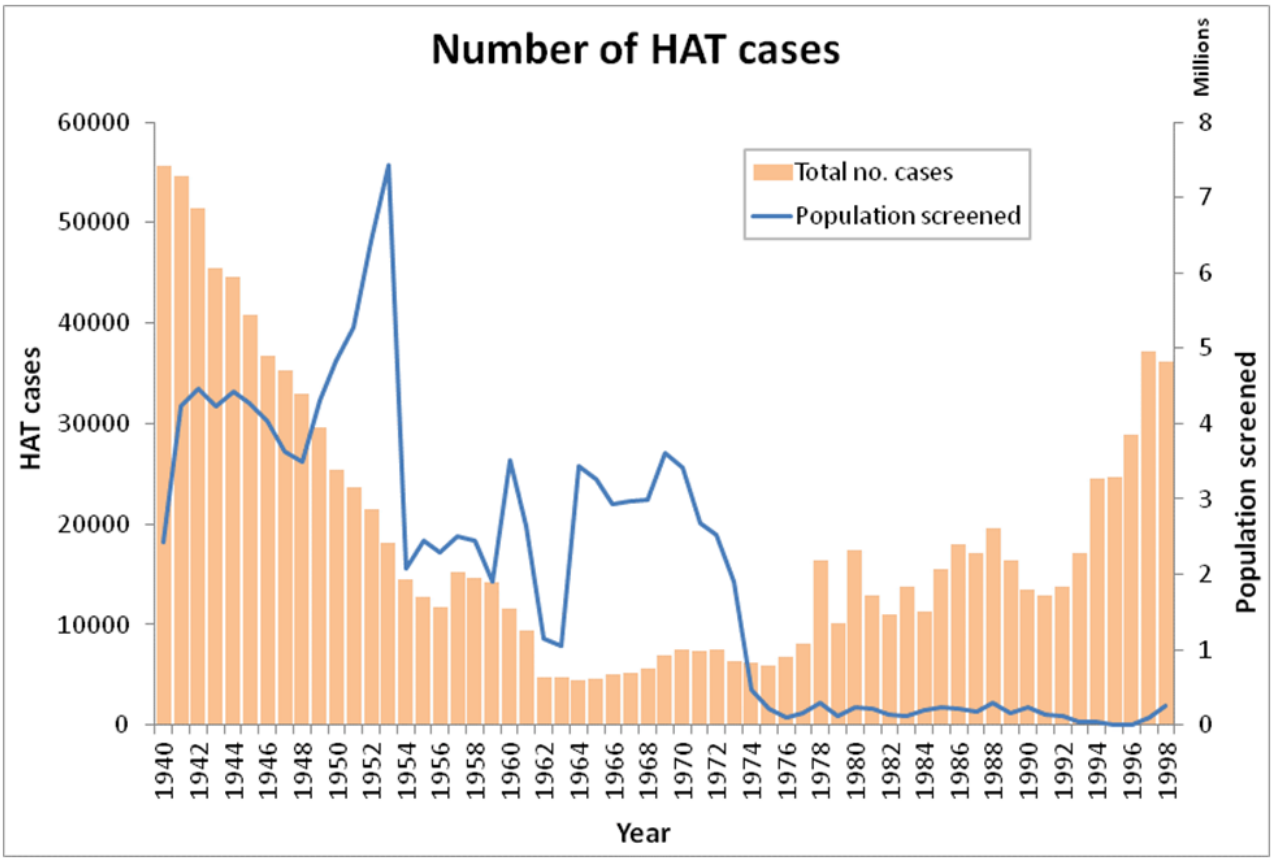


Figure 1-4: Total number of cases of sleeping sickness (orange) reported and population screened (active detection) worldwide between 1940 and 1998. Data extracted from WHO (2000)

HAT in the postcolonial era (1960s-mid 1990s)

By the end of the 1960s, the majority of HAT-affected countries became independent and were no longer supported by their former colonial powers. Health services were facing severe budgetary and operational constraints, and after a long period of sustained low endemicity, trypanosomiasis control was no longer a priority. Following independence, sleeping sickness re-emerged in Uganda (Fèvre *et al.*, 2005; Odiit *et al.*, 2005), DRC (Ekwanzala *et al.*, 1996; Van Nieuwenhove *et al.*, 2001), Sudan (Moore *et al.*, 1999), and Angola (Stanghellini *et al.*, 1994).

Economic decline, civil disturbance, war, population movements and refugees have been associated with resurgence and epidemics (Stanghellini *et al.*, 1994; Smith *et al.*, 1998; Moore *et al.*, 1999; Boelaert *et al.*, 2005; Fèvre *et al.*, 2005; Berrang-Ford, 2007). Active

screening, trypanocidal drugs distribution, and vector control interventions suffered the consequences of the civil unrest and lack of funds (Médecins Sans Frontières, 2001). When active screening resumed in the 1990s, the reported incidence of HAT had reached levels comparable to those of the 1930s (Figures 1-4 & 1-5).

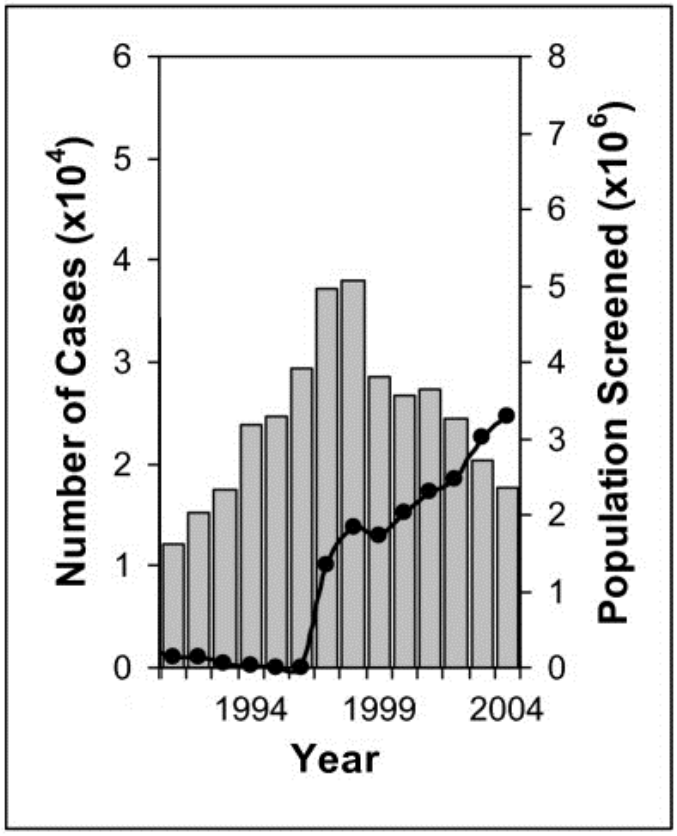


Figure 1-5: Number of reported cases of sleeping sickness (combined gambiense- and rhodesiense-HAT) and population screened, 1991-2004 across Africa. Grey columns: number of reported cases; black circles: population screened (Steverding, 2008)

Since 1962, WHO has assisted endemic countries to develop control programmes and mobilise the required resources. WHO encouraged the reinforcement of vector control where needed, emphasised the availability of drugs, stressed the importance of data collection, and advocated the expansion of inter-country, regional and international coordination under the auspices of WHO (WHO, 1998). However, prior to 2000, WHO progressively decreased its annual budget for trypanosomiasis field research, and reduced its regular staff devoted to trypanosomiasis control to one half-time individual (Ekwanzala *et al.*, 1996).

Sleeping sickness falls into the category of "most neglected" disease because of a failure of the market and of public policies (Médecins Sans Frontières, 2001).

HAT in recent years (mid 1990s-2000s)

With the new millennium, HAT was brought back onto national and international agendas. The adoption of the Health Assembly Elimination Resolution (WHO, 1997a) enhanced access to diagnosis and treatment, as well as the surveillance and control activities. Soon thereafter, the World Health Assembly called on member states to sustain the effort to eliminate the disease as a public health problem, creating the Programme Against African Trypanosomiasis (PAAT) (Simarro *et al.*, 2008). Efforts were made to coordinate national control programmes, non-governmental organisations, research institutions, and other concerned United Nations agencies. National structures were enhanced through financial and technical support from WHO, promoting intervention activities, and securing production and free distribution of drugs. In addition, in July 2000 the Organization of African Unity (now the African Union) launched in Lomé (Togo), the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), which is currently promoting interventions supported by the African Development Bank.

According to WHO (2006b), the control activities, focused mainly on the human reservoir, resulted in a reduction in the reported incidence from 36,585 new cases in 1997 to 11,382 new cases in 2006 for the *gambiense* form (97.5% of the total HAT reported cases), representing a 68.9% reduction (Table 1-4 & Figure 1-5).

A Countries	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
> 1,000 new cases/year										
Angola	8,275	6,610	5,351	4,546	4,577	3,621	3,115	2,280	1,727	1,105
DRC	25,094	26,318	18,684	16,975	17,322	13,853	11,481	10,369	10,269	8,023
Sudan	737	1,726	1,312	1,609	1,804	3,163	3,076	1,766	1,869	809
100-1,000 new cases/year										
Chad	122	134	187	153	138	715	222	483	190	276
CAR	730	1,068	869	988	717	570	538	737	666	460
Congo	142	201	91	111	894	1,005	682	859	398	300
Uganda	1,123	971	1,036	1,141	424	562	501	354	304	270
< 100 new cases/year										
Cameroon	10	54	32	27	13	32	33	17	3	15
Côte d'Ivoire	185	121	104	169	84	92	51	72	40	29
Equatorial Guinea	67	62	28	16	17	32	23	22	17	13
Gabon	11	6	38	45	30	25	26	48	53	31
Guinea	88	99	68	52	72	124	116	84	94	48
Nigeria	0	0	27	14	14	26	31	10	21	3
No new cases with control activities present										
Benin	0	0	20	72	83	8	3	0	0	0
Burkina Faso	1	15	15	8	8	2	3	2	0	0
Ghana	0	0	0	1	0	0	0	0	0	0
Mali	0	0	0	18	3	2	0	0	0	0
Togo	0	0	0	0	0	0	0	0	0	0
No new cases and no control activities										
Gambia	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Guinea Bissau	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Liberia	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Niger	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Senegal	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sierra Leone	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total	**36,585**	**37,385**	**27,862**	**25,945**	**26,200**	**23,832**	**19,901**	**17,103**	**15,651**	**11,382**

B Countries	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
100-1,000 new cases/year										
Tanzania	354	299	288	347	258	226	111	157	183	125
Uganda	217	283	283	266	426	328	321	318	479	245
< 100 new cases/year										
Malawi	7	10	11	35	38	43	70	47	41	58
Zambia	nd	nd	15	9	6	17	7	35	20	57
Sporadic new cases										
Kenya	5	14	22	12	14	13	0	0	0	1
Mozambique	nd	nd	nd	nd	nd	1	nd	1	nd	nd
Rwanda	nd	nd	nd	nd	8	27	5	22	nd	nd
Zimbabwe	9	nd	nd	nd	nd	nd	nd	nd	4	nd
No new cases										
Botswana	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Burundi	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ethiopia	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Namibia	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Swaziland	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total	**592**	**606**	**619**	**669**	**750**	**655**	**514**	**580**	**727**	**486**

Table 1-4: New sleeping sickness cases reported between 1997 and 2006. **A**: *T. b. gambiense* sleeping sickness. **B**: *T. b. rhodesiense* sleeping sickness (Simarro *et al.*, 2008). nd: no new cases reported

DRC, Angola and Sudan reported 89.9% of the new *gambiense* cases during the period 1997-2006 (87.7% of all the HAT new cases), and DRC alone 65.5% of the new *gambiense* cases (63.9% of all the HAT new cases).

Uganda and Kenya reported 89.0% of the *rhodesiense* new cases during the period 1997-2006 (5,514), although this represented only 2.2% of all the HAT new cases (Simarro *et al.*, 2008). Control activities based upon "active case detection and treatment" in humans for the *rhodesiense* form were considered insufficient, as it achieved only a marginal reduction in incidence, from 592 new cases in 1997 to 486 new cases in 2006 (Table 1-4 & Figure 1-5).

Despite successes in reducing the number of cases reported through 'case detection and treatment' during the last decade, the complexity of the treatments with the available drugs compromised the sustainability of HAT surveillance and control. Suramin, pentamidine and melarsoprol, three of the four currently approved drugs for the treatment of HAT, have been on the market for 60-90 years. Eflornithine (DL-alpha-difluoromethylornithin) is the only treatment that has been registered in the last 50 years (Legros *et al.*, 2002). Suramin is used for first-stage *rhodesiense*-HAT, pentamidine for first-stage *gambiense*-HAT, melarsoprol for the second stage of both forms of the disease, and eflornithine, is only effective in the second stage of the *gambiense* form. Over a hundred years after Forde's discovery, all of the current therapies are unsatisfactory for various reasons, including unacceptable toxicity, poor efficacy, undesirable routes of administration, and drug resistance (Fairlamb, 2003). Moreover, highly invasive diagnosis procedures, *i.e.* lumbar puncture, are still required to determine the stage of the disease. Lumbar puncture and the administration of treatments are not well tolerated by patients and require well-trained staff.

Achieving significant coverage at a sustainable cost poses a problem. On the one hand, primary health care systems are relatively well established in all the countries, and attend the population of remote rural areas; however, they lack trained staff and facilities to diagnose and treat HAT. On the other hand, centralised mobile teams are expensive, and they face difficulties in accessing remote areas.

The combination of feasible community-based measures with 'case detection and treatment' offers the possibility of tacklng the HAT problem from different fronts. Simple

technologies for tsetse control can be used at the community level, playing a role in reducing the transmission.

1.3. *Glossina* spp.

1.3.1. Description

Glossina spp. infest about ten million square kilometres of sub-Saharan Africa, extending from Mali and Ethiopia in the north to Angola and South Africa in the south (Torr *et al.*, 2007a)(Figure 1-6).

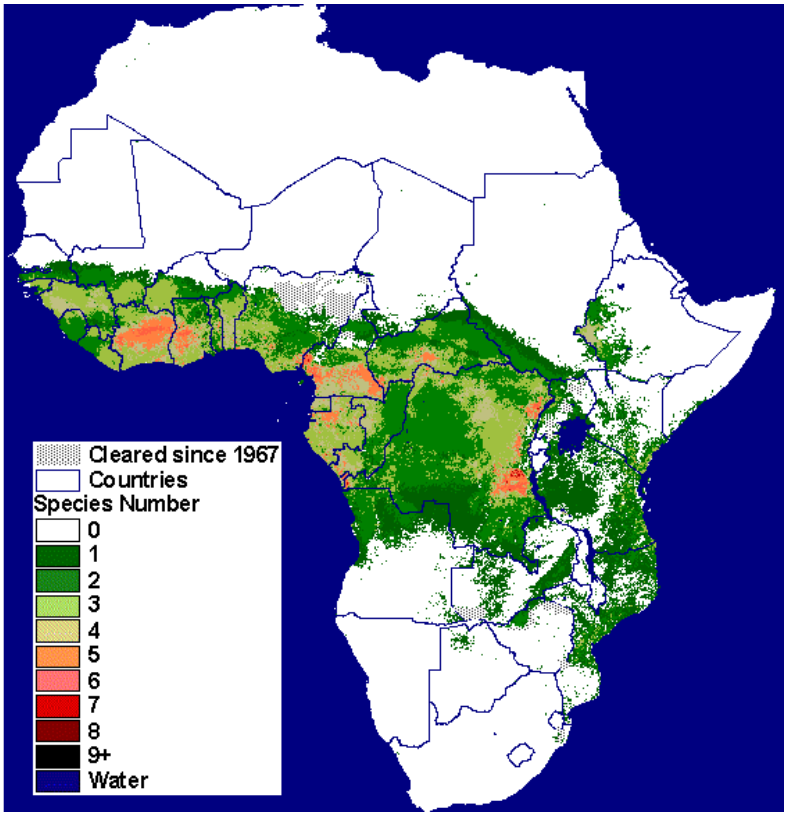


Figure 1-6: Distribution of *Glossina* spp (Torr *et al.*, 2003)

The genus *Glossina* is divided into three sub-genus, according to taxonomic differences. Each sub-genus is associated with different ecological habitats: (i) *Fusca*-group, subgenus *Austenina* Townsend, 1921: generally associated with deep forests in Central Africa; (ii) *Palpalis*-group, subgenus *Nemorhina* Robineau-Desvoidy, 1830: largely found in riverine

habitats of Central and West Africa; (iii) and *Morsitans*-group, subgenus *Glossina* Zumpt, 1935: it includes species found in the savannah regions across Africa (Table 1-5).

The forest flies	The riverine flies	The savannah flies
Subgenus *Austenia* Townsed, 1921	**Subgenus *Nemorhina* Robineau-Desvoidy, 1830**	**Subgenus *Glossina* Zumpt, 1935**
***Fusca*-group (forest flies)**	***Palpalis*-group (riverine flies)**	***Morsitans*-group (savannah flies)**
G. brevipalpis Newstead 1910	*G. caliginea* Austen, 1911	*G. austeni* Newstead, 1912
G. frezili Gouteux, 1988	***G. fuscipes fuscipes* Newstead, 1911**	*G. longipalpis* Wedemann, 1830
G. fusca congolensis Newstead & Evans, 1921	***G. fuscipes martinii* Zumpt, 1935**	***G. morsitans centralis* Machado, 1970**
G. fusca fusca Walker, 1849	***G. fuscipes quanzensis* Pires, 1948**	***G. morsitans morsitans* Westwood, 1850**
G. fuscipleuris Austen, 1911	*G. pallicera pallicera* Bigot, 1891	*G. morsitans submorsitans* Newstead, 1910
G. haningtoni Newstead & Evans, 1922	*G. pallicera newsteadi* Austen, 1929	***G. pallidipes* Austen, 1903**
G. longipennis Corti, 1895	***G. palpalis palpalis* Robineau-Desvoidy, 1830**	*G. swynnertoni* Austen, 1923
G. medicorum Austen, 1911	***G. palpalis gambiense* Vanderplank, 1911**	
G. nashi Potts, 1955	***G. tachinoides* Westwood, 1850**	
G. nigrofusca hopkinsi Van Emden, 1944		
G. nigrofusca nigrofusca Newstead, 1911		
G. schwetzi Newstead & Evans, 1921		
G. severini Newstead, 1913		
G. tabaniformis Westwood, 1850		
G. vanhoofi Henrard, 1952		

Table 1-5: Species and subspecies of tsetse (*Glossina* spp.) for the three subgenera *Austenia* (Fusca-group), *Nemorhina* (Palpalis-group) and *Glossina* (Morsitans-group). Within the HAT-vectors (in bold), *G. p. palpalis*, *G. p. gambiense*, *G. f. fuscipes* and *G. f. quanzensis* are responsible for the transmission of ~99% of cases (Brunhes *et al.*, 1994; WHO, 1997b; Torr *et al.*, 2003)

Differences in the ecological distribution of tsetse have important implications in the epidemiology of trypanosomiasis. Species of the Morsitans-group infest the main areas for cattle production across Africa, and hence play a major role in the transmission of AAT and *rhodesiense*-HAT. Conversely, species of the Palpalis-group tsetse are found in relatively dense riverine habitats, closer to human settlements, where *T. b. gambiense* occurs. Species of the *fusca*-group are found normally at low densities in forested areas, and they do not play an important role as vectors. Of the 31 species and subspecies of tsetse (Brunhes *et al.*, 1994), only nine are considered as potential vectors of HAT (WHO, 1997b) (Table 1-5), and only four subspecies of the Palpalis-group are significant vectors in the regions where about 99% of HAT-cases occur (Brunhes *et al.*, 1994; Simarro *et al.*, 2008). These are *G. p. gambiensis* (in Guinea and northern Côte d'Ivoire), *G. p. palpalis* (in Benin, Nigeria, western Cameroon, Equatorial Guinea, Gabon, south-western Republic of Congo, south-western Democratic Republic of Congo and western Angola), *G. f. fuscipes* (in eastern Cameroon, Central African Republic, western Republic of Congo, northern DRC, Sudan and Uganda), and *G. f. quanzensis* (in southern DRC and northern

Angola) (Brunhes *et al.*, 1994). During the period 1997-2006, 97.5% of the cases were caused by *T. b. gambiense* (Simarro *et al.*, 2008). These four riverine subspecies of tsetse were responsible for the transmission of virtually all the cases of the *gambiense* form of the disease, and the *rhodesiense*-HAT cases reported in Uganda – 51.0% of all the *rhodesiense*-HAT during the same period.

1.3.2. Life cycle of tsetse

Tsetse are regarded as *K*-selected species: from the age of 6 days, females produce a single egg, which develops within the uterus over a period of 7-12 days. The mature larva is then deposited in a suitable microhabitat where it burrows into the soil to pupariate, emerging about 20 days later as an adult. This process, in which eggs hatch inside the uterus and larvae are deposited immediately before pupating, is known as adenotrophic viviparity. The minimum period that a female can produce two larvae is approximately 25 days (Figures 1-7 & 1-8).

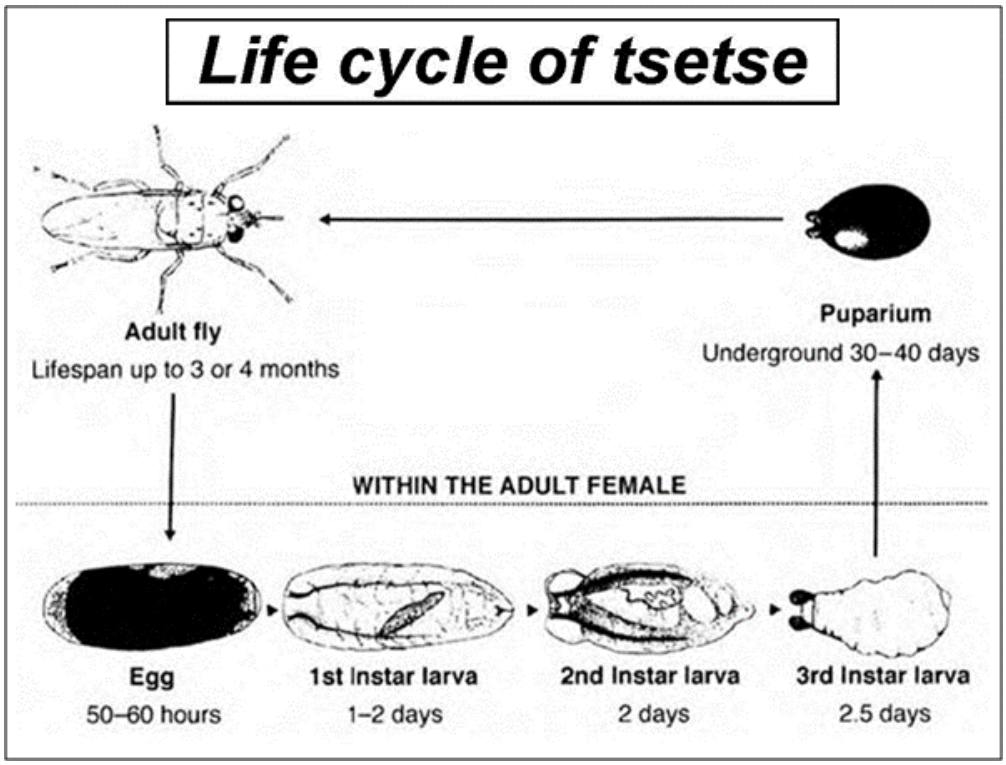


Figure 1-7: Life cycle of tsetse (Leak, 1998)

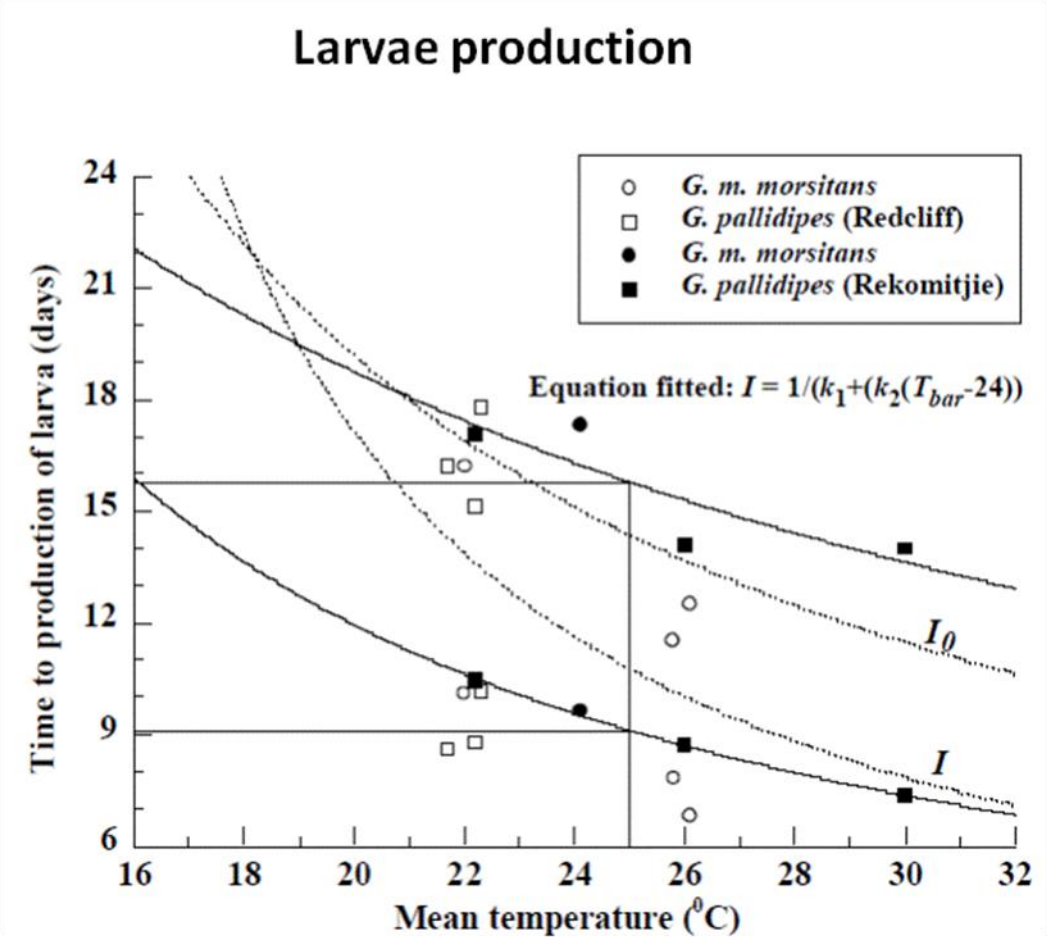


Figure 1-8: The relation between temperature and the observed and predicted times (I_0) to production of the first larvae and the duration (I) of subsequent inter-larval periods. Bold lines fitted to the data for flies collected at Rekomitjie Research Station, Zimbabwe (Hargrove, 1994). Estimated values, and standard errors, of the coefficients for the equation in the body of the graph were: For time to production (I_0) of the first pupa; k_1 = 0.061 ± 0.002, k_2 = 0.0020 ± 0.0009. For subsequent inter-larval periods (I); k_1 = 0.1046 ± 0.0004, k_2 = 0.0052 ± 0.0001. Faint lines show the predicted values from a laboratory study in Tanzania (East Africa High Commission, 1955). From Hargrove (2003b)

Unlike most haematophagous Diptera, both sexes of tsetse rely exclusively on blood for their development and maintenance. Therefore, every 3 days adult tsetse have to take a bloodmeal from their hosts. The combination of longevity and regular blood-feeding makes tsetse efficient cyclical vectors of *Trypanosoma* spp. However, their slow reproductive rate makes them particularly sensitive to control measures. A relatively low but persistent mortality rate in tsetse of about 3% of the adult females/day will drive a population of tsetse to elimination (Weidhaas & Haile, 1978; Hargrove, 1988; Vale & Torr, 2005)(Figure 1-9). Moreover, the absolute reliance of tsetse on feeding regularly from their hosts makes them vulnerable to interventions that exploit this behaviour.

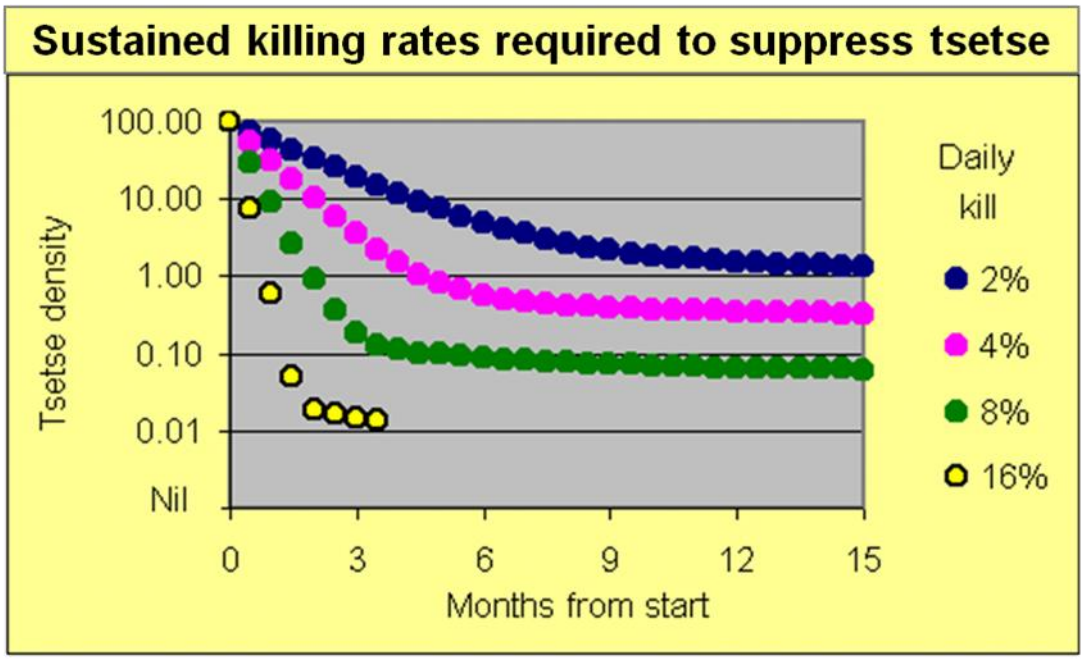


Figure 1-9: Required killing rates to suppress a tsetse population. Graph extracted from Tsetse Muse, software downloadable from tsetse.org (Vale & Torr, 2005)

1.3.3. Tsetse control

Vector control was first implemented soon after Bruce demonstrated the role of tsetse in the transmission of trypanosomiasis (Bruce, 1895; Bruce & Nabarro, 1903), and almost at the same time as the first trypanocides were available. Since then, several techniques of tsetse control have evolved over the years, contributing towards reducing the impact of trypanosomiasis, primarily AAT but also HAT. The suitability of the techniques in each situation varies according to the tsetse species, the features of the intervention area, the environmental impact, and the budgetary and technological strengths of each country or region. The main techniques are as follows: (i) bush clearing and game destruction; (ii) ground and aerial spraying; (iii) sterile insect technique (SIT); and (iv) living or artificial bait techniques. Extensive operations with different techniques were undertaken in Zimbabwe between 1980 and 1999, which allowed a comparative economical study for each technique. Thus, the estimated cost of ground spraying was US$265-390 per km^2, US$435-535 per km^2 for aerial spraying, US$220-385 per km^2 for targets and US$120 per km^2 for ITC (Shaw, 2004).

Game destruction

During the 1940s, the elimination of *G. swynnertoni*, *G. m. morsitans* and *G. pallidipes* was achieved in Shinyanga (Tanzania) by indiscriminate game destruction, which involved the slaughtering of over 8,000 animals (Hargrove, 2003a). The study area was isolated from invading tsetse and hosts from surrounding areas. Some years later, in the 1960s, the effect of selective game destruction upon the populations of *G. m. morsitans* and *G. pallidipes* was studied in the valleys of Nagupande, Busi, Sengwa and Lutope Rivers in Zimbabwe (Hargrove, 2003a). The hunting pressure was focused on warthog (*Phacochoerus africanus*), bushpig (*Potamochoerus larvatus*), bushbuck (*Tragelaphus sylvaticus*) and kudu (*Tragelaphus strepsiceros*), which constitute 75% of the tsetse diet in the area (Robertson, 1983). The experience in Zimbabwe showed that selective hunting was not going to eliminate tsetse (Hargrove, 2003a). First, the level of hunting was never sufficient to remove all of the favoured hosts. Secondly, even if all the favoured hosts were removed, any reduction in the hunting pressure thereafter would result in re-invasion by hosts, and subsequently by tsetse (Hargrove, 2003a).

Bush clearing

Bush clearing was suggested as an option for tsetse control in non-isolated areas, where game destruction cannot prevent surrounding tsetse from re-invading the area. The complete destruction of all the trees and shrubs in an area implies not only the destruction of the tsetse habitat, but also the destruction of the host habitat, and consequently, the reduction in the host availability. Concern about the gross ecological impact of this approach led to the development of, so-called, 'discriminative bush clearing'. For example, 3% of the vegetation was removed in a total area of around 725 km^2, between 1936 and the early 1950s in Mbala (Zambia). By the end of this period, the population of *G. m. morsitans* became undetectable (Hargrove, 2003a). Despite the success of this and other experiences, in the 1970s advances made with the cheaper ground spraying technique replaced the use of bush clearing to control tsetse.

Increased environmental awareness has made, in general, game and bush clearing obsolete, unpopular and, finally, unacceptable techniques for tsetse control (Vreysen, 2006).

However, extensive land use for agriculture or other development activities can render similar results indirectly, knocking down the tsetse population. In this case, ecological costs and benefits should be put into balance (Bourne *et al.*, 2001).

Ground spraying

Between 1955 and 1978, approximately 200,000 km^2 were cleared of tsetse in northern Nigeria, 94% of which was achieved by ground spraying and the remainder by helicopter spraying (Jordan, 1986). The technique was also used to eliminate tsetse from the Sabi-Lundi drainage system (south-eastern Zimbabwe) in the 1960s (Hargrove, 2003a). Other successful campaigns have been reported in Chad (Davies, 1981) and Kenya (Glover *et al.*, 1960)

Ground spraying is not always successful, and for example, reinvasions after treatments were reported in Central African Republic (Finelle, 1980; Itard, 1980) and Senegal (Touré, 1980).

Ground spraying is rarely used at present, due to the concerns over residual insecticides, alongside the high operational demands required.

Aerial spraying

The method, known as the sequential aerosol technique (SAT), involves spraying ultra-low volumes of non-residual insecticides, 10-15 m above the tree canopy by aircraft (Vreysen, 2006).

Aerial spraying achieves a rapid decrease in the tsetse population over large areas (Hargrove, 2003a), although its operational demands are high. It has virtually no residual effect (Hargrove, 2003a), and therefore it does not cause a significant adverse effect on the environment when the insecticide is applied at the correct dose (Takken *et al.*, 1976; Douthwaite *et al.*, 1981; Perkins & Ramberg, 2004). However, and for the same reason, aerial spraying does not prevent tsetse re-invasion, nor population recovery after the

intervention. SAT operations have been carried out in Zimbabwe, Côte d'Ivoire, Somalia, Nigeria, Uganda, Zambia, Kenya and Botswana.

The technique has proved effective when used in areas where the tsetse population is isolated. Thus, *G. pallidipes* was eliminated from Zululand (South Africa) in the 1940s, after an aerial spraying campaign (Du Toit, 1954). According to Hargrove (2003a), three factors probably contributed to the success of the campaign: (i) Zululand is located at the edge of the distribution of *G. pallidipes*; (ii) agricultural fields surrounded the intervention area, isolating the tsetse population; and, (iii) *G. pallidipes* was probably close to extinction after wild host hunting.

More recently, *G. m. centralis* was eliminated from the Okavango delta (Botswana) after the 2001-2002 campaign (Kgori *et al.*, 2006). The northern part of the delta was treated the first year, and the south during the second year. The success of the campaign was attributed to the application of an adequate dose of insecticide, and the use of a GPS-based navigation system, which ensured an even application of insecticide. A barrier of about 10 km was created between the northern and southern part of the intervention area using 12,000 deltamethrin-treated targets. The barrier stopped tsetse from re-invading the northern sprayed block before the southern one was treated.

Conversely, aerial spraying failed to control tsetse during the earlier campaign in the Okavango delta, 1973-1991 (Hargrove, 2003a), and in the Lambwe Valley campaign (Kenya), 1980-1981, (Hargrove, 2003a). In both campaigns, 99% of the tsetse populations were killed, but they recovered to pre-spray levels in about a year after the operations.

The use of aerial spraying is controversial because of the international community pressure to reduce worldwide reliance on pesticides (Allsopp, 2001). In addition, the technique is relatively expensive, and requires substantial economic and infrastructural support at national and international levels (Hargrove, 2003a).

Sterile insect technique (SIT)

SIT aims to release sufficient sterile males into a wild population so that the probability of a wild female being inseminated by a fertile wild male is drastically reduced. The smaller the wild population, the fewer sterile males need to be released to swamp the wild males. Therefore, it is necessary, in general, to reduce the target population as much as possible using other techniques before releasing sterile males.

In the late 1960s, SIT was used to eliminate a natural population of *G. m. morsitans* on Antelope Island in Lake Kariba (Zimbabwe) (Dame & Schmidt, 1970). Due to the size and location of Antelope Island, it offered excellent semi-controlled field conditions to test different techniques, first, with the original indigenous population, and later with re-introduced flies (Hargrove, 2003a). Prior to the release of the sterile males, aerial application of insecticide was used to suppress the tsetse population.

However, the elimination of *G. austeni* in Unguja Island (Zanzibar) during 1994-1997 (Vreysen *et al.*, 2000), has been probably the only experience where SIT achieved a genuine success under real conditions (Feldmann & Hendrichs, 2001). Prior to 1994, the tsetse population was reduced by means of insecticide-impregnated cattle and targets. By mid-1995, the sterile to indigenous male ratio was >50:1, and by the end of the same year it was increased to >100:1. The last trapped indigenous male and female flies were found in the first half of 1996, although SIT continued until the end of 1997.

The campaign in Unguja Island cost US$7,941,000 and the release of about 8.5 million sterile males (Msangi *et al.*, 2000) to eliminate a relatively low tsetse population of a single tsetse species – about 1000 flies estimated at the start of the release programme (Hargrove, 2003a). Doubts about the feasibility of using SIT against large and open populations in the African mainland have been raised (Hargrove, 2003a). Mathematical models show that, in general, controlling the tsetse population by increasing deaths is more appropriate than reducing births, which constitutes the basis of SIT (Vale & Torr, 2005)(Figure 1-10). Moreover, many African areas are infested with more than one species, which increases significantly the cost of SIT (Hargrove, 2003a).

Bait technologies

The techniques described above require centralised organisation, technical expertise and complex logistics. These factors have an impact on the operational cost and the sustainability of the operations. The search for simpler, cheaper and less damaging techniques has led to the development of bait technologies.

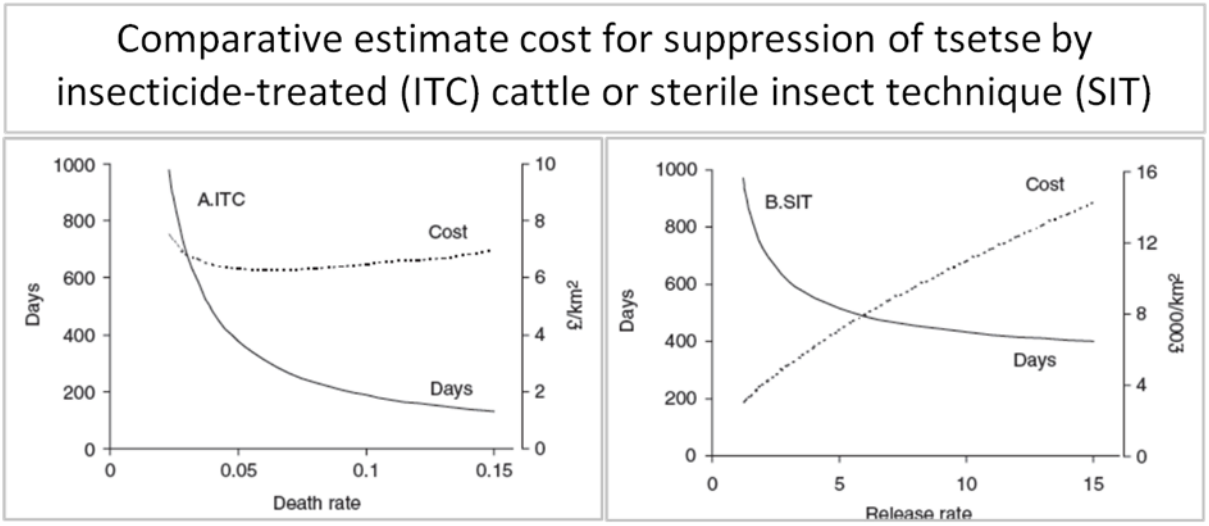


Figure 1-10: Days required for eradication (solid line) and costs (broken line), at various daily death rates imposed by insecticide-treated cattle (ITC) or various release rates with sterile insect technique (SIT). Each technique was used alone against an isolated population, without prior suppression. Cost scales use units for ITC and thousands for SIT (Vale & Torr, 2005)

Bait techniques can be based on natural, *i.e.* insecticide-treated cattle (ITC), or artificial baits, *i.e.* insecticide-treated targets and traps. Interventions based on bait technologies could overcome the present dependence on outside agencies, as they can be applied and afforded by local communities (ISCTRC, 2005). Bait technologies involve the use of long-lasting insecticides, but unlike ground spraying and SAT, they do not require the widespread application of large quantities of toxic chemicals, and therefore are more benign for the environment (Hargrove, 2003a).

Insecticide-treated cattle (ITC)

ITC involves treating parts or the full body of adult cattle with long-lasting insecticide. Tsetse landing on the treated animals are killed by the exposure to the insecticide. Where cattle and tsetse coexist, ITC provides a cheap, simple and effective means of tsetse control

(Hargrove *et al.*, 2000). Recent studies in Zimbabwe with *G. m. morsitans* and *G. pallidipes* have shown that the technique can be made even more cost-effective by applying insecticide to only the belly and legs of cattle at 2-week intervals, rather than the normal practice of treating the whole body of the animals (Torr *et al.*, 2007a). In this way, restricted application of insecticide reduces the cost by about 40%, improves the efficacy by 27%, and reduces the impact on non-target species. Another study, also in Zimbabwe and with the same tsetse species, showed that >89% of the flies fed on adult cattle, even though they represent 13% of the herd (Torr *et al.*, 2007b). Therefore, the cost of ITC can be further reduced by treating only the older/larger animals of the herd.

However, ITC presents two main constraints: (i) cattle are not present in many of the HAT-affected areas of West and Central Africa, *e.g.* Guinea, Southern Côte d'Ivoire, DRC, etc. (Wint & Robinson, 2007); and (ii) interventions based only upon ITC are likely to face problems with re-invasions, and therefore, they should be combined with other techniques to create barriers, such as insecticide-impregnated targets or aerial spraying (Warnes *et al.*, 1999).

ITC has been used in Zimbabwe (Thompson *et al.*, 1991; Thompson & Wilson, 1992a, b; Warnes *et al.*, 1999), Zambia (Chizyuka & Liguru, 1986), Tanzania (Fox *et al.*, 1993), Kenya (Stevenson *et al.*, 1991), Burkina Faso (Bauer *et al.*, 1992; Bauer *et al.*, 1999a; Bauer *et al.*, 1999b), and Ethiopia (Leak *et al.*, 1995; Rowland *et al.*, 2000), showing the advantages and limitations of the method. For example, in areas where there are large numbers of cattle, this is the cheapest, simplest and most effective method of vector control available (Hargrove, 2003a; Shaw *et al.*, 2013). Conversely, the use of ITC depends on cattle being present in tsetse-infested areas and in many of the HAT-affected areas of West Africa, cattle are not abundant (Wint & Robinson, 2007).

Targets and traps

Artificial bait technology is widely used to reduce or even eliminate tsetse. In addition, combined with ITC, it provides means to create barriers between the intervention and non-intervention areas, thereby preventing re-invasions.

The use of artificial baits has a long history. Maldonado's sticky panels contributed to the elimination of *G. p. palpalis* from the island of Principe, early in the 20th century (Da Costa *et al.*, 1916). The use of mobile sticky panels was however concurrent with the reduction of dog and wild pig populations, and hence it is difficult to assess the contribution of each method towards tsetse elimination (Leak, 1998).

Harris (1932, 1938) carried out the first large-scale control campaign using traps in the Umfolosi game reserve in Zululand (South Africa), where *G. pallidipes* imposed a severe problem for cattle. A density of 20-40 traps/km^2 was deployed between 1931 and 1938, reducing the apparent density from 100 flies/trap to 0.002 flies/trap (Hargrove, 2003a).

Since Harris' intervention, a number of traps have been developed for each group of flies and regions. Whereas biconical (Challier & Laveissière, 1973), or monopyramidal (Gouteux & Lancien, 1986) traps are effective to catch riverine species, for savannah tsetse Ngu (Brightwell *et al.*, 1987) or Nzi (Mihok, 2002) in east Africa, and Epsilon (Hargrove & Langley, 1990) in South Africa perform better.

To reduce the costs of control, traps were made simpler and cheaper resulting ultimately in targets. These consist of simple screens of cloth, impregnated with long-lasting insecticide. Tsetse landing on the targets are exposed to the insecticide deposits, and killed. Impregnated targets were designed for the Morsitans-group tsetse (Vale *et al.*, 1985; Vale *et al.*, 1986b), as well as for the Palpalis-group tsetse (Laveissière *et al.*, 1987a). Modern designs combine phthalogen blue and black cloth (see section 5.1).

Swynnerton (1933) and Lloyd (1935) observed that traps incorporating an animal hidden from view caught more *G. m. morsitans* and *G. sywnnertoni* than similar unbaited traps, suggesting that host odours could increase trap performance. The role of odours in the attraction of *G. m. morsitans* and *G. pallidipes* was demonstrated unequivocally by Vale (1974d, e). Subsequently the main kairomones present in host odour have been identified (Vale, 1979, 1980a; Hall *et al.*, 1984; Vale & Hall, 1985; Vale *et al.*, 1986a; Bursell *et al.*, 1988).

The use of odour-baited devices to control tsetse was first carried out in a semi-controlled trial on Antelope Island (Lake Kariba, Zimbabwe) between 1980 and 1984; the baits being

used against newly introduced populations of *G. m. morsitans* and *G. pallidipes* (Vale *et al.*, 1986b). Both species were eliminated on the island by the end of the experiment.

A trial in the Rifa Triangle (Zimbabwe, 1984-1985) assessed the effectiveness of odour baits against an open tsetse population. As few as five odour-baited targets per square km were sufficient to render undetectable the populations of *G. m. morsitans* and *G. pallidipes* in the area (Vale *et al.*, 1988b).

Bait technology has been tested in other African countries against tsetse of the Morsitans-group with similar results (Dransfield *et al.*, 1990; Willemse, 1991; Hargrove, 2003a). Savannah tsetse, particularly *G. pallidipes*, are highly responsive to host odours. Thus, insecticide-treated traps and targets, baited with synthetic attractants, and deployed at densities of about four targets/km^2, can eliminate populations of tsetse in just over a year's time. By contrast, no attractants have been identified convincingly for tsetse of the Palpalis-group, and consequently 30-40 traps/km^2 are required to control these riverine tsetse (Green, 1994).

The understanding of the cues, *i.e.* visual and olfactory, used by tsetse of the Palpalis-group would help to identify the mechanisms by which these flies locate their hosts. This information would serve to improve the bait technology against HAT-vectors, making it more effective. This goal underpins the current work (see section 1.4).

1.3.4. Host-orientated behaviour of savannah tsetse

Factors in host selection (Morsitans-group)

The frequency at which host species are fed on by tsetse species depends not only on their olfactory and visual attractiveness for the flies, but also on the frequency with which tsetse encounter the host species by chance, and by the opportunities to feed successfully on the hosts after approaching it (Baylis, 1996).

Approximately 80% of the identified bloodmeals in tsetse of the Morsitans-group were taken from Suidae and ruminants (Clausen *et al.*, 1998). Wild pigs, *i.e.* warthog (*Phacochoerus africanus*) and bushpig (*Potamochoerus larvatus*), and ungulates, *e.g.*

buffalo (*Syncerus caffer*) and bushbuck (*Tragelaphus sylvaticus*), are repeatedly identified as common hosts, whereas other relatively common mammals, such as primates, including humans, or domestic pigs– are virtually absent in their diet (Clausen *et al.*, 1998).

Host odour in relation to host selection has been investigated. Early studies suggested that semiochemicals present in human odour exhibit a repellent effect in tsetse of the Morsitans-group. Vale (1974e) found that the odour emanated from a man (74 Kg) attracted about. five-fold fewer *G. m. morsitans* and *G. pallidipes* than that of a goat (34 Kg). Furthermore, adding human odour to ox odour antagonised the attractiveness of the latter, and reduced the proportion of tsetse that subsequently alighted and fed (Vale, 1974e; Hargrove, 1976).

Field observations of bushpig (*Potamochoerus larvatus*) and warthog (*Phacochoerus africanus*) showed that tsetse landed predominantly near the eyes (Vale, 1974b). According to Vale (1974b), these results suggested the presence of specific kairomones in the pre-orbital secretion of Suidae. However, Torr (1994a) proved that the addition of natural warthog odour to a blend of synthetic attractants present in ox odour (*i.e.* carbon dioxide, acetone, octenol and phenols) did not increase the catch significantly, suggesting that warthog do not produce specific kairomones different to those already identified in cattle odour. Hence, the preferred landing response around the eyes of the host was due probably to visual cues, rather than olfactory ones (Torr, 1994a).

Differences in host selection were also observed among closely related hosts. For example, *G. m. morsitans* and *G. pallidipes* feed frequently on bushbuck (*T. sylvaticus*), cattle (*Bos* spp), and buffalo (*S. caffer*). However, bloodmeals from impala (*Aepyceros melampus*) or waterbuck (*Kobus defassa*) are rarely identified, despite both species being relatively abundant where *G. m. morsitans* and *G. pallidipes* exist (Clausen *et al.*, 1998). Bushbuck, cattle, buffalo, impala and waterbuck are all members of the Bovidae family, and have similar physiology.

With the exception of human odour, tsetse-host interactions mediated by species-specific semiochemicals have not been established consistently. Rather than host-specific chemicals, the amount of kairomones produced by hosts, particularly carbon dioxide, acetone, butanone, octenol and phenolic residues, seems to play a role in host-selection (Hargrove *et al.*, 1995). Vale (1974e) demonstrated that, in general, the numbers of tsetse

attracted to different hosts was correlated with their body-mass. He showed that the odour from an ox (450 Kg) attracted five times as many flies as that from a goat (about 32 Kg), whereas the number of tsetse attracted to an impala and a bushpig (both. 74 Kg) were similar. More recently, in dose response studies, Hargrove *et al*. (1995) showed that catches of *G. m. morsitans* and *G. pallidipes* increased as a power of cattle weight, with a 2.5-fold increase in the catch as the bait body-mass increased 10-fold.

In addition to odour-mediated responses, host selection is also strongly influenced by the degree of defensive behaviour by the host. For example, an impala attracted fewer flies than an ox, consistent with its smaller size, but no flies fed on it, whereas 35% of tsetse approaching an ox fed to completion (Vale, 1977a). Vale (1977a) suggested that, for equal host-biomass, impala and ox are equally attractive; however, the higher defensive response exhibited by impalas results in a lower overall feeding rate. Subsequent experiments with cattle showed a consistent correlation between age and feeding rates. Torr & Mangwiro (2000) observed that about 10% of tsetse attracted to calves fed, compared to 50-60% of tsetse attracted to adult cattle. The authors underlined a negative correlation between individual's rate of defensive leg movements, more intense in young cattle, and feeding rates. The result is consistent with previous studies, suggesting that small and/or young animals are less tolerant of biting insects (Vale, 1974e; Foil *et al.*, 1984; Torr, 1994a).

In support of this view, microsatellite DNA techniques, applied to bloodmeal extracts, were used to identify individual cattle within a herd (Torr *et al.*, 2001; Torr *et al.*, 2007b). The studies confirmed a bias in feeding rates towards large/old animals. Tsetse fed significantly more on adult cattle, even when smaller hosts were more numerous, or when large animals were at the centre of the herd. The studies confirmed previous results, concluding that: (i) there is a correlation between biomass and number of flies attracted to a herd (Hargrove *et al.*, 1995; Torr *et al.*, 2007b); (ii) tsetse land preferentially on large hosts, which produce higher rates of kairomones (Vale, 1974e; Hargrove, 1976; Vale, 1977b; Torr *et al.*, 2006; Torr *et al.*, 2007b); and (iii) young cattle exhibit stronger response to defend themselves from tsetse, which results in lower feeding rates (Vale, 1977a; Baylis, 1996; Torr & Mangwiro, 2000; Torr *et al.*, 2001; Torr *et al.*, 2007b).

Biting rates are most important parameters in the transmission of trypanosomiasis (Milligan & Baker, 1988; Rogers, 1988). Among other aspects, biting rates depend on the numbers of tsetse attracted to a host, and the proportion that subsequently land, probe and

feed (Torr & Hargrove, 1998). Tsetse feeding behaviour has important epidemiological implications. On the one hand, the lower probability of younger animals being bitten is consistent with reported lower prevalence in calves, compared with adult cattle (Torr & Mangwiro, 2000). On the other hand, adding cattle to a herd will increase the numbers of tsetse attracted, which will finally feed on large animals (Torr *et al.*, 2007b).

The distinctive feeding behaviour of tsetse has also some implications for control. For instance, it provides opportunities to improve the cost-effectiveness of ITC by selectively treating those animals that are effective baits, in general the largest/oldest members of the herd (Torr *et al.*, 2007b).

Responses of savannah tsetse to host cues

Tsetse must locate a distant food source that is mobile, frequently difficult to find, and which has evolved defences against insect attack (Gibson & Torr, 1999). Thus, a range of mechanisms for locating hosts has evolved in response to biotic and abiotic constraints.

Vale (1974d), working with *G. pallidipes* and *G. m. morsitans* in Zimbabwe, demonstrated that about90% of tsetse attracted to a stationary host, did so in response to the host's odour. Tsetse are able to perceive and respond to odour cues, leading them, eventually to land on a host (Vale, 1980b). The response of tsetse to host's odour was observed up to 90 m downwind of the source (Vale, 1977b).

For convenience, the set of tsetse odour-orientated responses, from resting to the final alighting on the host, have been classified in three phases (Gibson & Torr, 1999): (i) *activation*, which marks the initiation of host-orientated responses; (ii) *long-range responses*, which brings the activated fly to the vicinity of the host; and (iii) *short-range responses*, which culminates in 'landing' and 'feeding'.

Activation. Unlike other haematophagous Diptera, which use a metabolism based on carbohydrates (*i.e.* mosquitoes, blackflies, sandflies, etc.), tsetse rely on the amino acid proline to obtain the energy used in flight (Bursell *et al.*, 1974). This unusual metabolism allows tsetse to fly at a high speed, of 4 m/s (Griffiths *et al.*, 1995), but at a high energetic

cost: as 'sprinters', they are not able to sustain this effort for long. Due to this costly metabolism, their total daily flight is as short as < 30 min/day (Bursell & Taylor, 1980). During the remaining time of the day, they rest on branches and tree boles (Hadaway, 1977) or, when temperatures exceed 32°C, in 'refuges' such as holes in trees. They have two possibilities for locating hosts: either (i) "sit-and-wait" for the host to pass by, or (ii) "range" to increase the probability of encountering a suitable animal. Torr (1988a) showed that about 55% of flies were activated in apparent absence of any host stimuli, either visual or olfactory, presumably in response to their endogenous rhythm of spontaneous activity, modulated by nutritional condition, environmental temperature and falling light intensity (Brady, 1972; Brady & Crump, 1978; Torr & Hargrove, 1999). In another study, Vale (1980b) suggested that over 80% of the savannah flies range. Apparently, the activation of olfactory stimulation of resting flies is not an important precursor to host location, and 'ranging' seems to be the most common strategy (Vale, 1980b; Torr, 1988a). Video studies showed that in the absence of any host stimuli, tsetse flew with a downwind bias (Gibson *et al.*, 1991). This behaviour might imply an evolutionary advantage to maximise the chances of encountering the host odours. The hypothesis is supported by the fact that in typical tsetse habitat, variations in wind direction (Griffiths & Brady, 1995; Zollner *et al.*, 2004) are likely to create wide swathes of odour, which are more likely to be intercepted by flying up- or downwind, the latter being more energetically efficient (Sabelis & Schippers, 1989).

Long-range olfactory responses are defined as motor responses to host odours, which normally occur some distance away from the host (*i.e.* approximately 100 m), increasing the chances of encountering the odour source, *i.e.* upwind flight, and orthokinetic and klinokinetic responses to entering and losing odour, such as changes in flight speed, turning angle and angular velocity (Kennedy, 1977; Gibson & Torr, 1999).

Field studies showed that tsetse fly upwind in response to host odour (Vale, 1974d; Gibson & Brady, 1988; Torr, 1988c). When they lose contact with the odour, they execute a reverse turn to bring them back into the odour plume, where they turn upwind (Gibson & Brady, 1988; Torr, 1988c). Other field observations showed that after losing contact, tsetse land, wait for variations in the wind direction to bring the plume back to them, and take-off upwind when contact is re-established (Bursell, 1984). Whereas in the absence of vegetation, packets of air laden with odour are carried downwind in straight lines (David *et al.*, 1982), in areas with dense vegetation air does not travel straight through the flora, but

rather changes direction (Brady *et al.*, 1990). In the first scenario, the strategy of flying directly upwind, whenever the odour is detected, should lead a fly to its host. However, this ideal situation may not be applicable in the woodlands of Africa. In the bush, tsetse locate hosts, not using a precisely orientated navigation up an odour plume, but rather a 'quick-and-dirty' strategy of fast, mainly upwind, flight that rarely leads directly to the host (Griffiths *et al.*, 1995). In such situations, the vegetation and local topography constrain the direction of flight and hence flight directly towards the source may be frequently impossible. Game paths and gaps in bushes can be used by tsetse, where they may need only to estimate whether to fly up- or down trail, instead of in the precise direction of the host (Paynter & Brady, 1993).

Short-range responses. These are changes in behaviour within the visual range of hosts, *e.g.* increased tendency to circle or land on objects, changes in flight speed and turning angle (Gibson & Torr, 1999), which ceases with the insect alighting on the host. Odour-orientated responses bring the fly to the vicinity of the host, but the final location is largely a response to visual cues. Moreover, tsetse are unable to locate an odour source precisely without a visual target, and flies approaching an odour source can be diverted towards an odourless visual target (Vale, 1974e). Like other diurnal Diptera, the eye structures of tsetse contain a zone of high resolution, theoretically sufficient for the discrimination of cryptic host animals at high light intensities (Gibson & Young, 1991). The colour, shape and size of the target control the orientation towards targets (Hargrove, 1980a; Green & Flint, 1986; Torr, 1989). Indeed, phthalogen blue traps caught significantly more flies than any in an achromatic series, whereas yellow traps caught significantly fewer (Green & Flint, 1986); in addition, white and black were found to be the most favoured colours for landing (Green, 1986). Although close-range orientation is primarily visual, some host kairomones, *i.e.* CO_2, enhance the landing response (Vale, 1974c; Hargrove, 1980; Warnes, 1995).

Which chemicals elicit the host-orientated responses of savannah tsetse?

Attractants

Vale (1974c; 1977a) consistently demonstrated that the catches of savannah flies could be increased about 10-fold by baiting the collecting device with cattle odour. With this promising result, the new challenge was to isolate the main attractants contained in the natural host odour. Those chemicals could eventually be used to bait targets and traps, improving their cost-effectiveness significantly (Hargrove & Vale, 1978; Vale & Hall, 1985; Bursell *et al.*, 1988; Vale *et al.*, 1988b).

Attractants for tsetse were identified by analysing the electrophysiological responses of tsetse to the components of host odour, and chemical identification of these components, using gas chromatography linked with electroantennography (GC-EAG as detailed by Cork *et al.* (1990). The studies resulted in the identification of ten components of host odour that influence tsetse behaviour. The most active molecules were 1-octen-3-ol (henceforth termed octenol), carbon dioxide (CO_2), acetone and butanone, identified in ox (*Bos indicus*) breath (Hall *et al.*, 1984; Vale & Hall, 1985; Torr *et al.*, 1995), and of some phenolic compounds, isolated from buffalo (*Syncerus caffer*) and cattle urine (Hassanali *et al.*, 1986; Bursell *et al.*, 1988). These molecules were combined in a blend to bait traps and electric targets in the field. Collecting devices baited with the synthetic blend at a natural release dose, caught only half that of traps baited with natural ox odour, suggesting the presence of unidentified kairomones in cattle odour (Hargrove *et al.*, 1995; Torr *et al.*, 1995).

Repellents

Besides the attractants, other molecules might also protect humans and animals against tsetse, by antagonising 'attraction' or eliciting 'repellency'. Thus, lactic acid (Vale, 1979), acetophenone and 2-methoxyphenol (Vale *et al.*, 1988a) reduce trap catches of *G. pallidipes* and *G. m. morsitans*. Torr et *al.* (1996) investigated the responses of *G. pallidipes* to known and candidate repellents in detail. The authors found that low doses (*i.e.* 5-10 mg/h) of different combinations of 2-methoxyphenol, acetophenone, pentanoic and hexanoic acid reduced the catch of traps baited with synthetic attractants by up to 90%. Lactic acid was only repellent at high dispensing doses (about 100 mg/h), whereas 2-

methoxyphenol was the most potent halving trap catches. The repellent effect of 2-methoxyphenol was not enhanced by adding either pentanoic acid or acetophenone. This molecule is a natural product, found at low doses in cattle urine (Bursell *et al.*, 1988). Torr *et al.* (1996) suggested that repellents produced naturally by hosts might activate specific receptors that trigger other behavioural responses, *e.g.* to avoid competition or unsuitable hosts.

None of the repellents have an effect on landing response, and only pentanoic acid had a significant, but slight, effect on feeding (Torr *et al.*, 1996). The study concluded that these repellents do not provide any useful degree of protection to hosts. In the best scenario, baiting an ox with these chemicals would reduce the biting rate by about 60%, which is insufficient to prevent transmission (Torr *et al.*, 1996).

Gikonyo *et al*. (2000) compared the behaviour in laboratory conditions of individual *G. m. morsitans* exposed to both ox or waterbuck (*K. ellipsiprymnus*) odour. Although no difference was obtained in the landing rates, the authors observed that tsetse stayed longer on the ox, therefore, increasing the probability of probing and feeding (Gikonyo *et al.*, 2000). The results suggested that the difference in the feeding rates was due to unidentified short-range repellents, present in waterbuck odour. In subsequent GC-EAG studies, *G. m. morsitans* and *G. pallidipes* were exposed to ox, buffalo or waterbuck odour (Gikonyo *et al.*, 2002). The experiment showed electrophysiological responses of tsetse antennae for some molecules, unique to waterbuck odour, or present in trace amounts in the two other mammals. The electrophysiologically active chemicals found in waterbuck were: δ-octalactone, 2-methoxyphenol, series of methyl ketones, and 3-isopropyl-6-methylphenol, the latter only active for *G. m. morsitans* (Gikonyo *et al.*, 2002). Among these chemicals, only 2-methoxyphenol has shown moderate repellent effects for tsetse in the field, as explained above (Torr *et al.*, 1996).

Role of Carbon dioxide in the attraction of savannah tsetse

Carbon dioxide (CO_2) is naturally present in the environment at 300-400 p.p.m. during the day, rising to as much as 1000 p.p.m. at night (Gillies, 1980). This gas is the main substrate for plant photosynthesis and is released into the atmosphere through expiration by living organisms and decomposition of organic matter (Berry & Colls, 1990; Desjardins *et*

al., 1992). Short-term fluctuations about the diurnal variation are typically 1-5 p.p.m. and differ according to the time of the day, season, location and depth of the mixed layer (Reid & Steyn, 1997). Mechanical or convective turbulent motions in the atmospheric boundary layer, resulting from wind shear and surface heating, respectively, contribute further to these diurnal fluctuations (Sutton, 1953).

Before responding to the odour, tsetse must be able to identify the CO_2 produced by the host breathing over the competing CO_2 present in the background. Detection of carbon dioxide is not limited by the concentration of background carbon dioxide but, rather, its variability. Zollner *et al*. (2004) demonstrated that carbon dioxide, released at rates of 4-20 L/min, could be detected by an infrared gas analyser, placed up to 64 m downwind of the source. The resolution and sensitivity of this instrument is comparable to that of an insect. The results suggest that carbon dioxide is detectable by tsetse at 50-100 m (Zollner *et al.*, 2004).

CO_2 is a universal host kairomone that triggers a sequence of changes in tsetse behaviour, leading to the successful completion of taking blood from a host. It activates resting tsetse (Bursell, 1987; Torr, 1988b), elicits optomotor upwind anemotactic (Colvin *et al.*, 1989), klinokinetic and orthokinetic responses (Gibson & Brady, 1988; Warnes, 1990), and elicits alighting on a host animal (Vale, 1983; Vale & Hall, 1985). CO_2 acts synergistically with other attractants (Vale & Hall, 1985; Torr, 1990). For example, Torr (1990) observed that carbon dioxide and acetone dispensed individually at doses of 1200 L/h and 50 mg/h respectively, double the catch; in contrast, the catch was increased 16-fold when both odours were dispensed together.

1.3.5. Inter- and intra-specific variation in the responses of savannah tsetse to odours

Hitherto, most research on savannah tsetse has focussed on *G. m. morsitans* and *G. pallidipes* in Kenya and Zimbabwe. However, and although the literature for other species is not as complete, there is evidence of inter-specific variation. For example, *G. longipalpis* seems to behave similarly to *G. pallidipes*, being responsive to phenols, acetone and octenol (Späth, 1995). On the other hand, *G. swynnertoni*, like *G. morsitans*

subspp., responds to acetone and octenol, but not to any of the phenols (Brightwell & Dransfield, 1997). *G. austeni*, the least responsive species, only responds to carbon dioxide (Kappmeier-Green, 2001).

Differences in the response of the same tsetse species in different environments have been observed. For example, studies of *G. pallidipes* in Somalia showed that acetone or octenol were only effective in the presence of 4-methylphenol and 3-*n*-propylphenol, and that ox odour only doubles the catch in a trap (Torr *et al.*, 1989). Conversely, acetone, octenol and phenols are effective on their own for *G. pallidipes* in Zimbabwe (Vale, 1980a; Vale & Hall, 1985) and Kenya (Baylis & Nambiro, 1993). In Zimbabwe, ox odour increased the catch of *G. pallidipes* 10-fold (Vale, 1974e) compared to only a doubling in Somalia (Torr *et al.*, 1989). Tsetse-host interactions are mediated by a number of factors inherent to tsetse, *e.g.* physiological status, and to host, *e.g.* body-mass and defensive response (Torr & Hargrove, 1998). The distinctive olfactory responses observed for *G. pallidipes* in Somalia suggests that in addition to genetic factors, environmental factors also play a role in tsetse-host interactions, and that the same tsetse species respond differently to the same hosts, depending on abiotic conditions.

1.4. Objectives of the study

The chain of behavioural mechanisms, leading haematophagous Diptera to locate, approach, and land on a host, is modulated by olfactory and visual stimuli emitted by the host. Species of biting insects have evolved different mechanisms in response to abiotic and biotic constraints.

Abiotic factors. The daily solar cycle affects environmental conditions, and hence the type of host stimuli available. Biting insects have evolved to adapt their responses to the constraints and advantages at the time of the day when they are active (Table 1-6), and hence, diurnal Diptera include fast-flying flies, with relatively short peaks of activity to avoid extreme temperatures and low humidity, relying on both, olfactory and visual cues to locate hosts. By contrast, nocturnal Diptera have evolved host-orientated behaviour appropriate to feed on stationary hosts in low-light conditions, relying preferably on olfactory cues (Gibson & Torr, 1999).

	Disadvantages	Advantages
Day	• Increased risk of desiccation • Wind turbulence breaking up host-odour plumes • Increased risk from predators • Host mobility makes responses to odours more difficult • Increased host defensive response (hosts are often active)	• Good visual cues • High winds providing good directional cues in host plume • Reduced background noise of atmospheric CO_2 • Host mobility makes 'sit-and-wait' strategy feasible
Night	• Poor visual cues • Low wind speed implies poor directional cues of host-odour plumes • Increased background noise of atmospheric CO_2 • Host immobility makes 'sit-and-wait' strategy unfeasible	• Reduced risk of desiccation • Reduced wind turbulence (host-odour plumes travel farther) • Reduced risk from predators • Reduced host defensive response (hosts are often quiescent)

Table 1-6: Opportunities and constraints for haematophagous Diptera feeding during the day or night (Gibson & Torr, 1999)

Biotic factors. Habitat type, and host availability and defensive behaviour influence the strategies of biting Diptera to locate their hosts. The mechanisms used by biting insects to locate hosts are not well understood, particularly when the preferred hosts are concealed in dense vegetation, or encircled by other animals.

Although extensive work has been done to elucidate host-orientated behaviour of Morsitans-group tsetse, much less in known about Palpalis-group (see chapters 3, 4, 5 and 6). Accordingly, the overall objective of this project is **to explore the behavioural strategies *G. palpalis* and *G. fuscipes* use to locate their host**. Insights into the vector responses to olfactory and visual host cues are crucial in understanding the epidemiology of the diseases that they transmit, and will underpin the development of new methods of vector control. The study is divided into two parts, each of them with specific objectives:

Part I: **Host-mediated olfactory responses**

Experiments in Part I were designed *to assess responses of riverine tsetse to different host odours*.

Studies on the responses of riverine tsetse to host odours were carried out for *G. tachinoides* and *G. p. gambiensis* in Burkina Faso, *G. p. palpalis* in Côte d'Ivoire, *G. f. quanzensis* in DRC, and *G. f. fuscipes* in Kenya. Humans, cattle, and pigs were concealed in ventilated tents, and their odour exhausted through plastic pipes into various arrangements of trapping devices, where tsetse were collected. The relative number of tsetse collected with each treatment in relation to the control, provided a measure of the responses to odours. In Kenya only, colleagues assessed the responses of *G. f. fuscipes* to monitor lizard odour; these results were included in this thesis for comparison.

Responses of riverine tsetse to synthetic odours were investigated in the same countries, using similar arrangements of collecting devices. The lures used in the tests were known to be attractants for savannah tsetse, and included ketones, octenol, phenols and CO_2.

The role of odours in conditions of low visibility, *i.e.* dense vegetation, was investigated in Côte d'Ivoire and DRC by comparing the catches of partially concealed collecting devices with the catches obtained in visible sites, in the presence or absence of CO_2.

Part II: Host-mediated visual responses of G. palpalis and G. fuscipes

The specific objective in Part II was *to investigate the responses of riverine tsetse to visual cues, emphasising the importance of shape and size in the attraction*. The studies focussed on *G. p. palpalis* in Côte d'Ivoire and *G. f. quanzensis* in DRC. Visual responses of tsetse were assessed using electrocuting devices of different shape and size.

CHAPTER TWO

MATERIALS AND METHODS

2.1. Study area

Visual and olfactory responses of five species or subspecies of tsetse were studied in four countries; namely, *G. tachinoides* and *G. p. gambiensis* in Burkina Faso, *G. p. palpalis* in Côte d'Ivoire, *G. f. quanzensis* in Democratic Republic of Congo (DRC), and *G. f. fuscipes* in Kenya (Figure 2-1).

Figure 2-1: Partial map of Africa showing the countries where field sites were located: Burkina Faso (in red), Côte d'Ivoire (in blue), Democratic Republic of Congo (DRC, in green) and Kenya (in yellow). Obtained with SmartDraw 2012

2.1.1. Burkina Faso

Studies were undertaken along the Comoe River at Folonzo (approximately 09º 54'N, 04º 36'W) in the Comoe province of southern Burkina Faso (chapter 4). The area receives an annual rainfall of about 1100mm. Studies took place in the dry seasons between March to June 2007 and January to May 2008.

Study sites were located in a game reserve, where the tsetse habitats for riverine flies were found in typical Sudanese gallery forest[1] (Morel, 1983; Bouyer *et al.*, 2005) (Figure 2-2). There were several game species in relatively low abundance in the research area, including warthog (*Phacochaerus aethiopicus*), hippopotamus (*Hippopotamus amphibus*), monitor lizard (*Varanus niloticus*), hartebeest (*Alcelaphus buselaphus*), buffalo (*Syncerus cafer*), Buffon's kob (*Kobus kob*), bushbuck (*Tragelaphus scriptus*), waterbuck (*Kobus ellipsiprymnus*) and various species of monkey, snake and crocodile.

G. tachinoides and *G. p. gambiensis* occur sympatrically along the southern Comoe River. Two other tsetse species, *i.e. G. m. submorsitans* and *G. medicorum*, are also found in the area (Rayaissé *et al.*, 2009). Whereas *G. m. submorsitans* is present mainly in the savannah areas, *G. medicorum* is found exclusively in the thick bush. The Sudanese type gallery is more favourable for *G. tachinoides* (Bouyer *et al.*, 2005), which occurs at much higher densities than *G. p. gambiensis* (Rayaissé *et al.*, 2009). Therefore, in order to study *G. p. gambiensis*, additional studies were conducted at Solenzo (approximately 12º14'N, 03º83'W), in the Banwa province of western Burkina Faso, along the Mouhoun river. Climatic conditions are similar to those along the Comoe River, with an annual rainfall of 1000mm. Studies in Solenzo were undertaken between April - June 2007 and January - June 2008. Although the habitat along the river is classified as Sudanese gallery forest and theoretically favourable for *G. tachinoides* and *G. p. gambiensis*, only the latter is found in relative abundance. The vegetation on the banks forms a narrow corridor between agricultural fields and small patches of woodland, which is heavily degraded due to expansion of agricultural fields. Host species in the area include humans, cattle, goats and pigs.

[1] **Sudanese gallery forest** is defined as the dense linear habitat found along the river banks across the semi-arid ecoregion of the West Sudanian Savannah in the afrotropic ecozone, forming distinctive wooded canopies (Morel, 1983; Bouyer et al., 2005)

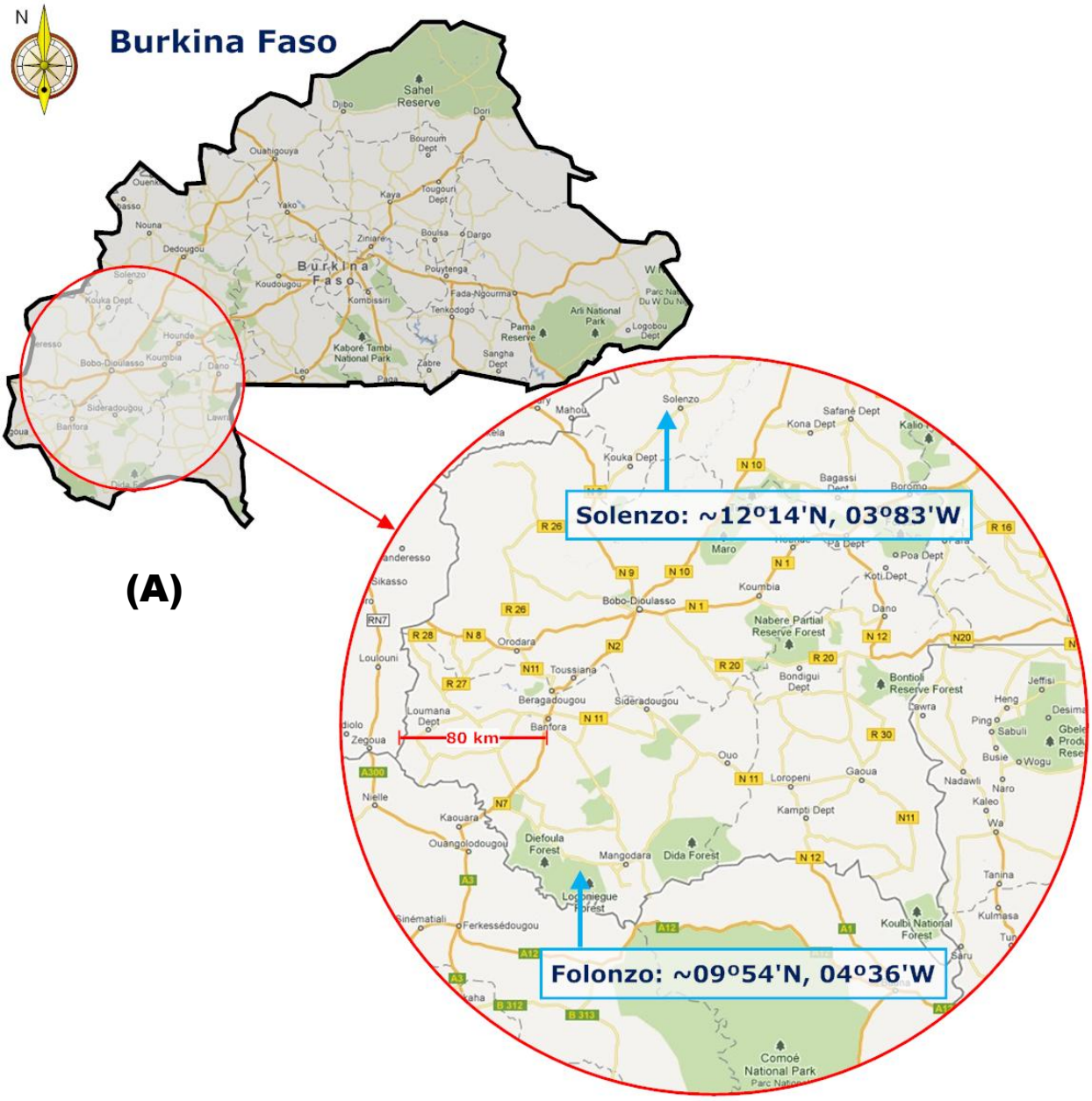


Figure 2-2: *Sites in Burkina Faso*: (A) Map of Burkina Faso, with details of the areas of Solenzo, in the West of the country, and Folonzo in the South-West (SmartDraw 2012). (B) View of the Comoe River flowing through Folonzo. (C) Treating a bull with trypanocides in Solenzo

The distinctive distribution of both species supports the idea that *G. palpalis s.l.* populations can extend into peri-urban areas (Späth, 2000; Courtin *et al.*, 2005; Cano Ortega, 2008), whereas *G. tachinoides* is more sensitive to land use and landscape degradation (Mahama *et al.*, 2005).

2.1.2. Côte d'Ivoire

Studies were carried out between February and April 2008 at sites near Bingerville (approximately 05°21' N, 03°50'W), approximately 25 km East of Abidjan, and between December 2008 and March 2009 at Azaguié (05°37' N, 04°06' W), approximately 45 km north of Abidjan (Figure 2-2) (chapters 4 & 6). Annual rainfall is about 1400 mm. Both areas comprise a mosaic of lagoons and farms, where tree crops such as banana, coffee, cocoa, rubber and oil palm are abundant. Scattered patches of the original primary forest are also found in the area. Humans, pigs and cattle are present at both sites but wild mammalian hosts are scarce. *G. p. palpalis* is the only species of tsetse present at these sites.

G. p. palpalis is relatively abundant in both areas, although low densities of *G. nigrofusca* are also found in Azaguié.

Figure 2-3: ***Sites in Côte d'Ivoire*****:** (A) Map of southern Côte d'Ivoire, and details of the areas of Bingerville and Azaguié (SmartDraw 2012). (B) Assistant deploying traps to select sites in Azaguié

2.1.3. Democratic Republic of Congo (DRC)

Studies were undertaken in a rural farming area *c.* 35 km south of Kinshasa city centre (4°29'S, 15°18'E) in June-August 2008, and July-September 2009 (chapters 3 & 5). Experimental sites were located in a hilly area, the valleys of which drain into the Lukaya River, and are occupied by small farms (Figure 2-4). Relatively small piggeries are common, containing around 15-30 animals each. The piggeries are often connected to large fishponds or dams, where *Tilapia* spp. and catfish are farmed.

Pockets of indigenous vegetation are still present in most valleys. Small crop fields and vegetable terraces are cultivated on the slopes and lower parts of the hills. Humans and livestock, principally pigs, are common in the area and are probably the main hosts of tsetse (De Deken *et al.*, 2005). Wild animals are rare. *G. f. quanzensis* was the only species identified during the studies.

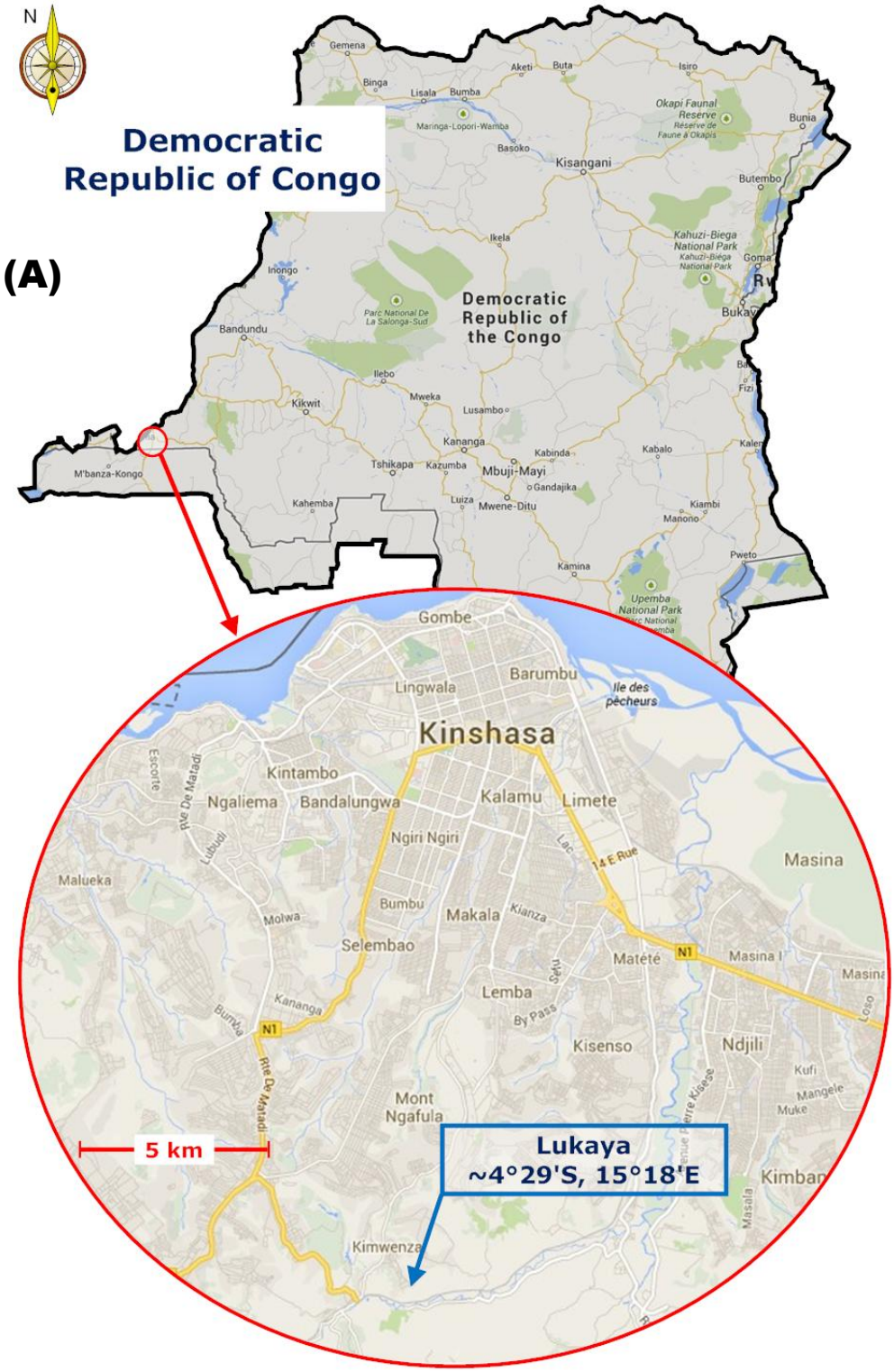


Figure 2-4: ***Sites in DRC*****:** (A) Map of DRC, and details of the areas in the valley of the Lukaya River (SmartDraw 2012). (B) Local assistants transporting a CO_2 cylinder along the fishponds

2.1.4. Kenya

Studies of *G. f. fuscipes* were undertaken in western Kenya, between July 2007 and December 2008, on the islands of Chamaunga (00°25'S, 34°13'E) with an area of about 0.5 km^2 and distanced 500 m from the mainland, Manga (00°21'S, 34°15'E) of about 0.4 km^2 area, and 300 m from the mainland, and the northern peninsula of Rusinga (00°21' S, 34°13' E) (chapter 3). Rusinga is essentially part of the mainland, connected by a causeway of 100 m in length. All islands are within 5 km of ICIPE Mbita Point Field station. A few experiments were also carried out in the mainland in either Kirindo (near Mbita, at 00°26' S, 35°15' E) or in Chakol Division of Teso District (00°30-32'N, 34°10-18'E), about 40 km north of Mbita Field station (Figure 2-5).

The islands of Rusinga and Manga are inhabited but Chamaunga is not, apart from occasional visits by fishermen and entomologists. The natural lacustrine vegetation at all of these sites has been degraded and fragmented by human activity. Monitor lizard, human, and domestic livestock, *i.e.* cattle, sheep and goats principally, are the main hosts within the area (Mohamed-Ahmed & Odulaja, 1997; Wamwiri *et al.*, 2007). Wild mammalian hosts, apart from hippopotamus, have been hunted out or driven away by destruction of the habitat.

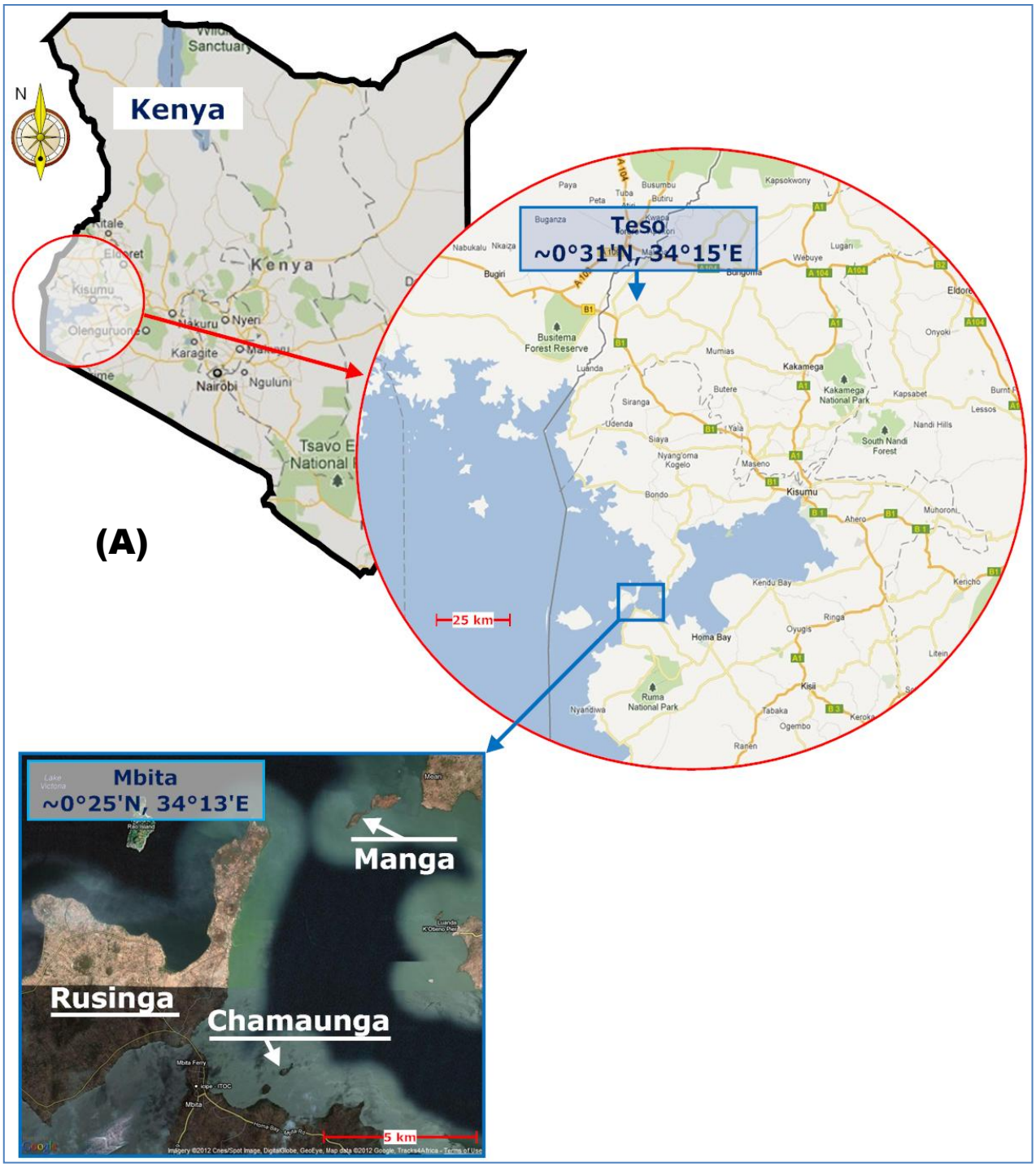


Figure 2-5: ***Sites in Kenya*****:** (A) Map of Lake Victoria, and details of the Mbita area with of Manga, Rusinga and Chamaunga islands, and Teso (SmartDraw 2012). (B) Field assistants transporting the equipment along the sites in Manga island

2.2. Natural host odours

In each country, local cattle, pigs or humans were used as sources of host odours (baits). In Kenya only, studies were also made of odour from monitor lizard. Cattle, humans or pigs were placed in rectangular PVC-coated tents in Burkina Faso, Côte d'Ivoire and Kenya (2×2×3 m) (Figures 2-6 B and D) or triangular PVC-coated tents in DRC (2×1.5×2 m) (Figure 2-6 A) (chapters 3 & 4).

Air from the tent was exhausted at *c*. 2000 L/min by a 12 V co-axial fan connected to a flexible PVC-coated tube (∅ 0.1 m), *c*. 15 m away, where various catching devices were placed. In this way, baits were not visible nor could they be bitten by approaching tsetse. Lizards (chapter 3) were unable to bask in a tent and, and being poikilothermic, the absence of basking might reduce their metabolic rate and, perhaps, the odour produced. Accordingly, they were placed in a chamber (2.4×2.4×2.5 m) with stainless-steel walls and a partially shaded glass roof, which allowed the lizards contained within it to move freely in and out of shade during the course of an experiment (Figure 2-6C).

Studies with *Morsitans*-group flies suggest that the effectiveness of odours from particular host species is related to their gross weight (Vale, 1974d; Hargrove, 1976). Accordingly, to match the weights of different mammalian host species, tents contained a single ox, two humans or three-to-four pigs. Given the average weight of the cattle (*c.* 150 kg), humans (*c.* 75 kg) and pigs (*c.* 50 kg) the gross weight of mammalian baits within the tent was 150-200 kg unless reported otherwise. Lizards (chapter 3) are considerably smaller and 5-6 lizards (ranging in individual weight from 2.5-7 kg and sex undetermined) with a total, combined weight of *c.* 30 kg were placed in the metallic chambers.

Cows and pigs (chapters 3 & 4) were provided by local farms and maintained under normal local conditions (Figures E and F). Lizards were trapped from the shores of Lake Victoria near Mbita, where they are abundant, by trained staff when required, held in cages, and provided with fish or beef on the evening of every third day. Lizards were used in experiments over a period of 12-14 days. Attempts to assess the olfactory responses of *G. p. palpalis* to dwarf crocodile (*Osteolaemus tetraspis*) odour were made in Côte d'Ivoire. However, due to the absence of responses in preliminary studies using specimens

borrowed from the Zoo of Abidjan, and the difficulties to capture and maintain the crocodiles in captivity, this line of research was ruled out.

In Kenya only (chapter 3), studies were also made of the responses to urine from lizards collected and dispensed as described by Mohamed-Ahmed (1998). Bacterial fermentation of host urine seems to have an effect on the responses of tsetse (Mohamed-Ahmed, 1998). Mohamed-Ahmed (1998) demonstrated that the addition of fermented urine increased the catch of *G. fuscipes* in an electrified trap 1.7 times. Attraction of tsetse to fermented urine is probably due the release of phenolic compounds caused by the bacterial catabolism of protenins (Okech & Hassanali, 1990).

To assess the effect of fermented urine, studies were made to compare the numbers of tsetse caught when fresh urine, or urine that had been fermented for two weeks were used as olfactory baits.

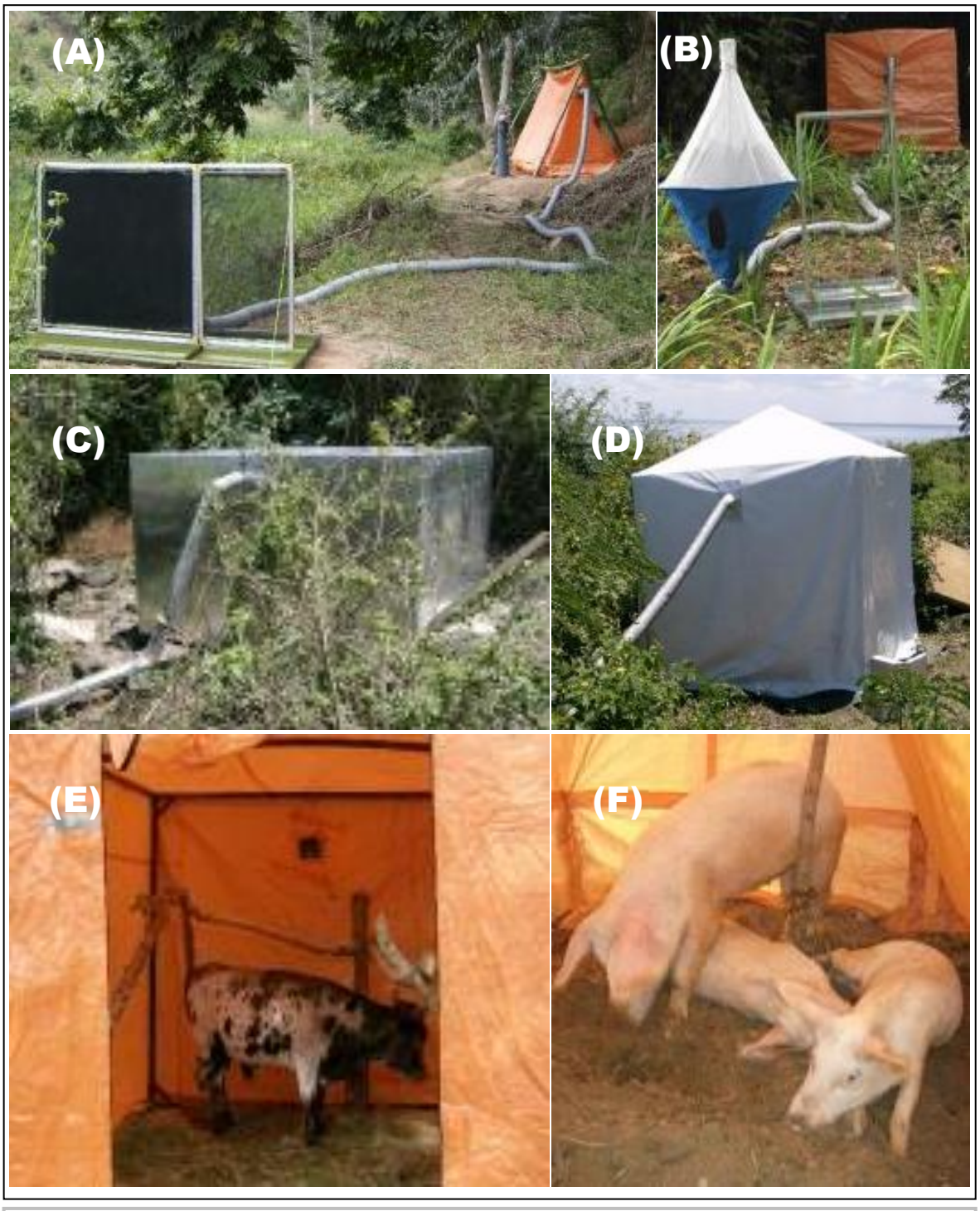


Figure 2-6: ***Examples of experimental setups:*** (A) Tent used in DRC with electric target and electric flanking net as collecting device; CO_2 provided by a pressurised cylinder used as bait (at the site of the tent). (B) Tent used in Burkina Faso and Ivory Coast with trap and electric flanking net as collecting device. (C) Metallic chamber for monitor lizards in Kenya. (D) Tent used in Kenya. (E) Bull in tent. (F) Three pigs in a tent.

2.3. Synthetic odours

Some of the experiments were designed to assess the responses of riverine tsetse to chemicals present in cattle odour (chapters 3 & 4). These chemicals have been identified as active ingredients, responsible for the attraction of tsetse of the Morsitans-group to cattle odour (Vale & Hall, 1985; Bursell *et al.*, 1988; Torr *et al.*, 1995; Torr & Mangwiro, 1996). They included acetone (*c.* 500 mg/h), octenol (*c.* 0.1 mg/h), 4-methylphenol (*c.* 0.4 mg/h), 3-*n*-propylphenol (*c.* 0.01 mg/h), and carbon dioxide (CO_2; 1-4 L/min).

Chemicals were dispensed individually or in various combinations. 4-Methylphenol and 3-*n*-propylphenol were dispensed individually or in combination with 1-octen-3-ol (henceforth termed 'octenol') from sealed sachets of 50 cm^2 surface and 150 μm thick, made from polyethylene lay-flat tubing. The blend consisting of acetone, octenol, 4-methylphenol and 3-*n*-propylphenol will be referred henceforth as POCA. In the POCA blend, 'P' stands for 3-*n*-propylphenol, 'O' for octenol, 'C' for *p*-cresol (4-methylphenol), and 'A' for acetone. In one experiment in Burkina Faso, collecting devices were baited with 'synthetic cattle odour', this being a blend of: acetone (*c.* 500 mg/h), octenol (*c.* 0.5 mg/h), 4-methylphenol (*c.* 1 mg/h), 3-methylphenol (*c.* 1 mg/h), 3-*n*-propylphenol (*c.* 0.1 mg/h), and CO_2 (2 L/min). In this case, the compounds were dispensed at doses similar to those produced naturally by a single ox (Torr *et al.*, 1995; Torr *et al.*, 2006). Due to the volatility and release dose required for octenol, when this chemical was dispensed alone, sachets of 300μm thickness were used. Glass vials with a hole of ∅7 mm in the lid were used as dispensers for acetone (Vale & Hall, 1985; Torr *et al.*, 1997).

CO_2 was provided from pressurised cylinders (chapters 3 & 4). The flow was controlled with a two-stage CO_2 regulator (BOC) and a "bead-and-tube" glass flow meter (Meterate tube, GPE Scientific Limited). The dose of synthetic CO_2 dispensed was estimated to match approximately the natural dose of CO_2 produced by natural baits. Hence, artificial and natural CO_2 were measured every hour at the distal end of the pipe, where the collecting devices were installed. Readings were made using an infrared gas analyser (EGM-1 or EGM-4, PP Systems, Hitchin, UK).

2.4. Collecting devices

2.4.1. Electric grids

Electric grids (E-grids) were used to assess responses of tsetse to visual and olfactory cues (Vale, 1974d) (chapters 3 to 6). E-grids are electrocuted devices made of metallic frames, and used to kill (by electrocuting shocks) and collect flies in behavioural experiments. They were mounted on metallic trays *c*. 5 cm deep, filled with soapy water. A bank of ∅0.2 mm copper wires was placed at each side of the grid, with both banks of wires being 8 mm apart, the same space as between two consecutive wires. These electrified wires are effectively invisible to tsetse (Packer & Brady, 1990). Electric grids were powered by a transformer with a DC input of 12V/3A and an output of *c*. 50 kV pulsing at *c*. 50 Hz. Flies were electrocuted as they collided with the electrified wires and fell, killed or stunned, into the soapy water contained in the trays. At the end of the experiment, flies collected in the trays were counted. Depending on the type of material inserted between the two banks of copper wires, e-grids were named electric target (E-target) or electric net (E-net) (Figure 2-7).

E-target: A panel of solid cloth was inserted between the two rows of wires of the e-grid, and used to catch flies as they landed. Unless stated otherwise, the E-targets in experiments of visual responses were 1×1 m and the cloth black (chapters 3 & 4); in chapters 5 & 6 E-targets adopted different configurations to assess visual responses of tsetse, and hence size, shape – *i.e.* vertical, horizontal or square – and the colour was modified accordingly (Figure 2-7).

E-net: E-nets were similar to E-targets, except that the solid cloth was replaced by fine black polyester net (Quality no. 166, Swisstulle, Nottingham, UK), which is effectively invisible to the flies. The black polyester net prevents tsetse from flying straight through wires of the grid. E-nets were placed side-by-side with the E-targets, and gave an estimation of the proportion of flies circulating the E-target, but not landing on it (Figure 2-7). Unless stated otherwise, E-nets were 1 m high × 0.5 m wide.

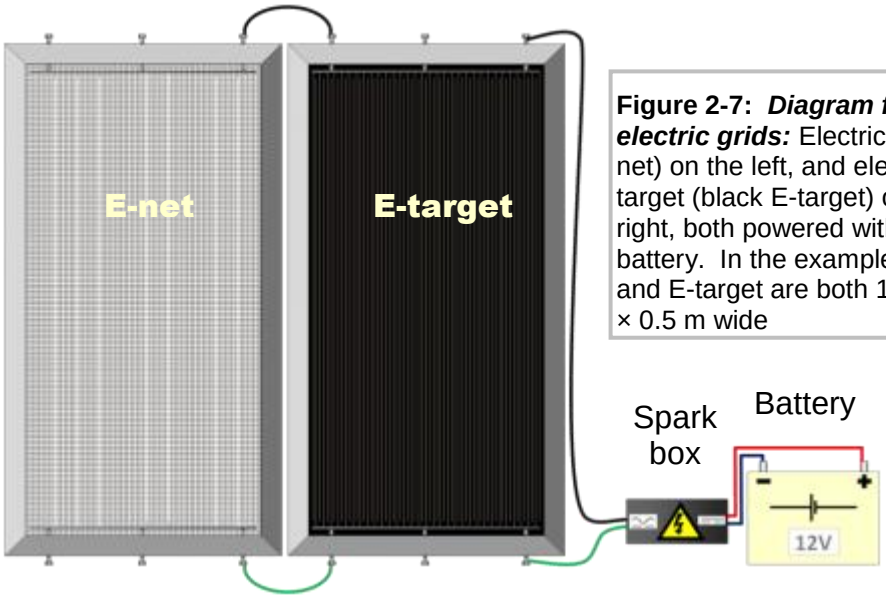


Figure 2-7: ***Diagram for electric grids:*** Electric net (E-net) on the left, and electric target (black E-target) on the right, both powered with a car battery. In the example, E-net and E-target are both 1 m high × 0.5 m wide

2.4.2. Inert targets

Studies of the numbers of tsetse attracted to and landing on small (*e.g.*, 0.1×0.1 m) E-targets face the problem that the framework, which supports the grid of wires, may itself be a source of visual stimuli (Figure 2-7). To overcome this, we conducted a series of experiments where we placed an E-net next to various panels of black cotton cloth mounted on a simple wire frame (*i.e.* 0.1×0.1 m, 0.25×0.25 m, 0.5×0.5 m, 0.75×0.75 m and 1×1 m; chapters 5 & 6). These panels were not enclosed in an electric grid, and hence, tsetse that landed on it were not caught. Instead, the catch from the flanking E-net provided a relative measure of the numbers of tsetse attracted to the target (Figure 2-8 for an example of 'inert targets'). These visual targets are referred to as 'inert targets' to distinguish them from the electrified E-targets.

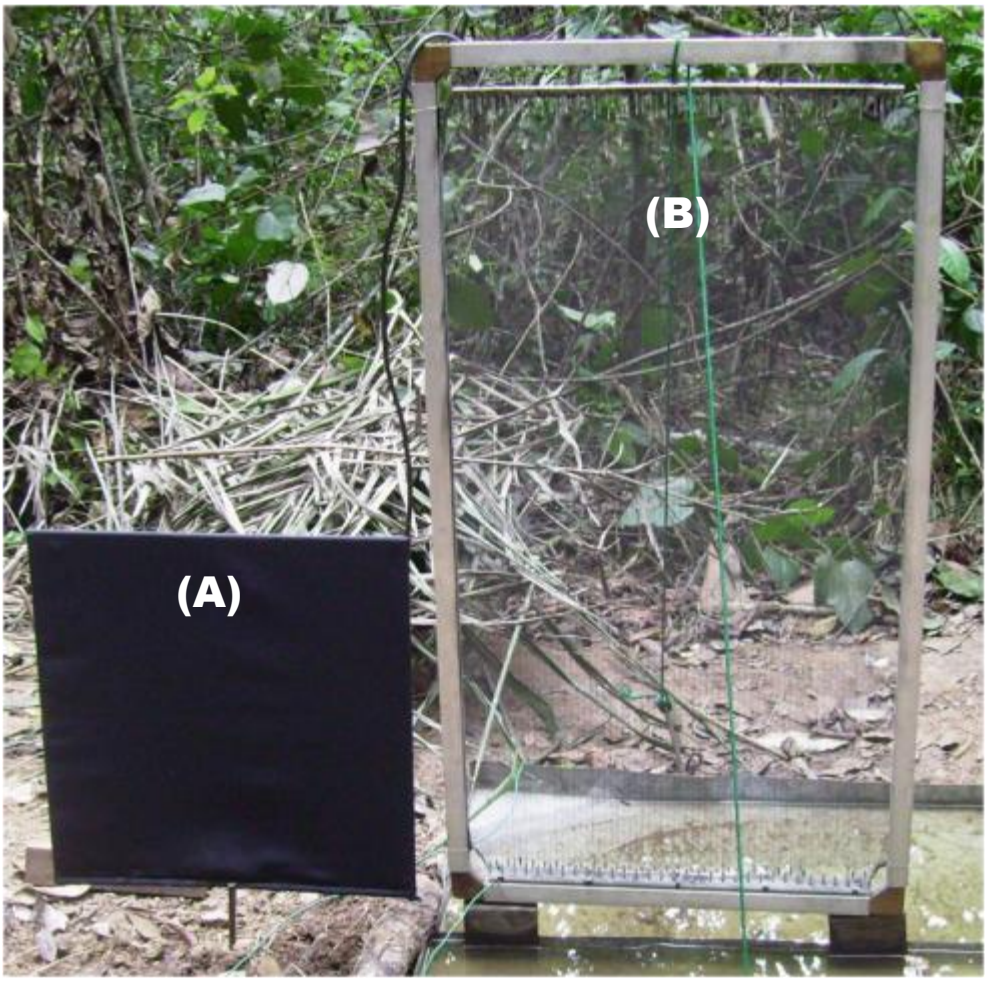


Figure 2-8: ***Example of 'inert target':*** 'Inert target of 0.5 m × 0.5 m (A) placed next to an electrocuting flanking net of 1 m high × 0.5 m wide (B)

2.4.3. Traps

Biconical traps (Challier & Laveissière, 1973) were used in all countries as the standard trap in Burkina Faso, Côte d'Ivoire and Kenya (chapters 3, 4, 5 & 6), whereas monopyramidal traps (Gouteux & Lancien, 1986) were used in DRC (chapters 3 & 5) as they are the model of trap used in the country. Phthalogen blue, with a reflectance spectral peak of 460 nm (Lindh *et al.*, 2009), and black cotton were the standard colours used throughout.

2.5. Attraction, landing responses and trap efficiency

Attraction

The numbers of tsetse attracted to the odours of different hosts was assessed with E-nets (0.5 m wide × 1.0 m high, unless stated otherwise). Tsetse do not orientate precisely to an odour source unless it is marked by a visual stimulus (Vale, 1974e). In the experiments, this visual stimulus was provided by a black E-target (1.0 × 1.0 m, unless stated otherwise), placed adjacent to the E-net.

Landing responses

The catch obtained on the E-target (*t*), expressed as a proportion of the total catch (E-net + E-target, N), provided an index of the strength of the landing response (Landing response = t/N).

Trap efficiency

The effect of host odours on trap-orientated responses was assessed by dispensing the odours at the base of the traps. The catch from a trap is the product of (i) the number of tsetse attracted to the vicinity of the trap, and (ii) the proportion of flies that subsequently entered it and were retained. This proportion is known as 'trap efficiency' (Vale & Hargrove, 1979). The effect of odours on the efficiency of the trap was estimated by setting an E-net (0.5 m width × 1.0 m height) adjacent to the trap. The total catch (E-net + trap) provided a measure of the numbers of tsetse attracted to the trap with or without host odours, and the catch from the trap, expressed as a proportion of the total catch, provided an index of trap efficiency.

2.6. Simulation of the effect of sites in visual attraction

Experiments in DRC and Côte d'Ivoire (chapters 5 & 6) were made to explore the effect that dense vegetation might have in obscuring the location of hosts. E-targets made with black and sky-blue cloths 0.5 m with × 0.25 m wide, flanked by a 1 m × 0.5 m with E-net, – baited with CO_2 (1 L/min, Figure 2-9D) or unbaited – were concealed in the centre of a palisade, and the catches compared with similar, but visible, devices. Palisades were

hexagonal in shape (∅3 m) with three openings of 30 cm each to allow the access of flies to the interior, where the grids were installed (Figures 2-9 A, B & C). The walls of the palisade were 1.5 m high and gaps in the walls covered with palm tree branches (Figures 2-9C & D). To balance potential visual stimuli in the different treatments, in experiments requiring CO_2 an empty cylinder was placed near the untreated control.

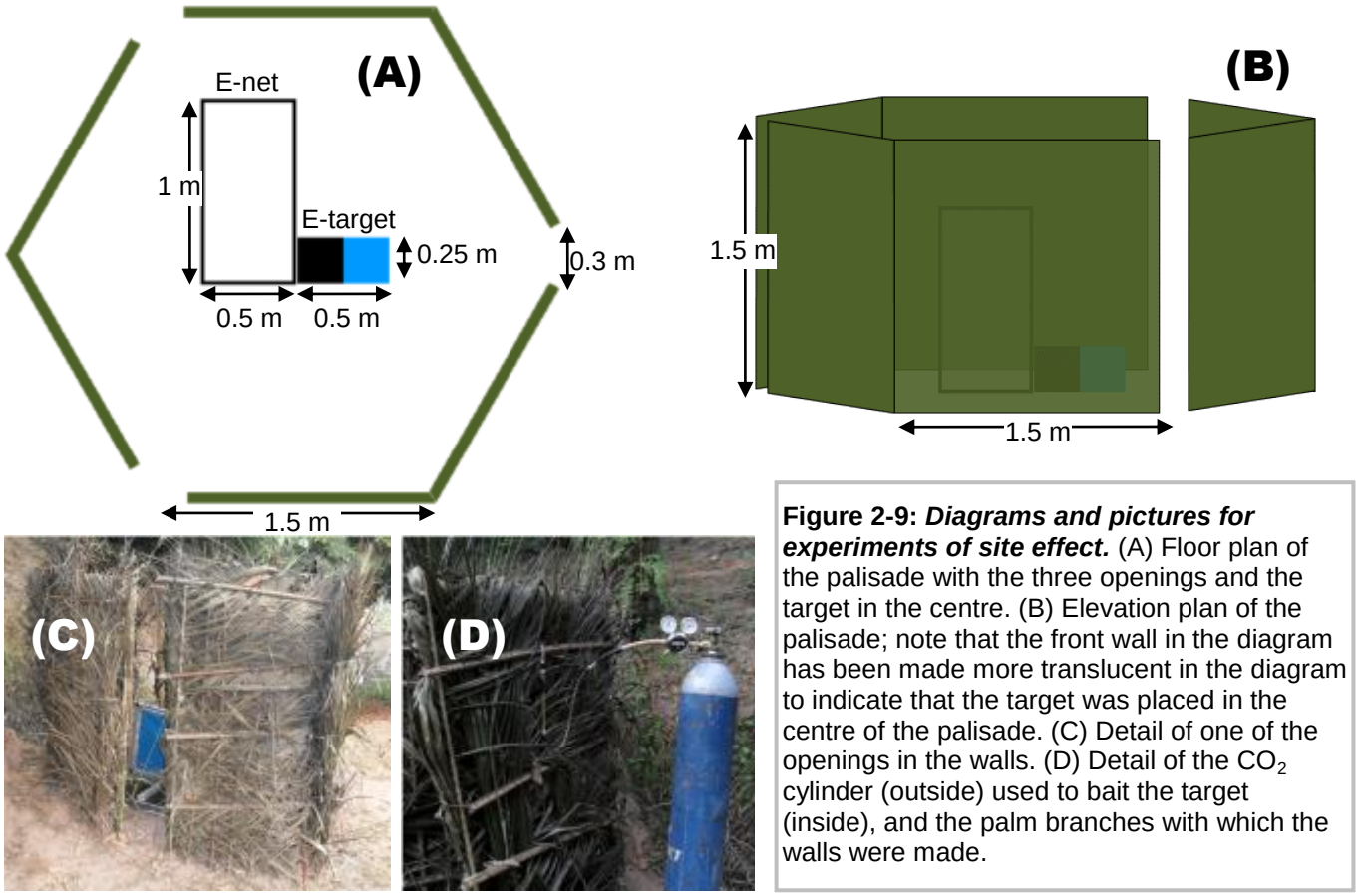


Figure 2-9: ***Diagrams and pictures for experiments of site effect.*** (A) Floor plan of the palisade with the three openings and the target in the centre. (B) Elevation plan of the palisade; note that the front wall in the diagram has been made more translucent in the diagram to indicate that the target was placed in the centre of the palisade. (C) Detail of one of the openings in the walls. (D) Detail of the CO_2 cylinder (outside) used to bait the target (inside), and the palm branches with which the walls were made.

2.7. Tsetse identification

Tsetse were identified up to species using the software edited by the French *Institut de Recherche pour le Développement* (IRD), entitled: "Les glossines ou mouches tsé-tsé. Un logiciel d'identification et d'enseignement" (Brunhes *et al.*, 1994). For confirmation, some specimens were sent to the Natural History Museum (London).

2.8. Experimental design and statistical analyses

Unless stated otherwise, experiments were carried out for 4 h, between 08:00 and 14:30 h, when Palpalis-group flies are most active (Crump & Brady, 1979; Mohamed-Ahmed & Odulaja, 1997). In general, odour baited devices (*i.e.* traps, electric nets, electric targets and combinations thereof) were compared with an unbaited control, in a series of replicated Latin squares of days × sites × treatments. The number of days that the experiments were repeated varied between 6 and 12 days. Experimental sites were 100-200 m apart.

The daily catches were normalized and variances homogenized using a $\log_{10}(n+1)$ transformation and then subjected to analysis of variance using GenStat 11 (version 11.1.0.1504). Differences between more than two means were assessed by the 'Bonferroni test'. Detransformed means are reported accompanied by their transformed means and standard errors of the difference (SED) between means. To provide a comparative index of the effect of the treatments, detransformed means of each treatment were divided by the detransformed mean catch of the control. Catch indices greater or less than unity indicate that the device caught more or less tsetse than the control, respectively.

Logistic regression with a logit link was used to analyse the effects of odours on the proportions that were caught landing on a target or entering a trap, as opposed to flies colliding with an E-net. Days, sites and treatments were specified as factors, and the statistical significance of differences in the proportion of tsetse landing on the target or entering a trap was assessed by removing the treatments factor from the full model (*i.e.*, days + sites + treatments). The catch from the target or trap was specified as the *y*-variable, and the pooled daily catches from E-target+E-net, or trap+E-net were the binomial denominator. The significance of changes in deviance was assessed by either a χ^2 test, or, if the data were overdispersed (*i.e.* residual deviance > residual degrees of freedom) an *F*-test following re-scaling by dividing Pearson's χ^2 by the degrees of freedom (Crawley, 1993). The SE is asymmetric about the mean, and thus, mean percentages are accompanied by the larger SE. For all analyses, the significance level was established at $P < 0.05$.

CHAPTER THREE

OLFACTORY RESPONSES OF *GLOSSINA FUSCIPES* S.L.

3.1. Introduction

3.1.1. Importance of G. fuscipes *as vectors of sleeping sickness*

During the period 1997-2006, out of the *c.* 240,000 cases of gambiense HAT reported worldwide, about 92% were diagnosed in Angola, DRC, Sudan or Uganda, where either *G. f. fuscipes* (northern DRC, Uganda and Sudan) or *G. f. quanzensis* (northern Angola, southern DRC) are the only significant vectors (Rogers & Robinson, 2004). In addition, about 51% of the *c.* 6000 reported cases of rhodesiense HAT during the same period were in southern Uganda, where *G. f. fuscipes* is the main vector. These figures suggest that >90% of cases of HAT start with a bite from a subspecies of *G. fuscipes*.

Despite their importance as vectors, campaigns against these tsetse subspecies to reduce HAT transmission have played a minor role, being undertaken occasionally to control the transmission of the zoonotic *T. brucei rhodesiense* (Lancien, 1991b; Maudlin, 2006), which is responsible for about 10% of sleeping sickness cases (Simarro *et al.*, 2008). Control of the transmission of *T. brucei gambiense*, responsible for over 90% of the HAT cases, is largely based on the detection and treatment of disease in humans (Simarro *et al.*, 2008).

This contrasts with the important role that vector control has played in tackling animal trypanosomiasis, mostly against tsetse of the Morsitans-group (Maudlin, 2006). As seen in chapter 1, modern methods of tsetse control include insecticide treated traps and targets, which can be baited with artificial lures to improve their cost-effectiveness. Baited targets and traps exploit the behaviour of tsetse responding to particular semiochemicals to locate their hosts. However, whereas the responses of Morsitans-group species to host odours is well established (see chapter 1), data on the olfactory responses of *G. fuscipes* is scant and synthetic lures have not been widely used for either control or monitoring purposes. The existing data on the host-oriented behaviour of *G. fuscipes* is reviewed in the following sections.

3.1.2. Feeding preference of G. f. fuscipes subspp

G. f. fuscipes and *G. f. quanzensis* are found near some populated areas of Uganda (Okoth, 1986) and DRC (De Deken *et al.*, 2005) respectively, where they are responsible for the transmission of HAT. In feeding studies of *G. f. fuscipes* in Uganda and Kenya, between 0% and 6% of the bloodmeals were identified as human (Waiswa *et al.*, 2006; Wamwiri *et al.*, 2007).

The monitor lizard (*Varanus niloticus niloticus*) is an important host of *G. f. fuscipes* in diverse ecosystems, representing over 65% of all the bloodmeals. For example, studies in Kamuli, Mukono and Tororo districts (Uganda), where livestock are relatively abundant, showed that 17-34% of bloodmeals in *G. f. fuscipes* were from monitor lizards (Waiswa *et al.*, 2003; Waiswa *et al.*, 2006). This percentage approached 100% along the shores of Lake Victoria, where monitor lizard are very abundant, and other potential hosts are rare (Mohamed-Ahmed & Odulaja, 1997; Wamwiri *et al.*, 2007).

Less information is available in relation to the hosts of *G. f. quanzensis*. In one of the few published reports, Simo *et al*. (2006) found that 27% of bloodmeals were from pigs in the peri-urban population of *G. f. quanzensis* around Kinshasa, and 68% were from humans (Simo *et al.*, 2006). The authors suggested that tsetse were concentrated in the riverine habitat and in the piggeries.

Feeding rates data do not prove whether tsetse are more attracted to particular hosts; rather they give an indication of the host species available in the habitat where tsetse occur. Accordingly, other experiments were undertaken to assess responses of tsetse to host odour.

3.1.3. Host-orientated behaviour of G. fuscipes *subspp*

In contrast with tsetse of the Morsitans-group, relatively few experiments have been carried out to assess olfactory responses of *G. fuscipes* sub species to host odours; data for *G. f. quanzensis* is particularly low. The results of these experiments are summarised in Table 3-1.

	Odour source		Country	Device	Catch index		Reference
	Source	Fraction			*G.f.fusc.*	*G.f.quan.*	
Natural odours	Cow	Urine	CAR	B	1.7		(Gouteux *et al*., 1995)
	Lizard	Whole	CAR	B	1.4-2		(Gouteux *et al*., 1995)
			Kenya	B	1.5		(Mohamed-Ahmed, 1998)
		Skin wash	Kenya	B, ET	1.5		(Mohamed-Ahmed, 1998)
Synthetic odours	CO_2		Uganda	B	n/s		(Rogers, 1970)
			Kenya	B, ET	2-3		(Mohamed-Ahmed & Mihok, 1999)
			Congo	B		40	(Frézil & Carnevale, 1976)
	Phenolic fraction		Uganda	B	n/s		(Rogers, 1970)
			Kenya	B	n/s		(Mwangelwa *et al*. 1995)
	Acetone		Kenya	B	n/s		(Mwangelwa *et al*. 1995)
	Octenol		Kenya	B	n/s		(Mwangelwa *et al*. 1995)

Table 3-1: Catch index for *G. p. palpalis* and *G. p. quanzensis* responding to natural and synthetic attractants. Catch index is the catch of a trap baited with the attractant expressed as a proportion of an unbaited trap ($p<0.05$); n/s = no significant increase in catch **Device: 'B'** stands for 'biconical trap' and **'ET'** stands for 'electrified trap' (trap designed by the authors).

G. f. fuscipes

Response of *G. f. fuscipes* to monitor lizard odour

Most of the behavioural studies on *G. f. fuscipes* have been conducted along the shores of Lake Victoria, where this tsetse species feed almost exclusively on monitor lizard (Mohamed-Ahmed & Odulaja, 1997; Wamwiri *et al.*, 2007). Consequently, several experiments were carried out to elucidate whether *G. f. fuscipes* responds to semiochemicals produced by lizards (Table 3-1).

Gouteux *et al.* (1995) observed that the odour from a concealed monitor lizard significantly increased the number of *G. f. fuscipes* trapped. Subsequently, Mohamed-Ahmed (1998) found that baiting electric grids with a cage containing three lizards doubled the catch significantly. The lizards in the cage were visible, and the effect of visual stimuli cannot be discounted. To avoid visual stimuli, Mohamed-Ahmed (1998) compared the catch of two electrified cylinders acting as traps, one empty and the other containing a monitor lizard. In this case, the numbers of *G. f. fuscipes* were doubled in the baited cylinder, although the differences were not significant for either males or females analysed separately. Lizard urine doubled the catch of electrified cylinders, and increased the number of tsetse male in a trap 1.4× compared with unbaited collecting devices. In summary, the effect of lizard odour in the catches of *G. f. fuscipes* was consistent but relatively small, albeit statistically significant at the $P<0.05$ level of probability.

Responses of *G. f. fuscipes* to host odours, others than monitor lizard

In addition to monitor lizard, *G. f. fuscipes* feed frequently on cattle (Clausen *et al.*, 1998). However, this species does not appear to be responsive to known attractants present in cattle odour, such as acetone, octenol or phenols, dispensed individually or as a blend (Mwangelwa *et al.*, 1995) (Table 3-1). Similarly, the odour of other potential hosts, such as human, crocodile, python, rabbit or chicken, did not increase the catch, suggesting that *G. f. fuscipes* respond to specific semiochemicals of monitor lizard (Mwangelwa *et al.*, 1995).

Responses of *G. f. fuscipes* to CO_2

Studies to assess the response of *G. f. fuscipes* to CO_2 have been carried out along the shores of Lake Victoria, with inconsistent results. Rogers (1970) reported that dry ice did

not increase significantly the catch of traps in Uganda (Table 3-1). In Kenya, Mohamed-Ahmed & Mihok (1999) reported that CO_2 doubled the catch of female *G. f. fuscipes* in a patch of dense vegetation, but did not have any significant effect in the riverine habitat, even when CO_2 was dispensed in the linear habitat at 5 L/min, doubling the dose of that in the dense vegetation (Table 3-1). In the 'dense forest', CO_2 doubled the number of female tsetse that landed on targets, but did not have any effect on the number of tsetse entering a trap (Mohamed-Ahmed & Mihok, 1999).

The authors suggested that carbon dioxide was ineffective for *G. f. fuscipes* along the river because the odour plume extended into areas outside the linear habitat – where tsetse were absent – and therefore the amount of CO_2 that was dispensed from the cylinder was latterly reduced in the linear habitat, where tsetse were present.

G. f. quanzensis

Response of *G. f. quanzensis* to CO_2

Only one paper describing olfactory responses of *G. f. quanzensis* was found in the literature. Frézil & Carnevale (1976) reported from their studies in the the zoo of Brazzaville (Congo) unusually high (20-fold) increases in the numbers of *G. f. quanzensis* caught with traps baited with dry ice, compared to unbaited traps (Table 3-1). The results were not conclusive as: (i) the density of *G. f. quanzensis* was very low, and the number of tsetse caught in unbaited traps was almost zero; and (ii) the release rate of CO_2 from the dry ice was not provided.

3.1.4. Aims of the study

The use of artificial baits to control tsetse of the Morsitans-group exploits the high response of these flies to host odours (Vale, 1974e; Vale, 1979; Vale & Hall, 1985). Insecticide-treated targets and traps, baited with synthetic blends of host odours, and deployed at low densities (*i.e.* ~4 targets/km^2) can eliminate populations of *G. pallidipes* and *G. morsitans* in about one year's time (Vale *et al.*, 1988b; Dransfield *et al.*, 1990; Willemse, 1991). Conversely, with the exception of the studies of lizard odours (Mohamed-Ahmed, 1998), there are no data on whether or not *G. fuscipes* use odours to

locate their hosts. Accordingly, this chapter reports the results from field studies undertaken in Kenya and the DRC to assess the responses of *G. f. fuscipes* and *G. f. quanzensis*, respectively, to natural odours from humans, cattle and pigs.

Various arrangements of electric nets were used to quantify the effects of odours on the specific behavioural responses, *i.e.* long-range attraction, landing, and trap entry.

Although experiments involving the responses of *G. f. fuscipes* to monitor lizards in Kenya are also reported here, these experiments were undertaken by Dr Maurice O. Omolo in a parallel study; the design of the experiments to assess responses of *G. f. fuscipes* to lizard odour was similar to those used for mammalian odours. However, I carried out the statistical analysis of these data to complement the studies regarding mammalian odour. Studies of olfactory responses of *G. fuscipes* to natural host odours in Kenya and DRC were carried out in collaboration with Dr Johan Esterhuizen. The majority of the results reported in this chapter were published in Omolo *et al.* (2009) (see Annex I).

3.2. Materials and methods

3.2.1. Study sites

Field studies of *G. f. fuscipes* and *G. f. quanzensis* were undertaken in Kenya and Democratic Republic of Congo (DRC) respectively. In Kenya, sites were selected on three islands of Lake Victoria (*i.e.* Chamaunga, Manga and Rusinga), except for a few experiments that were conducted in the mainland in Teso and Kirindo (experiments 4&9 and 13 respectively)(see section 2.1.4.); studies were undertaken between July 2007 and December 2008. Kirindo is located near the shores of the Lake Victoria, and although in the mainland, the habitat and environmental conditions are similar to those in the islands, and exposed to the influence of the lake. Conversely, Teso is located at about 40 Km from Mbita, and away of the influence of the Lake Victoria.

In DRC, experiments were carried out in valley of Lukaya, during the dry season between July and August 2009 and 2010 (see section 2.1.3.).

3.2.2. Natural host odours

Cattle, pigs and human volunteers were concealed in PVC-coated tents to provide natural host odours, as described in chapter 2 (see section 2.2). This chapter also includes responses of *G. f. fuscipes* to monitor lizard odour, although in this case data were collected by Dr Omolo (Omolo *et al.*, 2009). Unlike mammals, monitor lizards were placed in a metallic chamber as described in section 2.2.

Air from the tent or metallic chamber was exhausted at approximately 2000 L/min by a 12 V co-axial fan connected to a flexible PVC-coated tube (∅ 0.1 m), approximately 15 m away, where various catching devices were placed.

Additionally, responses of *G. f. fuscipes* to fresh or fermented urine from lizards were tested in Kenya, only. Fermented urine was obtained by incubating fresh urine in a sealed container for two weeks at room temperature.

3.2.3. Synthetic odours

Identified effective attractants for Morsitans-group tsetse, *i.e.* carbon dioxide, 1-octen-3-ol, 4-methylphenol (Vale & Hall, 1985; Bursell *et al.*, 1988; Torr *et al.*, 1995; Torr & Mangwiro, 1996), were dispensed from sealed sachets at the doses specified in section 2.3 (blend ratio 8:1:4 for 4-methylphenol, 3-*n*-propylphenol and octenol). The blend consisting of acetone, octenol, 4-methylphenol and 3-*n*-propylphenol will be referred henceforth as POCA. In the POCA blend, 'P' stands for 3-*n*-propylphenol, 'O' for octenol, 'C' for *p*-cresol (4-methylphenol), and 'A' for acetone.

Synthetic CO_2 was released from pressurised cylinders at 1-2 L/min as described in section 2.3. CO_2 dispensed inside the tent are likely to be diluted at the point where the collecting devices were placed at the distal end of the pipe, approximately 12 m away from the tent, compared to the concentration obtained from the cylinder. To test the effect in the catch of the dilution, CO_2 was dispensed, either inside the tent (*i.e.* similar to the natural host odours), or near the collection device.

To measure the dose of carbon dioxide produced by different hosts, the concentration (ppm) of carbon dioxide in the air being exhausted from the tents was measured using an infra-red gas analyser (EGM-1, PP Systems, Hitchin, UK). The velocity of air (m/s) was measure at the same point using a hot wire anemometer. These parameters allowed us to estimate the absolute volume of carbon produced by the test animals.

3.2.4. Collecting devices

Arrangements of electric grids (E-grids) were used to assess responses of tsetse to visual and olfactory cues (Vale, 1974d), as described in section 2.4.1. In some experiments, biconical traps (Challier & Laveissière, 1973) were also used in Kenya, whereas monopyramidal traps (Gouteux & Lancien, 1986) were used in some experiments in DRC (see section 2.4.3.).

3.2.5. Attraction, landing response and trap efficiency

Attraction

The numbers of tsetse attracted to the odours of different hosts were assessed with E-nets (0.5 m wide × 1.0 m high, at least stated otherwise), placed downwind of the source. Visual stimulus was provided by a black E-target (1.0 × 1.0 m), placed adjacent to the E-net (section 2.5).

Landing response

The catch obtained on the E-target (*t*), expressed as a proportion of the total catch (E-net + E-target, N), provided an index of the strength of the landing response (Landing response = t/N).

Trap efficiency

The effect of host odours on trap-orientated responses was assessed by dispensing the odours at the base of the traps. Trap efficiency was defined as the number of tsetse that entered a trap, expressed as a proportion of the total number of tsetse that were attracted to

the same trap (Vale & Hargrove, 1979). The effect of odours on the efficiency of the trap was estimated by setting an E-net (0.5 m width × 1.0 m height) adjacent to the trap. The total catch (E-net + trap) provided a measure of the numbers of tsetse attracted to the trap with or without host odours, and the catch from the trap, expressed as a proportion of the total catch, provided an index of trap efficiency.

3.2.6. Experimental design

Responses of *G. fuscipes* to odours were compared over 6-12 days in a series of replicated Latin squares of days × sites × treatments, as explained in chapter two (section 2.7). Experimental sites were at least 100 m apart. All experiments were carried out for 4 h, between 10:00 h and 14:00 h. Experimental setups for *G. f. fuscipes* and *G. f. quanzensis* are summarised in tables 3-2 and 3-3 respectively. All the experiments included an unbaited tent (*i.e.* no odour) as control.

Exp. number	Treat.	Location	Rep.	Collec. device
1	No odour	Manga	12	E-target
	Cattle			
	Human			
	Pig			
2	No odour	Manga	8	E-target
	Cattle			
	Human			
	Pig			
3	No odour	Rusinga	8	E-target
	Cattle			
	Human			
	Pig			
4	No odour	Teso	12	E-target
	Cattle			
	Human			
	Pig			
5	No odour	Chamaunga	12	E-target
	Cattle			
	Human			
	Lizard			
6	No odour	Chamaunga	8	Trap
	Cattle			
	Human			
	Pig			
7	No odour	Chamaunga	12	Trap + E-net
	Cattle			
	Human			
	Lizard			
8	No odour	Rusinga	10	E-target
	Cattle			
9	No odour	Teso	12	Trap
	Lizard			
10	No odour	Rusinga	12	E-target
	Lizard			
11	No odour	Rusinga	12	Trap + E-net
	Lizard			
12	No odour	Rusinga	6	E-target
	CO_2 - out			
13	No odour	Kirindo	9	E-target
	CO_2 - in			
	CO_2 - out			

Table 3-2: Experimental setups to explore olfactory responses of *G. f. fuscipes*

Exp. number	Treat.	Location	Rep.	Collec. device
1	No odour	Lukaya	12	E-target
	Cattle			
	Human			
	Pig			
2	No odour		12	E-target
	Human			
	Pig			
	CO_2 - in			
3	No odour		4	Trap
	Cattle			
	Human			
	Pig			
4	No odour		12	E-target
	Pig			
	CO_2 – in			
5	No odour		12	Trap
	POCA			

Table 3-3: Experimental setups to explore olfactory responses of *G. f. quanzensis*

3.2.7. Statistical analyses

Statistical analyses was conducted as described in section 2.7.

3.3. Responses of *G. f. fuscipes* to host odours

3.3.1. Attraction to odours

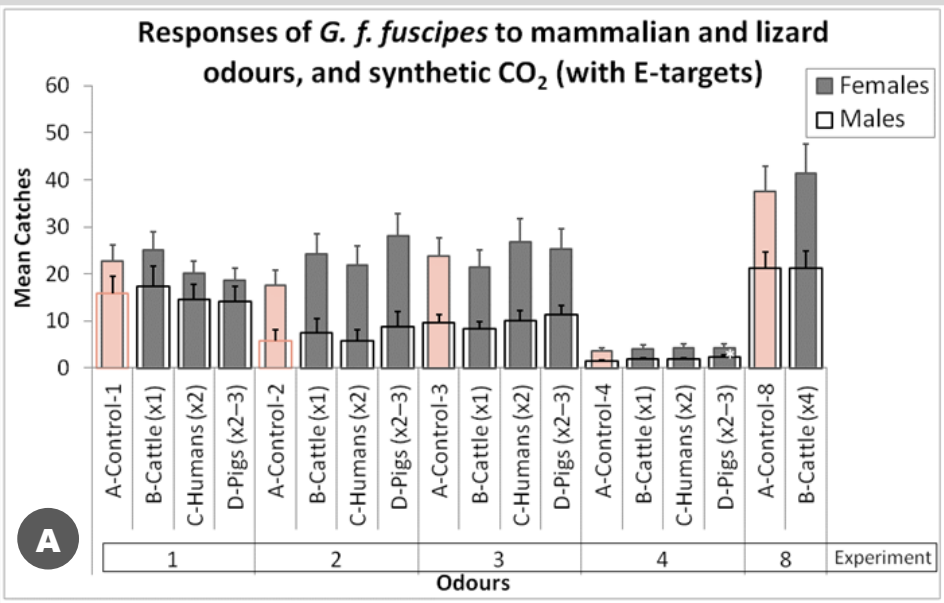
Baiting electrocuting devices (Figures 3-1A and 3-1B) or a trap (Figure 3-1C) with odour from cattle, humans or pigs had no significant effect on the tsetse catch rates, apart from one experiment where pig odour significantly increased the catch of males in one experiment carried out in Teso (experiment 4, Figure 3-1A). The geometric mean of the catch indices (*i.e.* mean catches obtained with baited collecting devices divided by mean catches of unbaited devices) for cattle, human and pig odour were 1.04, 1.08 and 1.25 respectively. The absence of a consistent and significant effect for mammalian odours was observed despite the natural CO_2 contained in the breath. The mean release rates of the

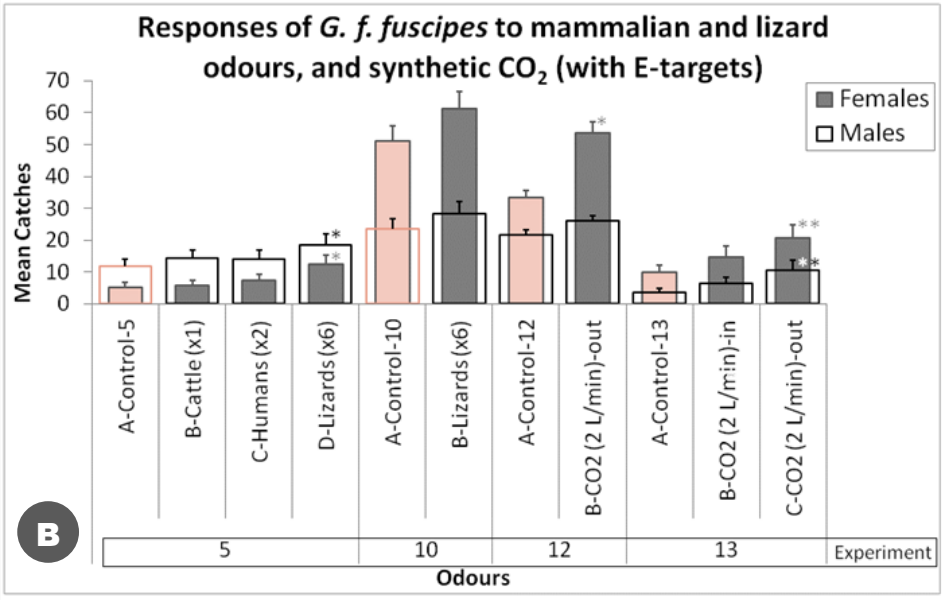
CO_2 produced by the hosts were about 1.1 L/min in the case of cattle odour, 0.6 L/min for human odour and 1.4 L/min for pig odour (Table 3-4).

Odour	A CO_2 in background (ppm)	B CO_2 at distal end of pipe (ppm)	B-C (ppm)	D Estimated CO_2 provided by host odour (L/min)
Control	416.5	448.4	31.9	0.1
Cattle (x1)	417.7	979.8	562.1	1.1
Human (x2)	410.4	726.3	315.9	0.6
Pig (x2)	416.5	1,092.4	675.8	1.4

Table 3-4: Estimate CO_2 release rates from host odours. (A) Atmospheric CO_2, measured in parts per million, detected by the infrared gas analyser in the background (*i.e.* 10 m upwind of the pipe). (B) CO_2, measured in parts per million, detected at the distal end of the pipe with different host odours. (B-A) CO_2, measured in parts per million, produced by the hosts, as the difference between the CO_2 detected at the distal end of the pipe and the atmospheric CO_2. (D) Estimated CO_2 released by the hosts, measured in L/min

Although the CO_2 release rate of four cattle was not measured in experiment 8 (Figure 3-1A), it was expected to be about 4 L/min, and yet the tsetse numbers were not significantly different from the control. Consistent with these results, baiting an E-target with synthetic CO_2, released at rates of 2 L/min inside the tent did not have any significant effect in the catch (experiment 13, Figure 3-1B). Conversely, when the synthetic CO_2 was dispensed at the same rate directly into the E-target it increased the catch 1.4 times, the difference being significant for females in the two experiments (experiments 12 and 13, Figure 3-1B) and for males in only one (experiment 13, Figure 3-1B).





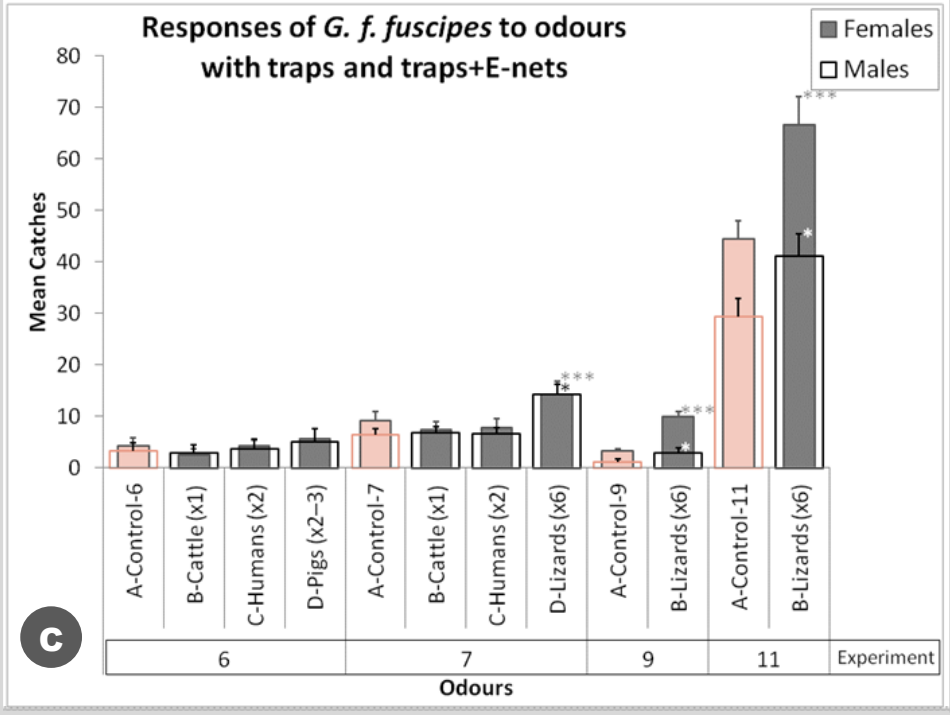


Figure 3-1: Responses of *G. f. fuscipes* to host odours. Detransformed means catches/day/site are represented in the ordinate axis +SED.

Asterisks indicate significant differences compared to the unbaited control treatment (in pink) at P<0.05 (*), P<0.01 (**) or P<0.001 (***).

(A) Mean catches of *G. f. fuscipes* caught with E-targets baited with mammalian odours. E-targets were 1×1 m. (B) Mean catches of *G. f. fuscipes* obtained with E-targets in experiments that involved different odours (i.e. mammalian and reptile odours, and CO_2). E-targets in experiments 5 and 10 were 0.5 m high×1 m wide; E-targets in experiments 12 and 13 were 1×1 m. (C) Mean catches of *G. f. fuscipes* obtained with biconical traps (experiments 6 and 9), or traps+E-targets operating simultaneously (experiments 7 and 11).

In contrast with mammals, odours from lizards increased the catch of males and females significantly in four out of five experiments (experiment 5 in Figure 3-1B and experiments 7, 9 and 11 in Figure 3-1C). However, baiting traps with fresh or fermented lizard urine had no significant effect in the catch. Biconical traps baited with fresh urine caught 14 (1.18±0.053, log-transformed mean±SED) males and 20 (1.31±0.038) females per day compared to 16 (1.22±0.053) males/day and 19 (1.31±0.038) females/day from an unbaited trap. Traps baited with fermented urine caught 10 (1.04±0.062) males and 15 (1.20±.051) females per day compared to 10 (1.03±0.062) males/day and 13 (1.150±0.051) females/day from an unbaited trap.

Analysis was also performed on the number of *Stomoxys calcitrans* when they were sufficiently abundant to allow analysis. The results showed that the absence of any response of *G. f. fuscipes* to cattle odour was not due to defects in the experimental design or sampling devices. For example, odour of one cow increased the catch of *Stomoxy* about 10-fold with a trap+E-net (P<0.001) (experiment 7), and about 7-fold when an E-target was used (P<0.001) (experiment 5), compared to unbaited collecting devices (Table 3-5). In experiment 8, the odour of four cattle increased the catch of *S. calcitrans* about 4 times greater (Table 3-5). No responses were observed for *S. calcitrans* with lizard or human odour (P<0.001) (Table 3-5).

Device	Exp	Odour	Catch	(m ± sed)	Index
Trap+E-net	7	Cattle (x1)	99.0	(2.00 ± 0.101)	11.0 ***
		Lizard (x6)	8.8	(0.99 ± 0.101)	1.0
		Human (x2)	9.0	(1.00 ± 0.101)	1.0
E-target	5	Cattle (x1)	37.0	(1.58 ± 0.122)	7.2 ***
		Lizard (x6)	4.8	(0.76 ± 0.122)	0.9
		Human (x2)	37.0	(1.58 ± 0.122)	7.2
E-target	8	Cattle (x4)	17.6	(1.27 ± 0.102)	4.3 ***

Table 3-5: Responses of *Stomoxys* to host odours. Detransformed mean daily catches (transformed mean and standard error of the difference (SED) shown in brackets) of *Stomoxys*. The detransformed mean daily catch of each odour-baited device is expressed as a proportion (Index) of that from an unbaited device; asterisks indicate that the index is significantly different from unity at the P<0.05 (*), P<0.01 (**) or P<0.001 (***) levels of probability

3.3.2. Landing responses

The results showed that odours from humans, cattle and pigs had no significant effect on the proportion of tsetse that were caught as they landed on the cloth panel of the E-target (experiments 3, 4 and 8, Figure 3-2). For all treatments, approximately 30% of males and 50% of females landed on the target.

Conversely, lizard odour increased the landing response of females significantly ($P<0.05$) compared to the unbaited E-target (24% *vs* 18%, respectively), although the increase was generally small and not always significant for males (40% *vs* 33%) (experiment 10, Figure 3-2).

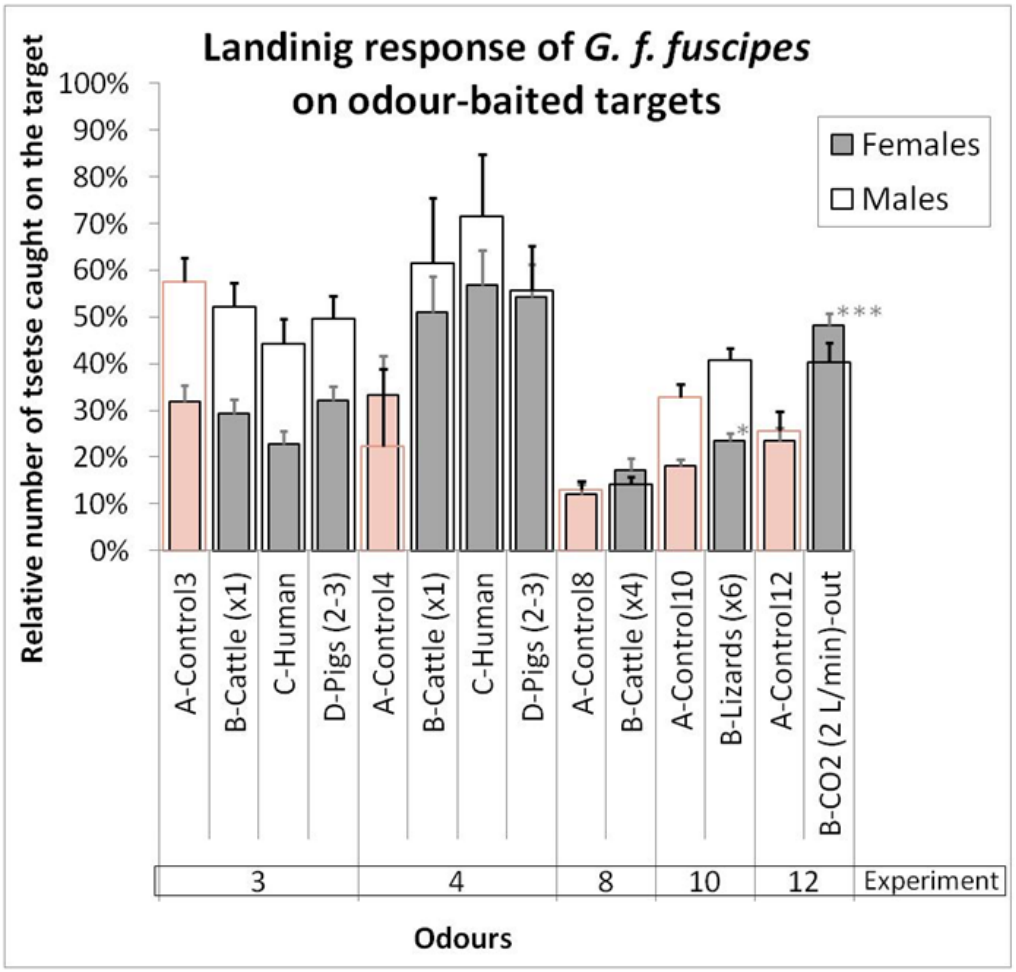


Figure 3-2: Effect of mammalian and lizard odour on landing response of *G. f. fuscipes*. Targets in experiments 3, 4, 8 and 12 were 1×1 m. E-targets in experiment 10 were 0.5 m high×1 m wide. E-targets operated simultaneously with an E-net placed at its side (0.5 m wide×1 m high). The landing response is the number of tsetse caught landing on the target expressed as a percentage of the total (landing+circling) catch. Lines on the top of the bars represent the +SE.

In one experiment (experiment 12, Figure 3-2) CO_2 dispensed outside a tent increased significantly (P<0.001) the proportion of female tsetse that landed on the target (48% *vs*. 23%) and had a similar, but not statistically significant effect for males (40% *vs*. 26%). In a second experiment comparing the effects of dispensing CO_2 inside and outside the tent (experiment 13), a similar trend was observed, although in this case the difference was not significant: 43% (±3.5) of females landed when CO_2 was dispensed outside, 34% (±3.8) when it was dispensed inside and 30% (±4.4) for an unbaited target. In accordance with previous results (section 3.3.1), these results suggest that the landing response increased when the concentration of CO_2 was greater (*i.e.*, dispensed near the collecting device, compared to the landing response obtained when CO_2 was dispensed within the tent).

3.3.3. Trap entry responses

In experiment 7 (Figure 3-3), the addition of odour from cattle, human or lizard had no significant effect on trap efficiency (Figure 3-3, experiment 7). Conversely, in experiment 11 (Figure 3-3) lizard odour increased the proportion of males (P<0.05) and females (P<0.001) entering the trap significantly. The variable results with lizard odour may merely reflect differences in the sample sizes, which allowed the detection of relatively small (~10%) increases in trap efficiency. Hence, the total catches of males and females from the lizard-baited trap for experiment 7 were 207 and 192, respectively, compared to 811 and 505 for experiment 11.

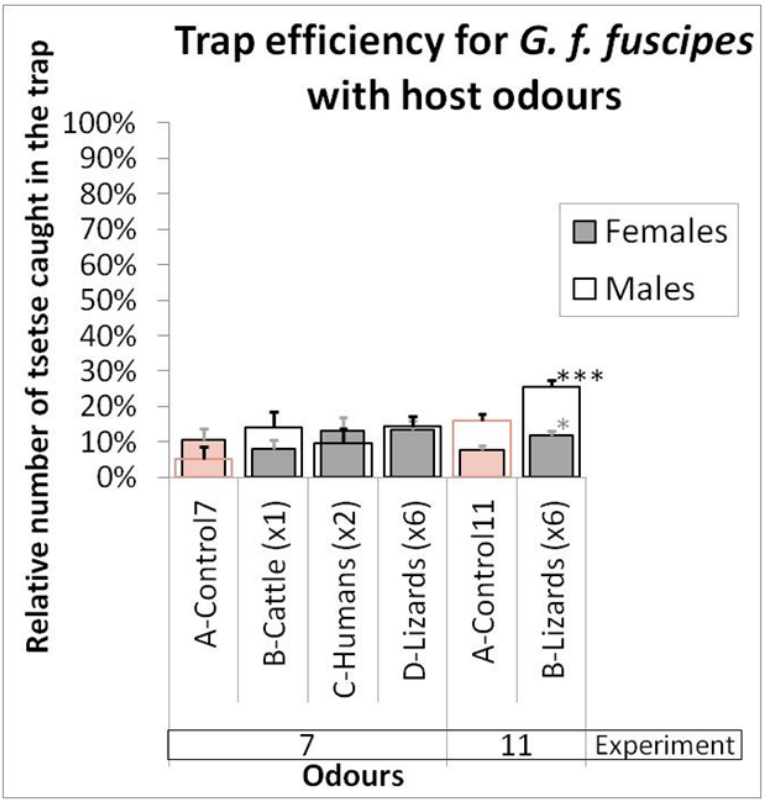


Figure 3-3: Effect of mammalian and lizard odour on trap efficiency for *G. f. fuscipes.* Trap efficiency was defined as the number of the tsetse caught in a biconical trap, expressed as a proportion of the total catch obtained in the trap and flanking E-net together. Lines on the top of the bars represent the +SE.

Analysis of experiments conducted when *S. calcitrans* were sufficiently abundant to allow analysis showed that cattle odour increased the landing response of *S. calcitrans* significantly. For instance, the landing response of *S. calcitrans* on a small E-target baited with cattle (58±3.0%) was significantly greater than that from lizard- (37±7.6%), human- (38±8.8%) or unbaited- (31±7.8%) E-targets. Baiting an E-net with odour from four cattle increased the landing response significantly from 21±9.8% to 55±4.9%.

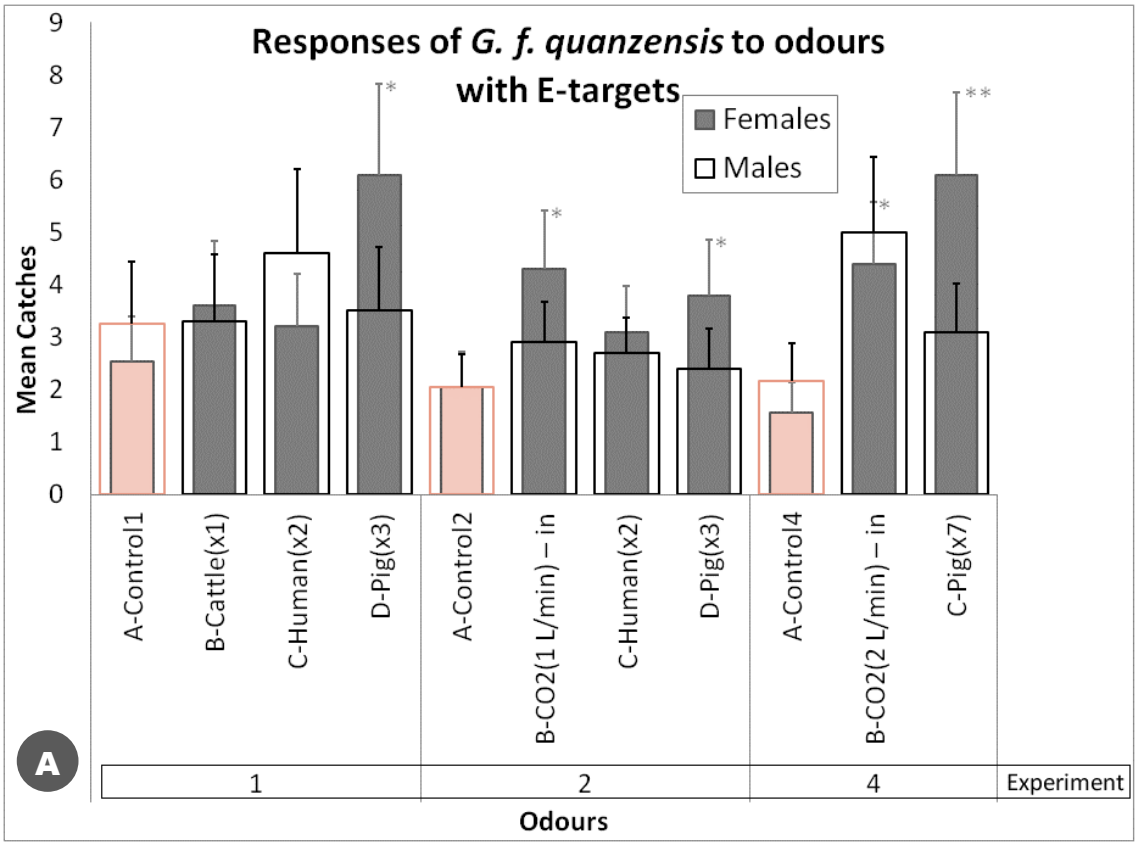
3.4. Responses of *G. f. quanzensis* to host odours

3.4.1. Attraction to odours

The E-targets baited with the odour of three pigs obtained mean catches of 6.1 *G. f. quanzensis*/day (0.85±0.096, *n*=522 in experiment 1 and 0.84±0.204, *n*=413 in experiment 2, both experiments replicated 12 times), significantly higher than the means obtained for the unbaited E-targets of 2.6 tsetse/day in experiment 1 (0.55±0.096) and 3.9 tsetse/day in experiment 2 (0.69±0.204)(Figure 3-4).. Analysis of the pooled data from the 24 days

(experiment 1+experiment 2) showed that pig odour doubled the female catches, from 2.3 (0.51±0.069) per day with the control unbaited target to 4.8 (0.76±0.081) per day with the pig-baited target (P<0.001); no effect was observed for males, with mean daily catches of 2.9 (0.59±0.076) tsetse/trap/day with pig odour *vs*. 2.6 (0.55±0.089) without odour. The odour from seven pigs (experiment 4, n=366, 12 rep.) increased the catch of females 3.9 times greater (P<0.01) from 2.1 tsetse/trap/day (0.46±0.098) in the unbaited E-target to 6.1 tsetse/trap/day (0.85±0.098) in the odour baited E-target, although it did not have any effect on males (experiment 4, Figure 3-4A).

CO_2 dispensed alone at 1-2 L/min within a tent also increased the catch of tsetse, with the increase being greater for females than males (experiments 1 and 4). Therefore, the effect of natural pig odour might be explained, at least in part, by CO_2 produced by the pigs. Accordingly, direct comparisons were made of the numbers of tsetse attracted to a target baited with either the pig odour or an equivalent dose of CO_2. In experiment 2 (Figure 3-4A) three pigs were compared to CO_2 dispensed at 1.4 L/min; in this case, 4.3 females (0.72±0.087) per day were caught with the CO_2-baited target *vs.* 3.8 females (0.68±0.087) per day with the pig odour. In experiment 4 (Figure 3-4A) the target baited with the odour of seven pigs caught 6.1 females (0.85±0.088) per day *vs.* 4.4 female (0.73±0.088) per day caught by the target baited with CO_2 dispensed at 2 L/min. In neither experiment was there a significant difference in the female catch from the pig- and CO_2-baited E-targets but both were significantly greater than that from an unbaited E-target.



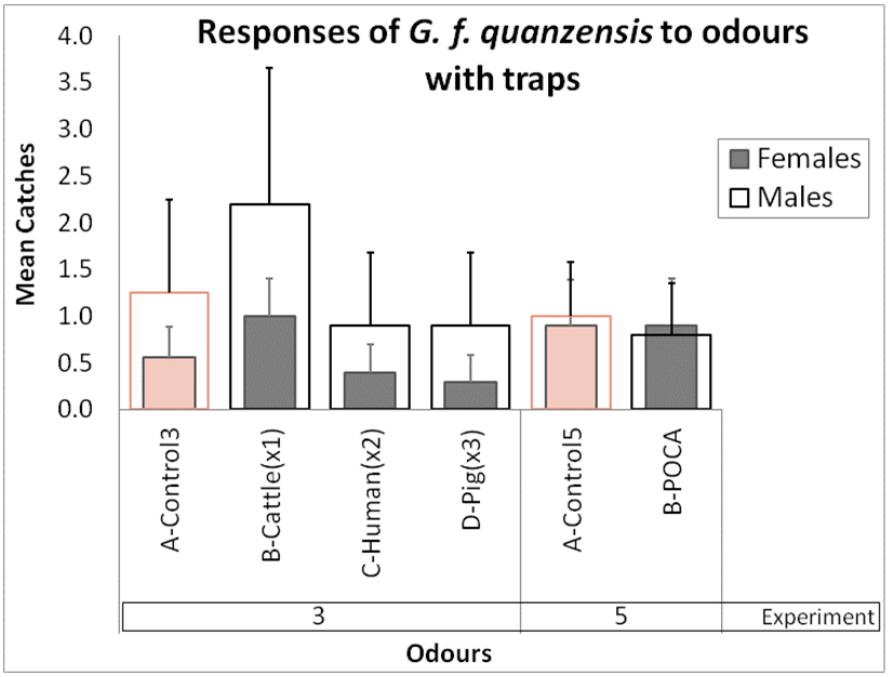


Figure 3-4: Responses of *G. f. quanzensis* to host odours. Detransformed means (catches/day/site) are represented in the ordinate axis +SED. Carbon dioxide was dispensed within the tent, only. Treatments with the same experiment number were incorporated into the same Latin square. Asterisks indicate significant differences compared to the unbaited control treatment (in pink) at P<0.05 (*), P<0.01 (**) or P<0.001 (***). (A) Mean catches of *G. f. quanzensis* caught with E-targets. Experiments were replicated 12 days. (B) Mean catches of *G. f. quanzensis* caught with biconical traps. Experiment 3 was replicated 4 days and experiment 5 was replicated 12 days

Odour from a single ox would produce doses of CO_2 similar to that produced by three pigs but did not have any significant increase in the catches (experiments 1 and 2, Figure 3-4A).

In experiment 3 (Figure 3-4B), baiting a trap with odour from cattle, human or pigs had no significant effect. However, the lack of a significant effect in the pig-baited trap might be due to the low samples size, as the experiment was replicated only 4 days. Therefore, the experiment showed no indication that a pig baited trap would catch more tsetse than the control.

Baiting traps with natural odours or a blend of acetone, octenol and phenols (POCA) had no significant effect on the catch of *G. f. quanzensis* (experiments 3 and 5, Figure 3-4).

3.4.2. Landing responses

The mean daily catches of *G. f. quanzensis* from an E-target in DRC were much smaller than the catches of *G. f. fuscipes* in Kenya. The geometric mean of the total (males+females) daily catches of *G. f. fuscipes* shown in Figure 3-1 is 23 tsetse/day (±0.9), compared to 5 (±0.2) tsetse/day for the catches of *G. f. quanzensis* shown in Figure 3-4. The small daily catches of *G. f. quanzensis* prevented analysis of landing rates from individual experiments. Accordingly, the data from all experiments were pooled and subjected to logistic regression. The results showed that there was no significant effect of host odours on the landing response (Figure 3-5).

However, the landing rate of females was consistently higher in the presence of pig odours. In the three experiments where pig-baited and unbaited E-targets were compared directly, the landing rates with pig odour were 43% (n = 176), 46% (n = 156) and 52% (n = 84) compared to 19% (n = 86), 35% (n =68) and 37% (n = 38), respectively, for an unbaited E-target. By contrast, there was no indication that CO_2 increased the landing rate.

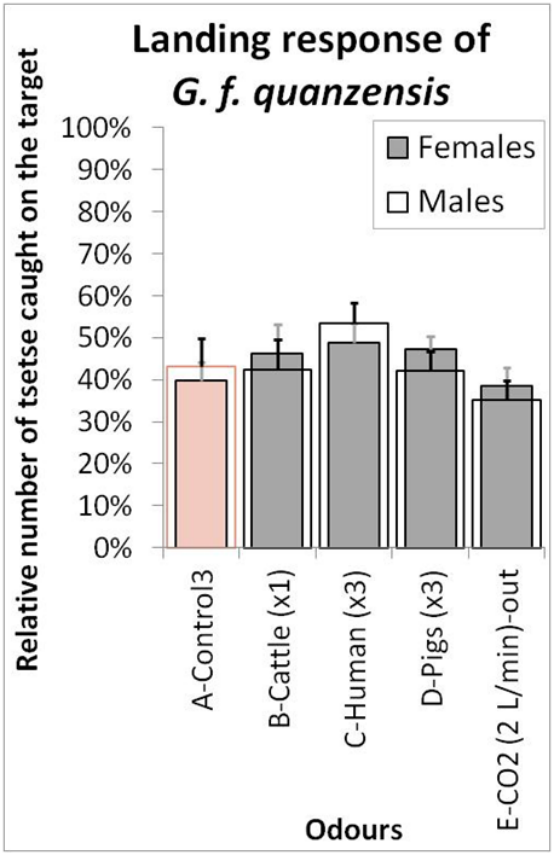


Figure 3-5: Effect of mammalian odour on landing response of *G. f. quanzensis*. E-targets operated simultaneously with an E-net placed at its side (0.5 m wide×1 m high). The landing response is the number of tsetse caught landing on the target expressed as a percentage of the total (landing+circling) catch. Lines on the top of the bars represent the +SE.

3.5. Discussion

This chapter showed that *G. fuscipes* responded to certain odours. For example, lizard odour doubled consistently the catches of *G. f. fuscipes* and the catch of *G. f. quanzensis* was increased slightly, but significantly, by baiting the E-targets with pig odour. Lizard odour also increased the number of *G. f. fuscipes* landing on the target. The effect of lizard odour was not due to the carbon dioxide released naturally in the respiration, as baiting the E-targets with CO_2 in the shores of Lake Victoria did not have any effect in the catches of *G. f. fuscipes*. Conversely, the effect of pig odour in the catch of *G. f. quanzensis* was

indistinguishable to that obtain with CO_2 at similar release rate. These results are discussed above.

3.5.1. Responses of G. fuscipes *to natural and artificial mammalian host odours*

This study showed that the addition of cattle, human or pig odour to different collecting devices did not increase the catches of *G. f. fuscipes*, and the catches of *G. f. quanzensis* were increased only with pig odour. In contrast, tsetse of the Morsitans-group, are highly responsive to cattle odour; for example, Vale (1974e) and Makumi *et al.* (1996) showed that cattle odour increases the trap catches of *G. morsitans*, *G. pallidipes* and *G. longipennis* up to 10 times greater; odours from members of the Suidae family (warthog and bushpig) are also highly attractive for *G. morsitans* and *G. pallidipes* (Vale, 1974e), and human odour seems to contain a mixture of attractants and repellents (Vale & Hargrove, 1979).

The lack of responses of *G. f. fuscipes* to kairomones effective for Morsitans-group tsetse (*i.e.*, acetone, octenol and phenols) have been previously demonstrated (Mwangelwa *et al.*, 1995). However, the present study also showed that these chemicals are also ineffective for *G. f. quanzensis*.

3.5.2. Effect of CO_2

Carbon dioxide is produced naturally by the metabolism of aerobic organisms, and it is considered to be an universal semiochemical for host-seeking haematophagous insects (Kline, 1994). For example, different field studies showed that CO_2 is a strong attractant for Morsitans-group species, notably *G. pallidipes* and *G. morsitans*, doubling the catch of both sexes (Vale, 1974e) and acting in synergy with other kairomones (Torr, 1990). In the present study, baiting targets with physiological doses of CO_2 inside a tent did not have any effect in the catches of *G. f. fuscipes* whereas CO_2 dispensed at the same dose outside the tent doubled the catches of *G. f. fuscipes*. The difference of these responses might be explained by the dilution of the odour with the air of the tent when the CO_2 is dispensed

inside the tent; when the CO_2 is dispensed outside the source concentration is 100% compared to 0.1% when dispensed inside the tent. Dispensing CO2 inside vs. outside does not have a significant effect on the catch of Morsitans-group tsetse (Vale, 1974e; Torr *et al.*, 1995). Zollner (2004) suggested that the diluting effects of atmospheric turbulence on the odour plume as it travels downwind, obscures the differences in source concentration. Why was this not the case in the present study? One possible explanation is the effect of a large body of water, such as the Lake Victoria, in the capacity of tsetse to recognise variations in the CO_2 concentration due to a host. The dispersion of CO_2 along the lakeshores is likely to be influenced by micro-meteorological factors, which in turn could depend on other factors, such as season, vegetation, topography and the time of day. Large bodies of water produce CO_2, and the thermal difference with the shore produces turbulences (Okubo *et al.*, 2002; Tremblay *et al.*, 2005). The second effect might have some particular importance, making it difficult for tsetse to detect the increase of CO_2 produced by the host above the CO_2 in the background. In Kenya, only one experiment was undertaken away from the lake, in Teso (experiment 4, Figure 3-1A). Although it was not conclusive, it was the only experiment where a mammalian host odour, pig-odour, increased significantly the catch of male *G. f. fuscipes*. Unfortunately, logistic problems to transport a cylinder to Teso prevented testing in that habitat the responses to synthetic CO_2 as in experiment 13 (Figure 3-1B). To support this hypothesis, in the experiments undertaken near the shores of the lake, *Stomoxys*, – which is considered to be highly responsive to CO_2 (Warnes & Finlayson, 1985; Alzogaray & Carlson, 2000) – responded to cattle odour, but not to pig or human odour with similar concentration of CO_2. Thus for this population of *S. calcitrans*, the olfactory response to cattle odour seems to be elicited by kairomone(s) other than CO_2, whereas studies conducted elsewhere suggest that carbon dioxide is the major kairomone produced by cattle that attracts *Stomoxys* (Vale, 1980a; Vale & Hall, 1985; Torr *et al.*, 2006).

In the experiments carried out in Lukaya (DRC) in 2007 and 2008 during the same period of the year, CO_2 doubled the catches of female *G. f. quanzensis*, but did not have any effect on males. In contrast, *G. f. quanzensis* did not respond to cattle and human odour, both of which contained CO_2 at similar concentrations as that released from the cylinder. Considering natural cattle and human natural odours contain carbon dioxide, the apparent lack of response from tsetse to human- and cattle-odour suggests that within the blend of cattle- and human-odour there are chemicals that act as repellents for *G. f. quanzensis*. (Vale & Hargrove, 1979).

The inconsistency in the responses of *G. f. fuscipes* and *G. f. quanzensis* to CO_2 are in agreement with Mohamed-Ahmed & Mihok (1999). They baited traps with CO_2 placed nearby, as 'outside the tent' in our case. In one experiment, they found that CO_2 dispensed at 5 L/min had no significant effect, whereas in a second experiment, with the carbon dioxide dispensed at a lower dose of 2.5 L/min, the catch of females was doubled, with no effect on males.

3.5.3. Responses of G. fuscipes *to lizard odour*

In agreement with previous studies (Gouteux *et al.*, 1995; Mohamed-Ahmed, 1998), *G. f. fuscipes* responded consistently to lizard odour. Mohamed-Ahmed (1998) also found that urine doubled the catch of female *G. f. fuscipes* in a electrocuting cylinder and increased by 1.5 times the catch of male tsetse in a trap. In this study, however, fresh or fermented urine did not have any significant effect on the catches. This was not surprising, considering that Mohamed-Amed's (1998) results were marginal: the increase in the catches with the electrocuting cylinder were not significant for either males or females analysed separately, and the increase with traps was only significant for males.

Carbon dioxide is considered a universal semiochemical for host-seeking haematophagous insects (Kline, 1994) and therefore, the responses of tsetse to host odour might be due, at least in part, to the CO_2 released naturally in the respiration of the host. However, in the experiments in Kenya, the CO_2 contained in lizard odour could not explain the responses of *G. f. fuscipes* to lizard odour. The biomass of lizard in the tent was about 20% of the mammalian hosts and they increased the concentration of CO_2 to about 0.2 L/min, only. Conversely, artificial CO_2 was released at 1-2 L/min, *i.e.*, ten times more than the dose produced by lizards. However, CO_2 released by the cylinder did not enhance the catches.

3.5.4. Responses of tsetse to host odours: G. fuscipes *vs. Morsitans-group*

Most of the experiments undertaken in this study were originally designed for tsetse of the Morsitans-group. For Morsitans-tsetse species, mammalian host odours (*e.g.* cattle-odour)

produced a 10-fold increase in the catches of *G. morsitans* and *G. pallidipes* (Vale, 1974d; Vale *et al.*, 1986a), whereas the best attractants used in this study only doubled the catches of *G. fuscipes*. Although according to the results host odours enhanced the catch of *G. fuscipes*, apparently *G. fuscipes* do not respond to odours the way that Morsitans-tsetse do. Differences could be genetic, as an adaptation to the environment, or determined by the large and relatively clear savannah habitats where Morsitans-species live, or by the restricted and bushy riverine habitats where Palpalis-tsetse are found. Odour plumes in the savannah habitat can be detected by tsetse up to 100 m (Zollner *et al.*, 2004), whereas it is disrupted by the vegetation and changes in wind direction much sooner in the bushy riverine habitats.

With the available data it is difficult to explain the difference in the responses of the two groups of *Glossina*. It is possible that *G. fuscipes* use host odours differently to savannah-tsetse and in accordance with the habitat where they live. For example, during the experiments in Kenya, we frequently observed tsetse resting on the ground near the host for extended periods of about 30 minutes, behaviour that has not been described for Morsitans-species (Gibson & Torr, 1999). Differences in the response of Morsitans- and Palpalis-tsetse species to host odours in relation with the habitat are discussed in 7.4.1.

It was intriguing the absence of response of *G. f. fuscipes* to CO_2 in Kenya, despite being considered a universal semiochemical for host-seeking haematophagous insects (Kline, 1994). Atmospheric CO_2 at the field sites in Kenya might be affected by Lake Victoria, by affecting the concentration CO_2 in the background and its variability. High variability in the concentration of background CO_2 might make it difficult for tsetse to detect the CO_2 released by a host. High-resolution measurements of carbon dioxide (Zollner *et al.*, 2004) would be required to test this hypothesis.

CHAPTER FOUR

OLFACTORY RESPONSES OF *G. PALPALIS* AND *G. TACHINOIDES*

4.1. Introduction

The use of artificial baits to reduce HAT transmission in West Africa was initiated during the second half of the 1970s with traps (Laveissière *et al.*, 1980), followed by the use of insecticide-impregnated targets early in the 1980s (Laveissière & Couret, 1981); both operations in Côte d'Ivoire. The authors showed that traps and/or insecticide-treated targets could be used to control populations of *G. palpalis*. In contrast with campaigns in eastern and southern Africa against tsetse of the Morsitans-group (Vale & Torr, 2004), the use of artificial baits in West Africa to control tsetse of the Palpalis-group lacked any attractant to increase the performance of the killing devices (Laveissière & Penchenier, 2000).

To assess host-orientated responses of *G. palpalis* and *G. tachinoides*, two series of studies were undertaken in West Africa: (i) to elucidate feeding preferences of these tsetse species; and (ii) to assess olfactory responses of *G. palpalis* and *G. tachinoides*, in order to explore the viability of using odour-baited artificial baits in West Africa.

4.1.1. Feeding preferences of G. palpalis *and* G. tachinoides

G. palpalis subspp, and particularly *G. p. palpalis*, show a remarkable capability to adapt their diet to different microhabitats, depending on host availability (Späth, 2000; Solano *et al.*, 2010). Thus, in natural habitats, *G. p. palpalis* feed largely on wild animals – *e.g.* bushbuck (*Tragelaphus scriptus*), warthog (*Phacochoerus africanus*) and monitor lizard (*Varanus niloticus*) – whereas in peri-urban areas domestic pigs (*Sus scrofa*) are the main host (Späth, 2000; Simo *et al.*, 2007).

Unlike savannah tsetse, *G. palpalis* subspp feed regularly on humans when they are available (Clausen *et al.*, 1998; Simo *et al.*, 2007). Depending on the availability of humans relative to other hosts, the location of the settlement and human activities, human bloodmeal rates in *G. p. palpalis* vary from 7% in degraded forest (Späth, 2000), to about 60% in dense forest (Njiokou *et al.*, 2004; Simo *et al.*, 2007). The proportion of human bloodmeals in *G. p. gambiensis* although still important, *i.e.* about 1%, are significantly lower (Späth, 2000).

The ability of *G. palpalis* subspp to live near human settlements (Courtin *et al.*, 2005), coupled with the fact that they feed on humans, makes this tsetse species an efficient vector of HAT.

G. tachinoides, although a member of the Palpalis-group, their host-range patterns are similar to that of the Mortitans group. For example, like *G. morsitans*, *G. tachinoindes* feeds frequently on wild mammals, *e.g.* bushbuck and hippopotamus (*Hippopotamus amphibious*), and less often on livestock. Studies of the feeding patterns of *G. tachinoides* report between 16% and 21% of bloodmeals being taken from bushbuck, and between 34% and 48% from hippopotamus, depending on host availability (Küpper *et al.*, 1990; Clausen *et al.*, 1998).

4.1.2. Host-orientated behaviour of G. palpalis *and* G. tachinoides

Olfactory responses of G. tachinoides

A summary of the of field olfactory responses of *G. tachinoides* to host odours can be found in Table 4-1.

	Odour source		Country	Device	Catch index		Reference
	Source	Fraction			*G.p.palp.*	*G. tachi.*	
Natural odours	Cow	Whole	Burkina Faso	ET		1.2	(Mérot *et al*., 1986)
			Burkina Faso	B		1.8⊕	(Filledier *et al*., 1988)
		Urine	Burkina Faso	B		3.3⊕	(Filledier *et al*., 1988)
			Burkina Faso	B		n/s	(Filledier & Mérot, 1989)
	Bushbuck	Skin wash	Burkina Faso	B		n/s	(Späth, 1997)
	Warthog	Skin wash	Côte d'Ivoire	B		1.5	(Späth, 1997)
	Pig	Whole	Burkina Faso	ET		1.2	(Mérot et al., 1986)
		Skin wash	Côte d'Ivoire	B		n/s	(Späth, 1997)
	Lizard	Whole	Côte d'Ivoire	B		n/s	(Späth, 1997)
		Skin wash	Côte d'Ivoire	B		1.3	(Späth, 1997)
Synthetic odours	CO_2		Burkina Faso	B		1.2	(Mérot et al., 1986; Galley *et al*., 1986)
	Phenolic fraction		Burkina Faso	B		1.4	(Mérot *et al*., 1988; Späth, 1995, 1997)
			Côte d'Ivoire	B		1.8	(Küpper *et al*., 1991; Späth, 1995, 1997)
			Liberia	B	n/s		(Cheke & Garms, 1988)
	Acetone		Burkina Faso	B		1.2	(Späth, 1995)
			Côte d'Ivoire	B		1.2	(Küpper *et al*., 1991; Späth, 1995)
			Liberia	B	2		(Cheke & Garms, 1988)
	Octenol		Burkina Faso	B		1.3	(Späth, 1995)
			Côte d'Ivoire	B		1.3	(Küpper *et al*., 1991; Späth, 1995)
			Liberia	B	2		(Cheke & Garms, 1988)

Table 4-1: Catch index for *G. p. palpalis* and *G. tachinoides* responding to natural and synthetic attractants. Catch index is the catch of a trap baited with the attractant expressed as a proportion of an unbaited trap ($p<0.05$); n/s = no significant increase in catch. ⊕ No *P* provided by the reference. Devices: B: biconical trap; ET: E-target

Den Otter (1991) reported strong electrophysiological responses to 3-ethylphenol and 3-methylphenol, and moderate responses to 4-methylphenol. These phenolic compounds were extracted from buffalo (*Syncerus caffer*) urine.

Mérot *et al.* (1986) compared the numbers of *G. tachinoides* attracted to odour from a human (*Homo sapiens*, *c*. 60 kg), a pig (*Sus scrofa*, *c*. 60 kg) or a cow (*Bos primigenius*, *c*.

150 kg). They found small but significant increases in catch of 1.1 times when the electric grid was baited with human odour, and 1.2 times with either cow or pig odour, compared with the catch of an unbaited grid. When the grid was baited with the odour of four cows, the catch increased 1.8-fold (Mérot *et al.*, 1986) (Table 4-1).

Filledier et *al*. (1988) compared the numbers of *G. tachinoides* attracted to two different breeds of cattle: one trypanotolerant, Baoulé, and one trypanosensitive, Zebu. No compelling evidence was obtained to prove a correlation between the breed and the number of *G. tachinoides* caught.

Mérot *et al*. (1986) and Galey *et al.* (1986) observed that collecting devices baited with CO_2, at release rates <3 L/min caught 1.2 times more flies than unbaited devices, increasing up to 3.3-fold when the release rate was 20 L/min. These experiments demonstrated that the responses of *G. tachinoides* to host odours are due, at least in part, to CO_2 contained in the breath (Table 4-1). Activated charcoal filters were used subsequently to intercept chemicals contained in cattle odour but not CO_2 (Mérot *et al.*, 1986). Traps baited with filtered odour caught significantly fewer tsetse than traps baited with unfiltered odour, suggesting the presence of semichemicals in cattle odour, other than CO_2 (Table 4-1).

The role of semiochemicals, other than CO_2, was farther investigated, by studying the responses of *G. tachinoides* to different factions of host odour. For example, traps baited with skin washings, obtained from monitor lizard and warthog, increased significantly the catch 1.3-fold and 1.5-fold respectively, compared with unbaited traps (Späth, 1997). However, the effect vanished when the odours were released at high doses. Küpper *et al.* (1991) observed similar effects using synthetic baits, and suggested that some of the molecules contained in host odours, *i.e.* octenol and acetone, are attractants at physiological doses but repellents at higher release rates. This assumption is consistent with the variability in the results for octenol and acetone, which in some experiments increased the catch (Küpper *et al.*, 1991; Späth, 1995), and had no effect or decreased the catch of *G. tachioides* in others (Mérot *et al.*, 1988; Späth, 1995) (Table 4-1).

Conversely, baiting traps with the phenolic fraction of cattle urine increased consistently the catch from 1.4- to 1.8-fold, compared with unbaited traps (Mérot *et al.*, 1988; Küpper *et al.*, 1991; Späth, 1995, 1997) (Table 4-1). Within the phenolic fraction, 3- and 4-

methylphenol where the two compounds that produced the highest response (Filledier & Mérot, 1989; Küpper *et al.*, 1991; Späth, 1995) (Table 4-1). Some authors suggested that octenol acts synergistically with the phenolic compounds, reinforcing the response of *G. tachinoides* (Mérot *et al.*, 1988; Späth, 1995).

Olfactory responses of G. palpalis

Despite the importance of *G. palpalis* as vectors of HAT in central and western Africa (Sané *et al.*, 2000; Melachio *et al.*, 2011), hitherto, only one small field trial was undertaken to assess the response, in this case, of *G. p. palpalis* to host odours (Cheke & Garms, 1988). Cheke and Garms baited biconical traps with different synthetic chemicals known to enhance the catch of *G. pallidipes* and *G. morsitans*: *i.e.* acetone, octenol and a blend of various phenolic compounds – *i.e.* 4-methylphenol, 3-methylphenol, 3-*n*-ethylphenol, 3- and 4-propylphenol and 2-methoxyphenol (Table 4-1). The authors found that traps baited with acetone or octenol, caught twice as many flies as unbaited traps, although no significant effect was observed when both chemicals were used simultaneously. No significant effect was reported for the phenolic blend.

4.1.3. Aim of the study

Hitherto, there are no comprehensive studies of the olfactory responses of the main HAT vectors in West Africa, *G. palpalis*. The present work aimed to address this gap by undertaking field studies of the behavioural responses of *G. p. palpalis* in Côte d'Ivoire, and *G. p. gambiensis* in Burkina Faso to natural and synthetic olfactory cues are reported. *G. p. gambiensis* and *G. tachinoides* occur sympatrically on the southern Comoe River, and particularly in our field sites in Folonzo; therefore, and despite the secondary role as vector of sleeping sickness of the latter (Brunhes *et al.*, 1994), results for *G. tachinoides* are also described in this chapter.

Collecting devices consisting of arrangements of electrocuting devices and biconical traps were baited, either with natural – *i.e.* cattle, pig or human – or artificial odours – *i.e.* CO_2, POCA, etc – concealed PVC-coated tents (Vale, 1974d), or directly following the methods

described in Torr *et al.* (1995). The effects of odours on the specific behavioural responses, *i.e.* long-range attraction, landing, and trap entry, were quantified.

Experiments of responses of *G. tachinoides* and *G. p. gambiensis* to natural host odours in Burkina Faso were carried out in collaboration with Drs Johan Esterhuizen and Jean-Baptiste Rayaisse. Experiments with synthetic cattle odour were undertaken by Drs Johan Esterhuizen and Jean-Baptiste Rayaisse. However, I conducted the statistical analysis to complete the study. The majority of the results reported in this chapter were published in Rayaisse *et al.* (2010) (see Annex II)

4.2. Materials and methods

4.2.1. Study sites

Studies in Burkina Faso were carried out during the dry season, between March to June 2007 and January to May 2008. Some of the studies were conducted in southern Comoe River, where *G. tachinoides* and *G. p. gambiensis* are sympatric (see section 2.1.1). Complementary studies took place at Solenzo, where *G. p. gambiensis* is predominant (see 2.1.1).

G. p. palpalis was studied in Côte d'Ivoire near Bingerville town between February and April 2008, and between December 2008 and March 2009 at Azaguié (see section 2.1.2).

4.2.2. Natural host odours

As in chapter 3, human volunteers, cattle and pigs provided the natural host odour for the experiments. Hosts were concealed in PVC-coated tents, from which the air from the tent containing the host odour was exhausted 12-15 m from the tent, where the collecting devices where placed (see section 2.2).

4.2.3. Synthetic odours

As described in section 2.3, acetone, octenol, 4-methylphenol (a.k.a. *p*-cresol) and 3-*n*-propylphenol (POCA blend, 'P' standing for 3-*n*-propylphenol, 'O' for octenol, 'C' for *p*-cresol, and 'A' for acetone) were dispensed individually or in various combinations from sealed polyethylene sachets of 50 cm^2 surface area per side and 150 μm thick. These chemicals, have been identified as effective attractants for Morsitans-group tsetse (Vale & Hall, 1985; Bursell *et al.*, 1988; Torr *et al.*, 1995; Torr & Mangwiro, 1996). In some experiments, synthetic odours were dispensed inside the tent. Conversely, some other experiments did not require the use of the tent and synthetic odours were dispensed directly underneath a trap.

Synthetic CO_2 was released from pressurised cylinders at 1-2 L/min either inside the tent or near the collecting device, as explained in section 3.2.3. When all the synthetic odours (*i.e.* POCA blend plus CO_2 released at 1 L/min) were dispensed together, the odour was called 'synthetic cattle' (Torr *et al.*, 1995; Torr *et al.*, 2006).

4.2.4. Collecting devices

Arrangements of electric grids (E-grids) were used to assess responses of tsetse to visual and olfactory cues (Vale, 1974d), as described in section 2.4.1. All the traps used in the experiment were the biconical model designed by Challier and Laveissière (1973) (see section 2.4.3.).

4.2.5. Attraction, landing and trap efficiency

Attraction

The numbers of tsetse attracted to the odours of different hosts were assessed with E-nets (0.5 m wide × 1.0 m high), placed downwind of the source. Visual stimulus was provided by a black E-target (1.0 × 1.0 m), placed adjacent to the E-net (section 2.5).

Landing response

The catch obtained on the E-target (*t*), expressed as a proportion of the total catch (E-net + E-target, N), provided an index of the strength of the landing response (Landing response = t/N).

Trap efficiency

The effect of host odours on trap-orientated responses was assessed by dispensing the odours at the base of the traps. Trap efficiency was defined as the number of tsetse that entered a trap, expressed as a proportion of the total number of tsetse that were attracted to the same trap (Vale & Hargrove, 1979). The effect of odours on the efficiency of the trap was estimated by setting an E-net (0.5 m width × 1.0 m height) adjacent to the trap. The total catch (E-net + trap) provided a measure of the numbers of tsetse attracted to the trap with or without host odours, and the catch from the trap, expressed as a proportion of the total catch, provided an index of trap efficiency.

4.2.6. Air entrainments

Glass tubing (∅5 mm), containing a porous polymer (Porapak Q 50/80 (50mg), Supelco, Bellefonte, USA) was used to collect samples of host odours and controls (chapters 4 & 5). Porapak™ filters hung in the middle of the tents, above the host, and were connected to a pump placed in the exterior. A sample of the air was passed through the filters at a rate of 1 L/min for four hours. After collection, samples were stored in sealed glass tubes, and sent to Rothamsted Research (UK) for chemical characterization by gas chromatography (GC) and mass spectrometry (MS).

4.2.7. Experimental design

Experiments were carried out for 4 h, between 08:00 h and 12:00 h in Burkina Faso, and between 10:00 and 14:00 h in Côte d'Ivoire, when *G. p. gambiensis* and *G. tachinoides* (Challier, 1976; Filledier *et al.*, 1988) and *G. p. palpalis* (Crump & Brady, 1979) are more active. Responses to odours were compared over 6-12 days in a series of replicated Latin

squares of days × sites × treatments, as explained in chapter two (section 2.7). Experimental sites were at least 100 m apart. Experimental setups with tents for *G. p. palpalis*, *G. p. gambiensis* and *G. tachinoides* are summarised in tables 4-2, 4-3 and 4-4 respectively. Experimental setups when the odours were dispensed directly underneath a trap (*i.e.* no tent required in these experiments) are summarised in tables 4-5 and 4-6. All the experiments included an unbaited treatment (*i.e.* no odour) as control.

Exp. number	Treat.	Location	Rep.	Collec. device
1	No odour	Bingerville	8	E-target
	Human			
2	No odour	Bingerville	8	E-target
	Pig			
3	No odour	Bingerville	8	E-target
	Cattle			
4	No odour	Bingerville	8	E-target
	CO_2-in (1L/min)			
5	No odour	Azaguié	12	E-target
	Cattle			
	Human			
	Pig			
6	No odour	Azaguié	12	E-target
	Human			
	Pig			
	CO_2-in (2L/min)			
7	No odour	Azaguié	12	E-target
	CO_2-in (2L/min)			
	CO_2-out (2L/min)			
8	No odour	Bingerville	8	Trap + E-net
	Pig			
9	No odour	Bingerville	8	Trap + E-net
	Human			

Table 4-2: Experimental setups to explore olfactory responses of *G. p. palpalis*. Except for the treatment 'CO_2-out', baits were placed inside a PVC-coated tent, and odours exhausted 12-15 m to the fly collecting device

Exp. number	Treat.	Location	Rep.	Collec. device
1	No odour	Folonzo	8	E-target
	Cattle			
	Human			
	Pig			
2	No odour	Folonzo	8	E-target
	Cattle			
	Pig			
	POCA			
3	No odour	Folonzo	9	E-target
	Cattle			
	CO_2-in (1L/min)			
4	No odour	Solenzo	10	E-target
	Cattle			
	Human			
	Pig			
5	No odour	Solenzo	8	E-target
	Cattle			
	Human			
	Pig			
8	No odour	Folonzo	10	Trap
	Cattle			
9	No odour	Folonzo	10	Trap
	Human			
11	No odour	Folonzo	10	Trap + E-net
	POCA			
12	No odour	Solenzo	8	Trap + E-net
	Cattle			
	Human			
	Pig			

Table 4-3: Experimental setups to explore olfactory responses of *G. p. gambiensis*. Except for the treatment 'CO_2-out', baits were placed inside a PVC-coated tent, and odours exhausted 12-15 m to the fly collecting device

Exp. number	Treat.	Location	Rep.	Collec. device
1	No odour	Folonzo	8	E-target
	Cattle			
	Human			
	Pig			
3	No odour	Folonzo	9	E-target
	Cattle			
	CO_2-in (1L/min)			
6	No odour	Folonzo	10	E-target
	Cattle			
7	No odour	Folonzo	8	E-target
	Cattle			
	Pig			
	Synthetic cattle			
8	No odour	Folonzo	8	Trap
	Cattle			
9	No odour	Folonzo	12	Trap
	Human			
10	No odour	Folonzo	8	Trap + E-net
	Cattle			
	CO_2-in (1L/min)			
	POCA			

Table 4-4: Experimental setups to explore olfactory responses of *G. tachinoides*. Except for the treatment 'CO_2-out', baits were placed inside a PVC-coated tent, and odours exhausted 12-15 m to the fly collecting device

subspp	Exp. number	Treat.	Location	Rep.
G. p. palpalis	1	No odour	Bingerville	40
		POCp		
		A		
		O		
	2	No odour	Azaguié	36
		POCpA		
		POCp		
		A		
		O		
		PCp		
G. p. gambiensis	7	No odour	Folonzo	8
		POCpA		
	8	No odour	Solenzo	20
		POCpA		
	9	No odour	Folonzo	16
		POCpA		
	10	No odour	Solenzo	12
		Ocp		
		PCp		
		PO		
		POCp		
		POCpA		
	11	No odour	Solenzo	12
		A		
	12	No odour	Solenzo	12
		POCp		
		POCpA		
	13	No odour	Solenzo	12
		Cp		
		O		
		P		
		POCpA		

Table 4-5: Experimental setups to explore responses of *G. palpalis* to synthetic odours. Odours were dispensed underneath the traps (tents were not used in these experiments). The initials of the treatments stand for:

- P: 3-*n*-propylphenol
- O: 1-octen-3-ol
- Cp: 4-methylphenol (*p*-cresol)

spp	Exp. number	Treat.	Location	Rep.
G. tachinoides	1	No odour	Folonzo	12
		POCmCpA		
	2	No odour	Folonzo	12
		POCmA		
		POCpA		
	3	No odour	Folonzo	12
		POCpA		
	4	No odour	Folonzo	3
		POCpA		
		POCp		
	5	No odour	Folonzo	8
		A		
		POCp		
		POCpA		
	6	No odour	Folonzo	12
		A		
		POCp		

Table 4-6: Experimental setups to explore responses of *G. tachinoides* to synthetic odours. Odours where dispensed underneath the traps (tents were not used in these experiments). The initials of the treatments stand for:

- P: 3-*n*-propylphenol
- O: 1-octen-3-ol
- Cm: 3-methylphenol (*m*-cresol)
- Cp: 4-methylphenol (*p*-cresol)

4.2.8. Statistical analyses

Statistical analyses was conducted as described in section 2.7.

4.3. Responses of *G. p. palpalis* to host odours

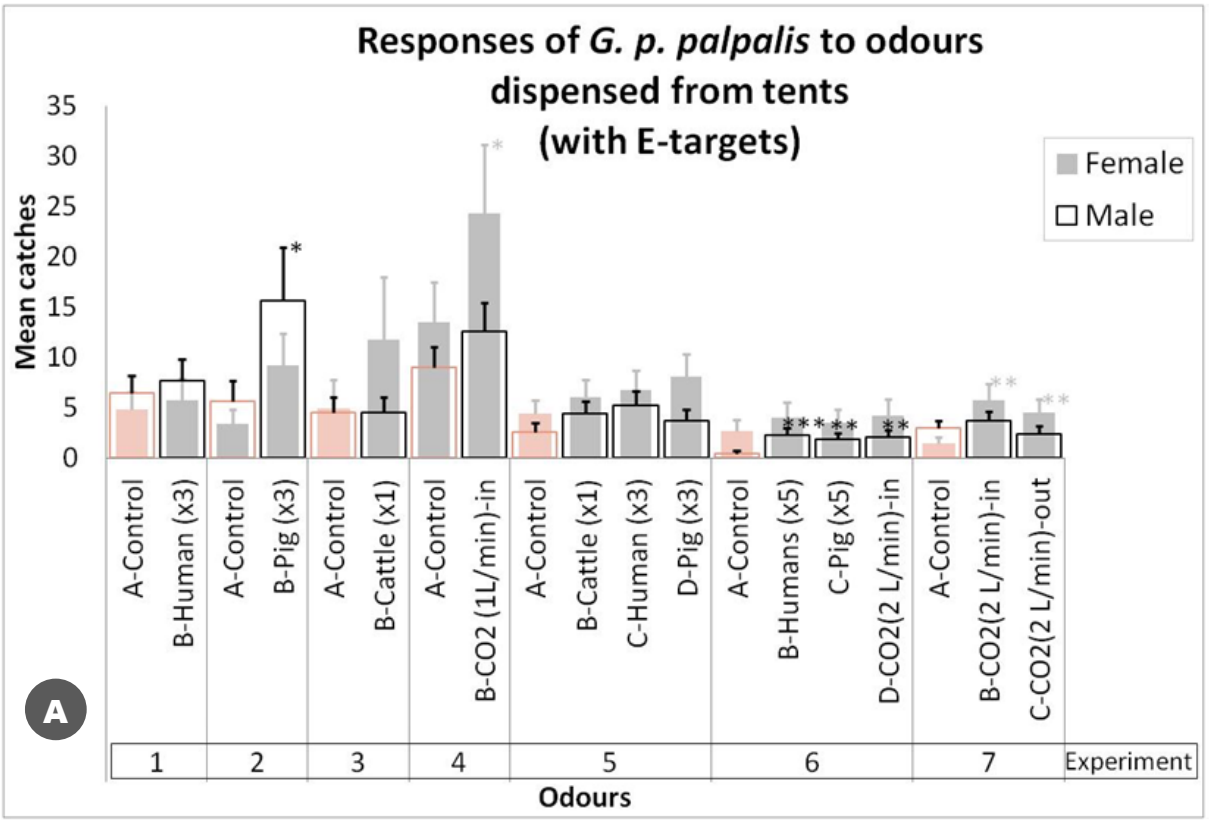
4.3.1. Attraction to odours

Responses to natural odours

The results showed that carbon dioxide, dispensed inside the tent, enhanced the catch of E-targets (Figure 4-1). Increasing the dose of carbon dioxide resulted in an increase of the catch of tsetse. For example, dispensing CO_2 at 1 L/min resulted in about 1.5-fold increase in the catches (1.4-fold for males and 1.8-fold for females, experiment 4, Figure 4-1A); when the CO_2 was dispensed at 2 L/min the increase in the catch was approximately four-fold for both males and females. This increase was observed for males in two experiments (experiments 6 and 7, Figure 4-1A) and only in one experiment for females (experiment 7, Figure 4-1A). The increase in the catch of female was about 1.5-fold and not significant in experiment 6 where, as in experiment 7, CO_2 was released in the tent at 2 L/min (Figure 4-1A). No significant difference was observed when the CO_2 was dispensed directly into the E-target – *i.e.* outside the tent (experiment 7, Figure 4-1A).

Consistent with the above results, increasing the dose of natural pig and human odours resulted in an increased catch of *G. p. palpalis*, although in this case significant differences were obtained only for males. For example, odours from five humans increased significantly the male catch from E-targets five-fold, whereas no significant difference in the catch was observed with the odour from three men. Similarly, male catches increased four-fold when the E-target was baited with the odour from five pigs; when only three pigs were used, the male catches increased in 2.8-fold in one experiment (experiment 2), and the difference was not significant in another one (experiment 5) (Figure 4-1A).

In experiments using a combination of biconical traps and E-nets as collecting devices, no significant differences in the catches were observed for pig or human odour (Figure 4-1B).



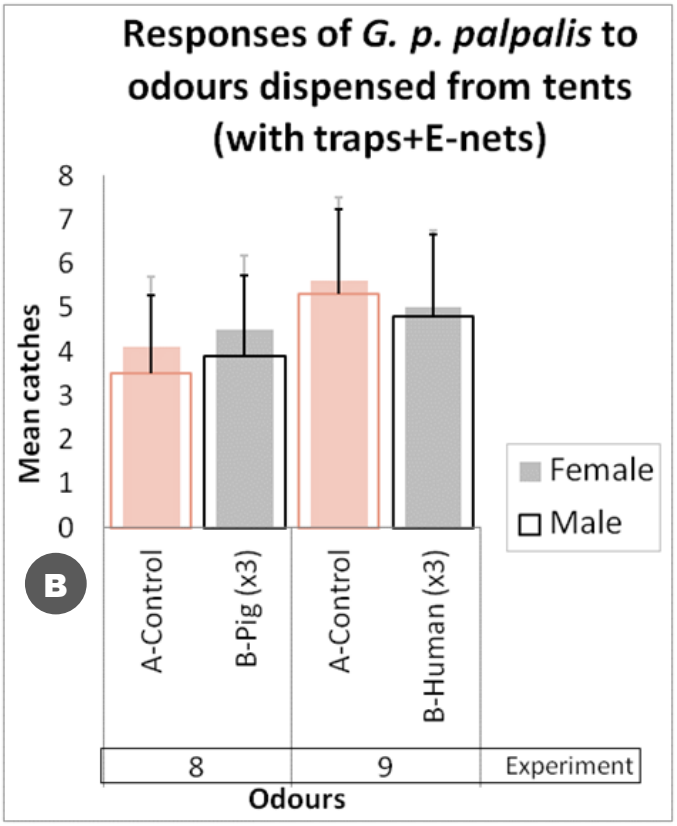


Figure 4-1: Responses of *G. p. palpalis* to host odours. Detransformed means catches/day/site are represented in the ordinate axis +SED.

Carbon dioxide was dispensed within ('in') or outside ('out') the tent. Asterisks indicate significant differences compared to the unbaited control treatment (in pink) at P<0.05 (*), P<0.01 (**) or P<0.001 (***).

Treatments with the same experiment number (Experiment) were incorporated into the same Latin square.

(A) Mean catches of *G p. palpalis* caught with E-targets baited with mammalian natural odours or CO_2. E-targets were 1×1 m. Experiments 1-4 were replicated 8 days and carried out in Bingerville. Experiments 5-7 were replicated 12 days and carried out in Azaguié

(B) Mean catches of *G p. palpalis* obtained with biconical traps +E-nets operating simultaneously. Experiments were carried out in Bingerville and replicated 8 days each

Synthetic odours dispensed directly into biconical traps

Different blends of 3-*n*-propylphenol, octenol, 4-methylphenol and acetone, components of natural cattle odour (Torr *et al.*, 1995; Torr *et al.*, 2006), increased the catches of male *G. p. palpalis*. Although small, the increase (about 1.5-fold) was significant in some cases. Only a blend of 3-*n*-propylphenol, octenol and 4-methylphenol in experiment 1 increased significantly the female catch (about 1.5-fold) (Figure 4-2B).

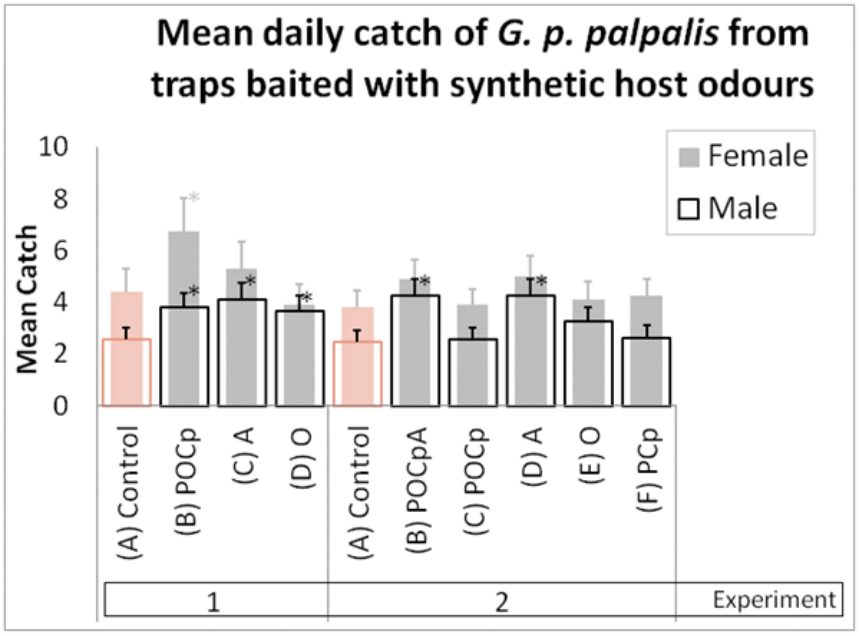


Figure 4-2: Responses of *G. p. palpalis* to synthetic odours. Detransformed means catches/day/site are represented in the ordinate axis +SED.

Asterisks indicate significant differences compared to the unbaited control treatment (in pink) at P<0.05 (*)

Treatments with the same experiment number (Experiment) were incorporated into the same Latin square.

Experiment 1 was undertaken in Bingerville (40 replicates), and experiment 2 in Azaguié (36 replicates).

The initials of the treatments stand for:

- P: 3-*n*-propylphenol
- O: 1-octen-3-ol
- Cp: 4-methylphenol (*p*-cresol)
- A: acetone

4.3.2. Landing response and trap efficiency

Analyses of the effect of odours on landing response was conducted for 12 experiments. No difference in the number of tsetse that landed on the target, as a proportion of the total number of tsetse caught – *i.e.* target+net – was observed for any of the odours tested. Two

examples are shown in Figure 4-3 (experiments 5 and 6); similar results were observed in other experiments.

Similarly, odours did not increase significantly the proportion of tsetse that entered into a trap (experiments 8 and 9, Figure 4-3). The percentage of *G. p. palpalis* caught with a trap ranged between 8 and 27%.

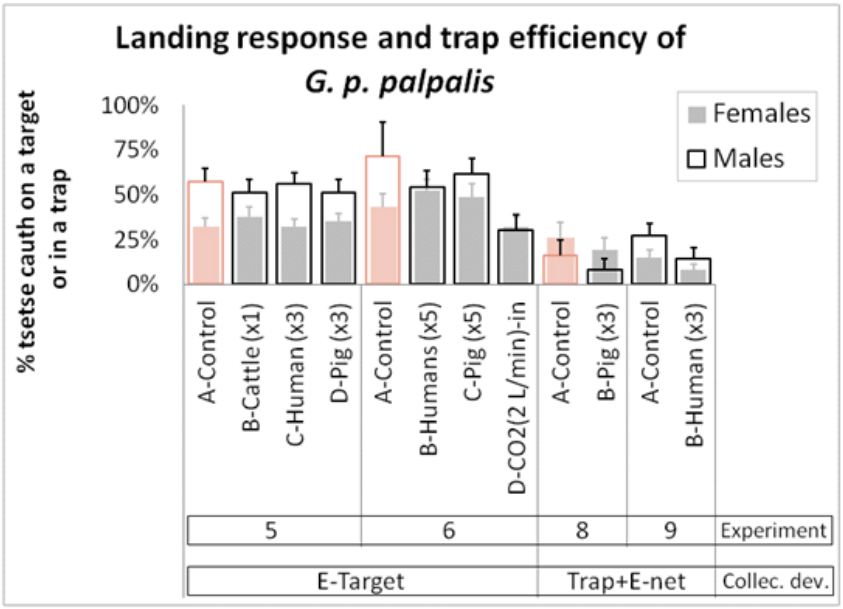


Figure 4-3: Effect of odours on landing response and trap efficiency of *G. p. palpalis*. E-targets (1×1 m, experiments 5 and 6) operated simultaneously with an E-net (0.5 m high×1 m wide) placed at its side. Traps (experiments 8 and 9) operated with an E-net. The landing response is the number of tsetse caught landing on the target expressed as a percentage of the total (landing+circling) catch. Trap efficiency was defined as the number of the tsetse caught in a biconical trap, expressed as a proportion of the total catch (i.e. trap+flanking E-net)

4.4. Responses of *G. p. gambiensis* to host odours

4.4.1. Attraction to odours

Natural and synthetic odours dispensed from tents

While cattle odour consistently increased the female catches of E-targets, the difference was only significant (2.4-fold) in one experiment. Differences for all the other treatments were not significant (experiment 1, Figure 4-4A). Similarly, no significant difference in the catches was observed for any of the treatments when a trap operating with an E-net was used as collecting device (experiments 11 and 12, Figure 4-4B). In contrast, when the trap operated alone, cattle odour increased the catches of males and females 2.8-fold and 6.2-fold respectively, and human odour increased the male catches 4.4-fold and 2.2-fold the female catches (experiments 8 and 9, Figure 4-4B).

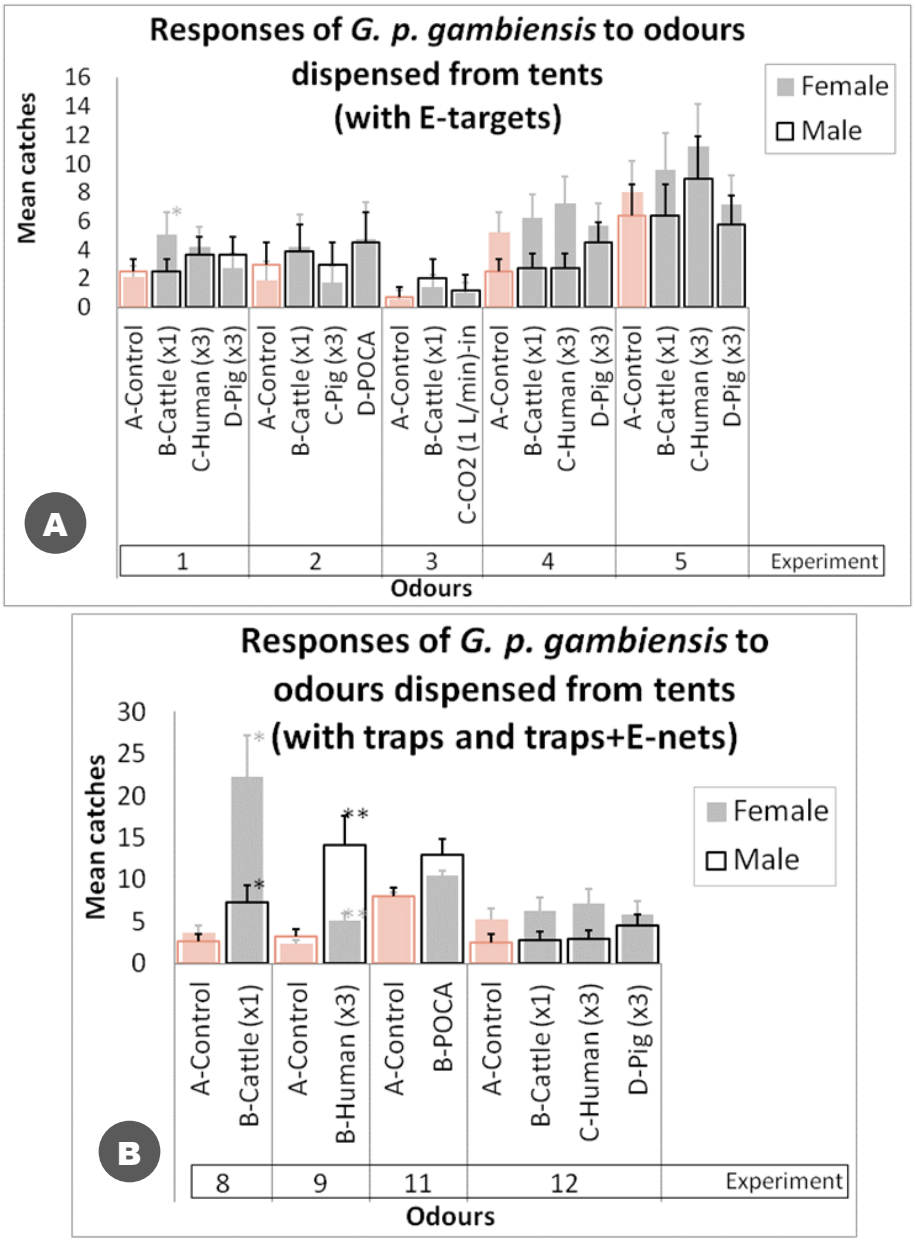


Figure 4-4: Responses of *G. p. gambiensis* to natural and synthetic odours. Detransformed means catches/day/site are represented in the ordinate axis +SED. Carbon dioxide was dispensed within the tent. Asterisks indicate significant differences compared to the unbaited control treatment (in pink) at P<0.05 (*), and P<0.01 (**). Treatments with the same experiment number (Experiment) were incorporated into the same Latin square.

(A) Mean catches of *G p. gambiensis* caught with E-targets. Experiments 1 (8 rep.), 2 (8 rep.) and 3 (9 rep.) were undertaken in Folonzo, and experiments 4 (10 rep.) and 5 (8 rep.) were undertaken in Solenzo

(B) Mean catches of *G p. gambiensis* obtained with biconical traps +E-nets operating simultaneously. In Folonzo, traps alone were used in experiments 8 (10 rep.) and 9 (10 rep.), and traps+E-nets in experiment 11 (10 rep.). In Solenzo , traps+E-nets were used in experiment 12 (8 rep.)

Synthetic odours dispensed directly into biconical traps

Contrary to the results obtained when odours were dispensed from a tent (Figure 4-4), POCA enhanced the catches of *G. p. gambiensis* when the odours were dispensed adjacent to a trap (Figure 4-5). Combining all the data in a pooled analysis (78 replicates) showed that the catch increased significantly in 2.2-fold for males, from 2.3-fold (transformed mean 0.51±0.050 SE) males/day to 5.1 (0.78±0.050) males/day, and by 1.8-fold for females, from 3.7-fold females/day (0.67±0.063) without odour to 6.1-fold (0.85±0.063) females/day with POCA. The same blend without acetone, *i.e.* POC, increased the male catch 3.4-fold and the female catch 2.2-fold in experiment 12, but had no significant effect in experiment 10 (Figure 4-5).

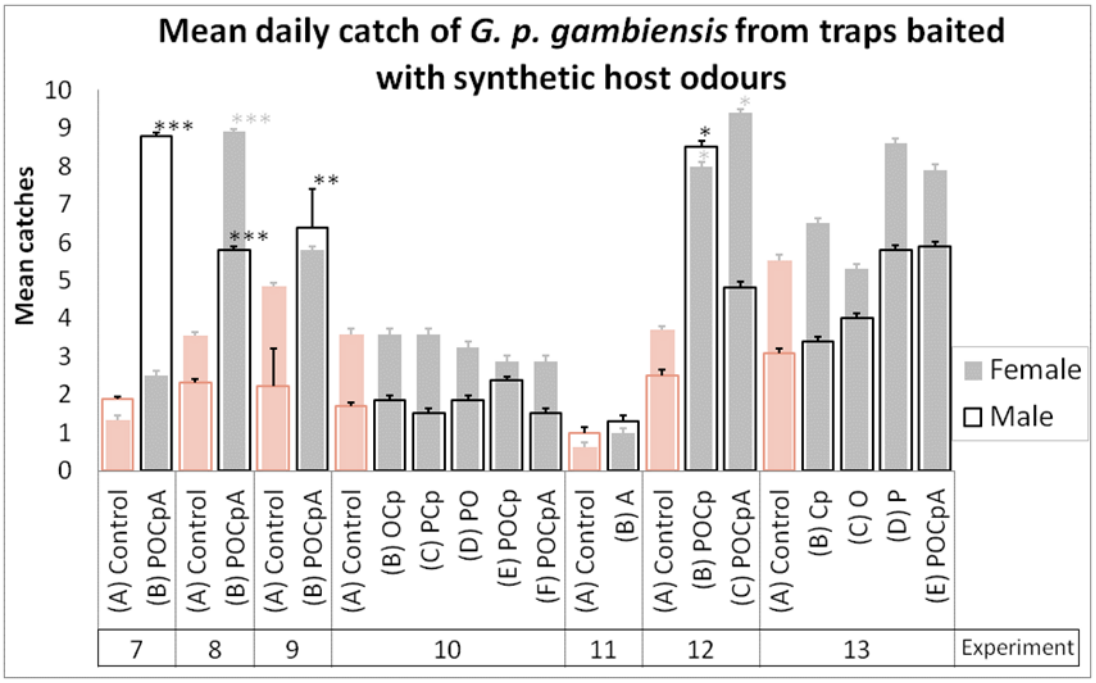


Figure 4-5: Catches of *G. p. gambiensis* obtained with traps baited with synthetic odours. Detransformed means catches/day/site are represented in the ordinate axis +SED.

Asterisks indicate significant differences compared to the unbaited control treatment (in pink) at P<0.05 (*), P<0.01 (**) and P<0.001 (***). Treatments with the same experiment number (Experiment) were incorporated into the same Latin square.

Experiments 7 (8 replicates) 9 (16 replicates) were undertaken in Folonzo, and experiments 8 (20 replicates), 10 (12 replicates), 11 (12 replicates), 12 (12 replicates) and 13 (10 replicates) were in Solenzo

The initials of the treatments stand for:

- P: 3-*n*-propylphenol
- O: 1-octen-3-ol
- Cp: 4-methylphenol (*p*-cresol)
- A: acetone

4.4.2. Landing response and trap efficiency

None of the odours in 14 experiments analysed had any significant effect on the landing response of *G. p. gambiensis*. Two examples are shown in Figure 4-6 (experiments 1 and 7). Similar results were obtained for the trap efficiency (experiments 11 and 12, Figure 4-6).

The absence of significant difference obtained with traps+E-targets, as opposed to traps alone, suggest an experimental artefact: the E-net may have killed circling flies that would eventually enter the trap (Figure 4-4B). Accordingly, in Folonzo we also assessed trap efficiency for *G. p. gambiensis* using the alternative protocol of comparing catches from traps with or without a flanking E-net in the presence or absence of cattle odour (10 replicates). The result showed that host odour had no significant effect, but placing an E-net adjacent to a trap increased the detransformed mean daily catch of both sexes significantly from 2 males and 4 females to 10 males and 13 females. Thus, the catch from the trap alone was 20-25% of that from the trap+E-net

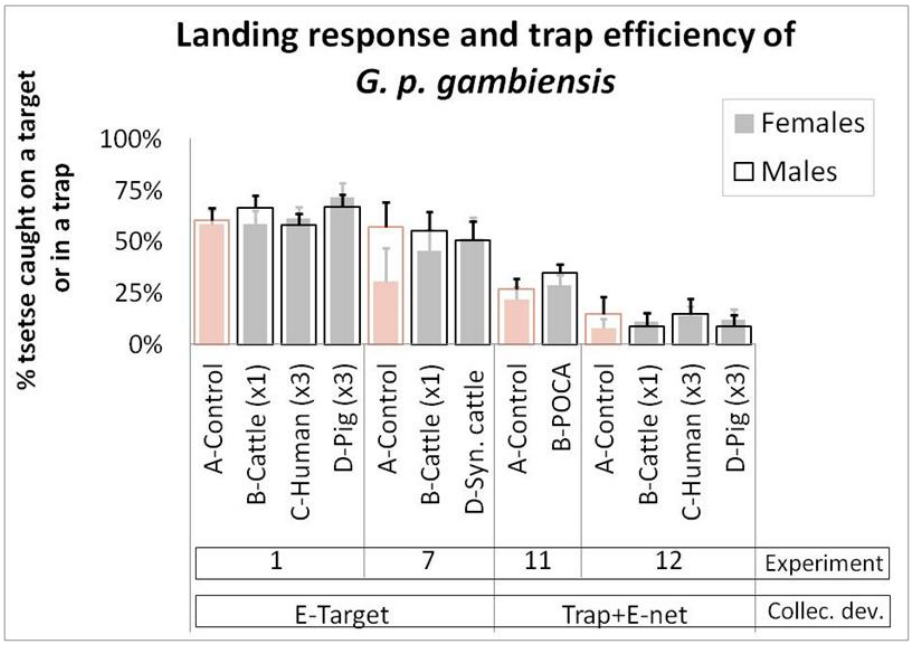


Figure 4-6: Effect of odours on landing response and trap efficiency of *G. p. palpalis*. E-targets (1×1 m, experiments 1 and 7) operated simultaneously with an E-net (0.5 m high×1 m wide) placed at its side. Traps (experiments 11 and 12) operated with an E-net. The landing response is the number of tsetse caught landing on the target expressed as a percentage of the total (landing+circling) catch. Trap efficiency was defined as the number of the tsetse caught in a biconical trap, expressed as a proportion of the total catch (i.e. trap+flanking E-net). Syn. cattle corresponds with a blend of 3-*n*-propylphenol, octenol, 1-octen-3-ol, 4-methylphenol and acetone and CO_2 (2 L/min) (Torr *et al.*, 1995; Torr *et al.*, 2006)

4.5. Responses of *G. tachinoides* to host odours

4.5.1. Attraction to odours

Natural and synthetic odours dispensed from tents

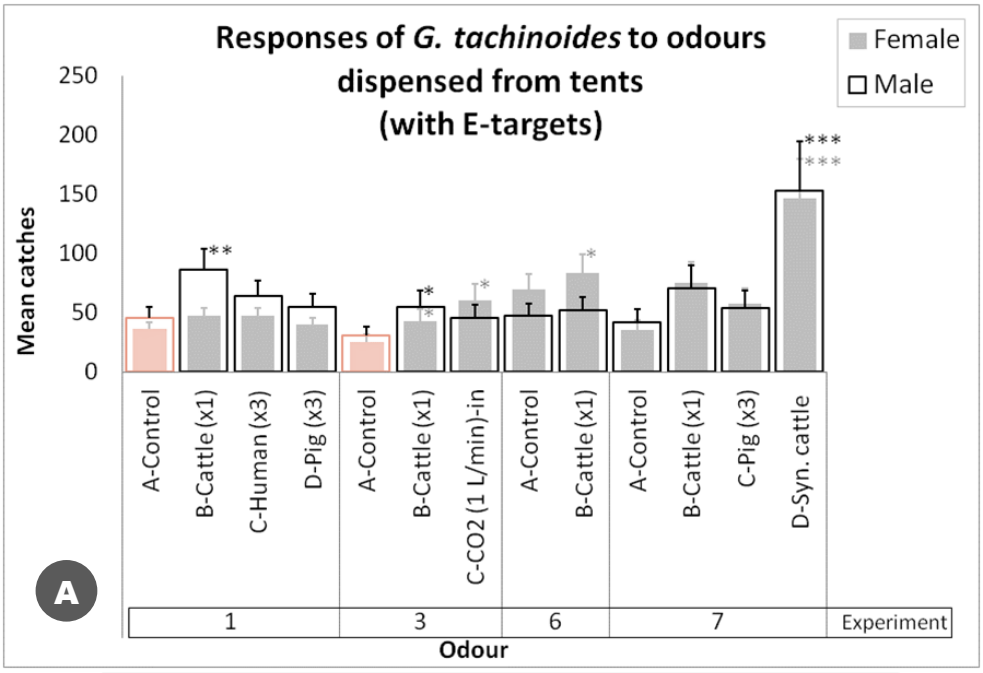
Natural odours

Only cattle odour increased the catches of male and female *G. tachinoides* from E-targets, although not in all experiments (experiments 1, 3 and 6, Figure 4-7A). There was no evidence that males were more responsive than females, and overall the catch increase with cattle odour was 1.6-fold, compared to 1.2-fold and 1.3-fold for human and pig odour, respectively.

In the only experiment carried out with traps alone, cattle odour increased significantly the male and female catches about 5-fold (experiment 8, Figure 4-7B), whereas no effect was observed for human odour (experiment 9, Figure 4-7B). When an E-net operated with the trap, no effect was observed with cattle odour.

Synthetic cattle odour

Experiments with natural host odours did not clarify whether responses were due to the CO_2 or other attractants. Accordingly, some experiments were designed to assess the responses of tsetse to CO_2 alone, or in combination with POCA blend, dispensed at natural doses (Torr *et al.*, 1995; Torr *et al.*, 2006). The results showed that CO_2 increased significantly the catch of female *G. tachinoides* at E-targets 1.7-fold (experiment 3, Figure 4-7A), but not traps (experiment 10, Figure 4-7B). Carbon dioxide dispensed in combination with POCA (synthetic cattle odour) increased the male and female catches of E-targets approximately 4-fold, the difference being highly significant (P<0.001). POCA without CO_2 increased the male catches from traps from 30.2 males and 25.4 females without odour to 53.8 and 50.8, respectively, with POCA (P<0.05 for both sexes) (experiment 10, Figure 4-7B).



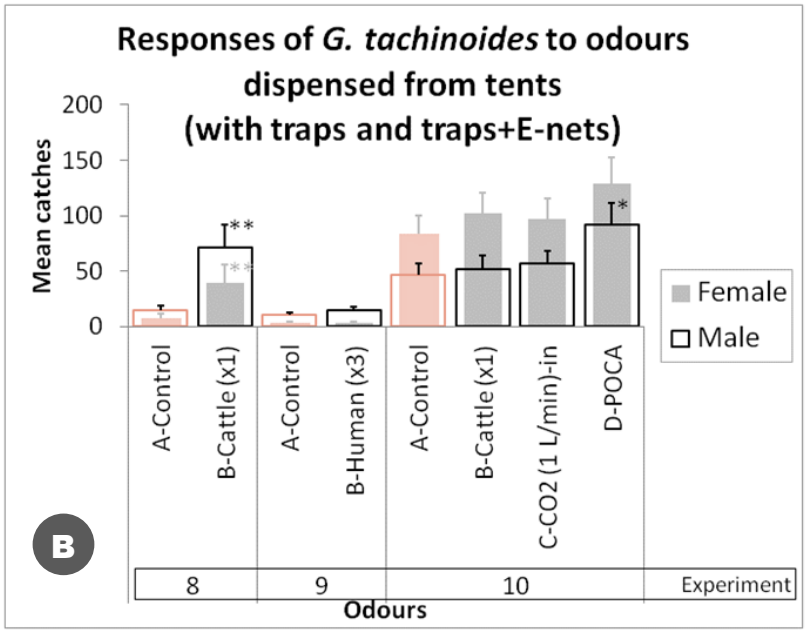


Figure 4-7: Responses of *G. tachinoides* to natural and synthetic odours. Detransformed means catches/day/site are represented in the ordinate axis +SED. Carbon dioxide was dispensed within the tent. Asterisks indicate significant differences compared to the unbaited control treatment (in pink) at P<0.05 (*), P<0.01 (**) and P<0.001 (***).Treatments with the same experiment number (Experiment) were incorporated into the same Latin square.

(A) Mean catches of *G tachinoides* caught with E-targets. Experiments 1 and 7 were replicated 8 days, exp. 3 was replicated 9 days and experiment 6 was replicated 10 days. Syn. cattle corresponds with a blend of 3-*n*-propylphenol, octenol, 1-octen-3-ol, 4-methylphenol and acetone and CO_2 (2 L/min) (Torr *et al.*, 1995; Torr *et al.*, 2006)

(B) Mean catches of *G tachinoides* obtained with biconical traps alone (experiment 8, 8 rep.; and experiment 9, 12 rep.), or traps+E-nets operating simultaneously (experiment 10, 8 rep.)

Comparative release rates of natural and synthetic cattle odour

Samples of the air inside the tents were entrained onto porous polymers (Porapak Q 50/80, Supelco, Bellefonte, USA), and analysed by personnel in Rothamsted Research, UK, using gas chromatography (GC). The results showed that the release rates of octenol and phenols were greater in the synthetic rather than the natural cattle odour (Table 4-7). This suggests that the responses of *G. tachinoides* to natural cattle odour can be explained by the combination of CO_2, octenol and phenols; the greater response to the synthetic cattle probably correlates with the higher release rate of octenol and phenols of the artificial blend.

		Release rates [mean mg/h (±SE)]		
Odour	**(location)**	**Octenol**	**4-Methylphenol**	**-Propylphenol**
Cattle (x1)	(Folonzo)	30.9 (±0.4)	55.0 (±0.7)	22.3 (±2.6)
Cattle (x1)	(Solenzo)	30.5 (±0.2)	55.5 (±1.2)	16.5 (±0.1)
Synt. Cattle	(Solenzo)	129.0 (±6.0)	332.0 (±11.0)	66.0 (±2.0)

Table 4-7: Release rates of chemicals from natural and synthetic odour sources. Data provided by Rothamsted Research, UK

Synthetic odours dispensed directly into biconical traps

Different blends of 3-*n*-propylphenol, octenol, 4-methylphenol and acetone, increased the trap catches of *G. tachinoides*, although the differences were not significant in all the experiments (Figure 4-8). Pooled analysis for the data of all the experiments with the full blend (31 replicates) showed that POCA increased male catches in 4-fold, from 2.1 (0.50±0.104) males/day to 8.5 (0.98±0.104) males/day, and female catches increased 6-fold, from 1.3 (0.36±0.114) females/day to 7.5 (0.93±0.114) females/day (P<0.001 for difference between means for both sexes).

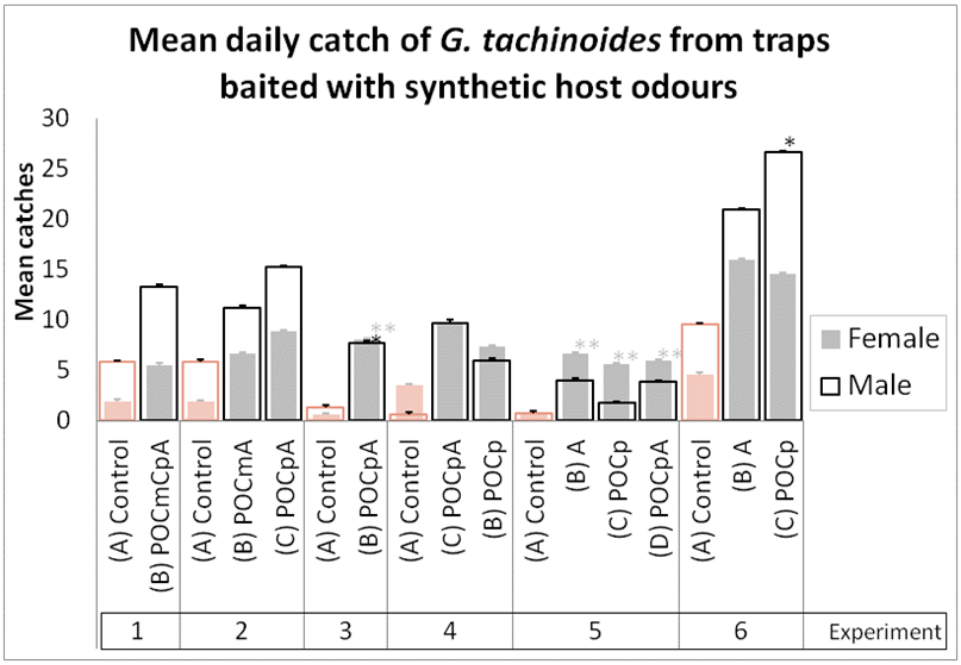


Figure 4-8: Catches of *G. tachinoides* obtained with traps baited with synthetic odours. Detransformed means catches/day/site are represented in the ordinate axis +SED.

Asterisks indicate significant differences compared to the unbaited control treatment (in pink) at P<0.05 (*), and P<0.01 (**). Treatments with the same experiment number (Experiment) were incorporated into the same Latin square.

Experiments 1, 2, 3 and 6 were replicated 12 days. Experiments 4, 3 days. Experiment 5, 8 days.

The initials of the treatments stand for:

- P: 3-*n*-propylphenol
- O: 1-octen-3-ol
- Cm: 3-methylphenol (*m*-cresol)
- Cp: 4-methylphenol (*p*-cresol)
- A: acetone

4.5.2. Landing response and trap efficiency

Among the nine experiments analysed, only in experiment 1 did human and cattle odour increase significantly the landing response of male *G. tachinoides* (Figure 4-9). Two examples are shown in Figure 4-9 (experiments 1 and 7).

No significant increase in the proportion of *G. tachinoides* entering a trap was observed for cattle, CO_2 or POCA (experiment 10, Figure 4-9). The results showed a marked difference in the trap entry response between male and female *G. tachinoides*, with 30-38% of males being caught in the trap, compared to 11-16% of females (experiment 10, Figure 4-9).

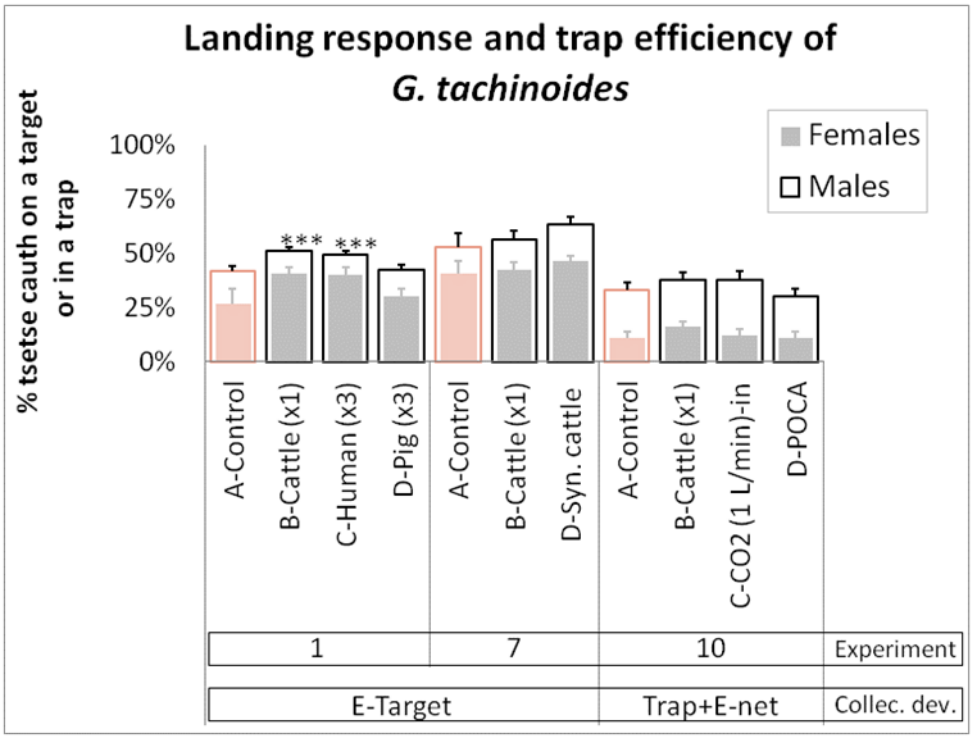


Figure 4-9: Effect of odours on landing response and trap efficiency of *G. tachinoides*. E-targets (1×1 m, experiments 1 and 7) operated simultaneously with an E-net (0.5 m high×1 m wide) placed at its side. Traps (experiment 10) operated with an E-net. The landing response is the number of tsetse caught landing on the target expressed as a percentage of the total (landing+circling) catch. Trap efficiency was defined as the number of the tsetse caught in a biconical trap, expressed as a proportion of the total catch (i.e. trap+flanking E-net). Syn. cattle corresponds with a blend of 3-*n*-propylphenol, octenol, 1-octen-3-ol, 4-methylphenol and acetone and CO_2 (2 L/min) (Torr *et al.*, 1995; Torr *et al.*, 2006). Asterisks indicate significant differences compared to the unbaited control treatment (in pink) at P<0.001 (***).

4.6. Discussion

As in Chapter 3, *G. tachinoides* and *G. palpalis* responded to certain olfactory stimuli, although differences in the behavioural responses of these species compared to the Morsitans-group tsetse were observed. The main findings in this chapter were:

i. **Responses to natural host odours:**
   a. ***G. p. palpalis:*** Pig and human odour increased slightly but significantly the catch of *G. p. palpalis*, the effect being greater at high doses of odours (*i.e.* five pigs or five men, as opposed to three); the effect of natural host odours for *G. p. palpalis* might be explained by the CO_2 contained in the host odours.
   b. ***G. p. gambiensis:*** The catches of biconical traps baited with cattle or human odour where 3-4 times greater than unbaited traps. Conversely, the effect of natural host odours using E-targets were normally not statistically significant.
   c. ***G. tachinoides:*** As for *G. p. gambiensis*, the effect of odour in the catch of *G. tachinoides* was greater for the biconical trap than for the E-target: the odour of one cow increased the catches of a biconical trap by five-fold, but only by 1.6-fold when E-targets were used.

ii. **Responses to artificial odours:** A blend of 3-*n*-propylphenol, octenol, 4-methylphenol and acetone (POCA) increased the captures of tsetse, *i.e.* about five-fold the catches of *G. tachinoides*, it doubled the catches of *G. p. gambiensis* and increased the catches of *G. p. palpalis* about 1.5-fold, compared to unbaited traps. Comparable catch ratios were obtained when acetone was removed from the blend (POC). Acetone is the most volatile chemical in the POCA blend, and therefore the most difficult to use when targets must be baited for 6-12 months. In control operations, POC would give results comparable to those obtained with POCA and it would be more practical to use.

iii. **Inter-specific differences in the response to odours of *G. p. gambiensis* and *G. tachinoides:*** *G. p. gambiensis* and *G. tachinoides* are sympatric in Folonso (southern Burkina Faso). Despite sharing the same habitat, CO_2 increased significantly the catches of *G. tachinoides*, whereas no effect was observed for *G. p. gambiensis*. In addition, while cattle odour was the only natural bait that enhanced the catches of *G. tachinoides*, *G. p. gambiensis* showed a preference for human and pig odour.

4.6.1. Responses of G. palpalis *and* G. tachinoides *to natural host odours*

The results from traps for *G. p. gambiensis* with natural odours contrast with those obtained with E-targets for the same species. Whereas the odour from three men or one cow increased the catch of *G. p. gambiensis* from traps the same odours did not have any effect with E-targets. Significant effects in the catches of *G. p. palpalis* with E-targets required higher dose of odour. For example, natural odours from five pigs or five humans increased the catch of *G. p. palpalis* from electrocuting devices but studies with lower numbers of hosts were ineffective.

The discrepancy between the results obtained with traps and E-targets suggests that there might be some interactions between olfactory and visual stimuli. In addition, significant differences might have been obscured by the relatively low catches of *G. p. gambiensis*. To support the hypothesis, the trend in the catch index obtained with cattle odour for *G. p. gambiensis* was similar (about twice as much as the control, see Figure 4-1A, experiments 3 and 5) to that of *G. tachinoides* (see Figure 4-7); however, the trend in the catch index of *G. tachinoides* was usually significant, whereas for *G. p. gambiensis* it was not. In both cases the catch index was far from the ten-fold increase in the catches of tsetse of the Moristans-group observed with cattle-baited collecting devices (Vale, 1974e). The results for *G. tachinoides* confirm previous studies on the response of this species to cattle odour (Filledier *et al.*, 1988).

Intriguingly, the three species in the study showed a preference for certain natural host odours, whereas no effects were observed for others with similar CO_2 release rate. This might suggest the presence of natural repellents within some host odours; for example, human and cattle for *G. p. palpalis*, pig for *G. p. gambiensis*, and human and pig for *G. tachinoides*. This phenomenon was observed by Vale (1974e) with tsetse of the Morsitans-group, who proposed that human odour contains natural repellents for those tsetse.

4.6.2. Responses of G. palpalis and G. tachinoides to synthetic odours

Synthetic cattle odour produced greater increases in *G. tachinoides* catches (approximately four-fold) than that of natural cattle odour (approximately two-fold). There are two possible explanations: (i) the release rate in the synthetic cattle was about five times greater

than that in the natural odour, enhancing a higher response; and/or (ii) natural cattle odour contains chemicals that 'repel' a proportion of the tsetse.

Combinations of acetone, octenol, 3-*n*-propylphenol and 4-methylphenol, originally developed for the control of *G. pallidipes* and *G. m. morsitans* (Vale *et al.*, 1988a), were implemented to increase the performance of traps and insecticide-treated targets to monitor and control various Morsitans- and Fusca-group species of tsetse (Gibson & Torr, 1999). The results in the present work confirmed those of earlier studies (Mérot *et al.*, 1988) showing that POCA blend, would increase the *G. tachinoides* catches from artificial baits. Our data suggest that the incorporation in the blend of 4-methylphenol is about twice as effective as 3-methylphenol (Figure 4-8). In agreement with earlier studies (Filledier & Mérot, 1989; Späth, 1995), a blend of POC might increase the killing rate of artificial baits almost as much as POCA, and would avoid the use of large volumes of acetone, with the consequent logistic and economical benefits.

Results from the experiment to assess the effect in the catches of *G. p. palpalis* and *G. p. gambiensis* with synthetic odours confirm Cheke & Garms' (1988) findings showing that acetone, octenol and a combination of these two chemicals with phenols can increase the catch index, slightly (about 1.5-fold), but consistently. However, due to the low catch index achieved, it is not clear whether synthetic lures would improve the cost-efficiency of any control campaign against *G. palpalis* using artificial baits. Economical studies to compare the use of odours against an increased number of targets would be required (see chapter 7).

It is worth mentioning the fact that whereas the catch in Azaguié with POC-baited traps was about 1.5 time greater than the unbaited traps (experiment 1), the differences with the control were not significant in Bingerville (experiment 2). The apparent difference in the behaviour of *G. p. palpalis* in the two locations might be explained by the difference in the habitat. Azaguié and Bingerville are both located in southern Côte d'Ivoire, about 40 Km apart, but unlike the former, Bingerville is on the shores of the eutrophicated Ebrié Lagoon near Abidjan, with high content of organic material (Pagano & Saint-Jean, 1993). In this location, the environment is likely to contain a relatively high concentration of volatiles, such as propylphenols, resulting from the bacterial-mediated breakdown of proteins in the detritus (Okech & Hassanali, 1990; Jeong *et al.*, 2003; Borhan *et al.*, 2012). The high

content of phenolic compounds in the background atmosphere could make it difficult for tsetse to detect synthetic kairomones, such as 3-*n*-propylphenol and 4-methylphenol.

Pig odour and carbon dioxide increased the catches of *G. p. palpalis* in a similar proportion; no significant difference was observed in any experiment between these two odours. This suggests that natural CO_2 contained in pig odour might explain the responses of this species to pigs.

CO_2 did not have any significant effect in the catch index of *G. p. gambiensis*. Contrary to *G. f. fuscipes* in the previous chapter, environmental conditions can hardly explain the lack of response: *G. p. gambiensis* and *G. tachinoides* share the same habitat along the Comoe River at Folonzo, where the same experiments proved that *G. tachinoides* respond to CO_2.

4.6.3. Landing response and trap efficiency

Our results suggested that the three species exhibit a relatively high landing response (40-50%), which in our experimental conditions, was not modulated by host odours. Most likely, exhausting the odours from a long tube has an effect on the proportion of chemicals that arrived at the distal end, compared with the proportion of chemicals in the tent. Low volatile molecules might not reach the distal end of the tube, and therefore, we cannot know with our experimental design whether they play any role in enhancing the landing response. Warnes (1995) demonstrated that electrified targets impregnated with cattle sebum caught more flies than targets without it, although he did not assessed whether the cattle sebum increased the number of tsetse approaching the target (*i.e.* increase in the attractioin) or the number of tsetse that finally landed on it (*i.e.* increase in the landing response). In addition, previous studies demonstrated that CO_2 enhanced the landing response of Morsitans-group of tsetse (Vale, 1974c; Hargrove, 1980; Warnes, 1995). In our studies, CO_2 did not have any effect in the landing responses of *G. p. palpalis*, *G. p. palpalis* and *G. tachinoides* on targets.

For *G. p. gambiensis* odours were more effective when they were used to bait traps rather than E-targets. This suggests that visual cues play an important role in the behavioural responses of this species to feed on hosts. For example, *G. p. gambiensis* could be more attracted to odour baited 3D structures (*i.e.* traps) than to 2D panels (targets)(Lindh *et al.*, 2009). Further experiments comparing 3D and 2D catching devices would be required to

assess the visual effect of the structures in the attraction. Odours did not have any apparent effect in either the landing (Figure 4-6, experiments 1 & 7) or entry responses (Figure 4-6, experiments 11 & 12).

4.6.4. Inter-specific differences in the response to odours

The olfactory responses of the species studied in this section resemble those described in chapter 3: low overall increases in catch index, landing and entry responses. This differs notably with responses found for savannah-tsetse, for which the catches of E-targets baited with cattle odour were about 10 times greater than unbaited E-targets (Vale, 1974e; Hargrove *et al.*, 1995).

In accordance with *G. fuscipes*, *G. palpalis* occupy bushy habitats where, perhaps, orthokinetic or orthotactic responses to odours might be more adequate in their environment than the anemotactic responses displayed by savannah tsetse. With this notion, among the Palpalis-group species studied here, *G. tachinoides* is the one that exhibits the highest response to natural (*i.e.* cattle odour) and artificial odours (*i.e.* CO_2 and POCA). This is consistent with previous observations describing *G. tachinoides* behaviour, ecology and feeding preferences as intermediate between the savannah-dwelling Morsitans-group of tsetse, and the more riverine Palpalis-group species (Küpper *et al.*, 1990; Clausen *et al.*, 1998; Leak, 1999; Laveissière *et al.*, 2003). As a 'transition' species between Palpalis- and Morsitans-tsetse, *G. tachinoides* might exhibit lower responses to odours than species of the Morsitan-group of tsetse, but greater responses to odours than other species of the Palpalis-group (Mérot *et al.*, 1986; Filledier *et al.*, 1988; Mérot *et al.*, 1988).

Yet, we need to understand better how the Palpalis-group species locate their hosts, the real role that the olfactory cues play in the location of a host, landing and final feeding, and how those olfactory cues combine with the visual cues.

CHAPTER FIVE

VISUAL RESPONSES OF *GLOSSINA FUSCIPES*

5.1. Introduction

5.1.1. Visual responses of Palpalis-group of tsetse to differences in colour, shape and size

The use of natural (ITC) or artificial baits (traps and insecticide-treated targets, sometimes baited with odour) are the only techniques that might be applied by local communities (Laveissière *et al.*, 1994; Shaw *et al.*, 2001; Shaw *et al.*, 2006) but their wider use is constrained by the low densities of livestock in HAT-affected areas (Kuzoe & Schofield, 2004), and/or the poor performance of artificial baits for Palpalis-group tsetse. In contrast to Morsitans-group tsetse, Palpalis-group species are less responsive to host odours (see chapters 3 & 4), and hence, artificial baits must be deployed at densities that are not affordable or sustainable. For example, Shaw *et al*. (2006) estimated that a campaign with traps against the savannah tsetse *G. pallidipes* in Uganda would cost US$ 400-500/km^2, whereas the expenses of a similar campaign against *G. f. fuscipes* would be around US$ 900.

However, recent results have revived the prospects for the use of cost-effective baits against HAT. First, the performance of artificial baits can be enhanced by the use of odours (Omolo *et al*., 2009; Rayaisse *et al*., 2010; see chapters three and four). Second, studies suggest that significant improvements in cost-effectiveness of baits for vectors of

HAT might be achieved through the exploitation of the visual responses to hosts (Lindh *et al.*, 2009). Targets are designed to reproduce artificially a host-oriented response; however, different authors estimated that only 10-40% of the tsetse approaching a target landed on it, whereas virtually all the tsetse landed on a host after coming near it (Hargrove, 1980a; Mérot & Filledier, 1985; Green, 1988, 1989, 1990). The difference in landing responses elicited by natural (hosts) and artificial (targets) baits suggests that the design of the latter can be improved.

Which parameters should be modified in order to improve the performance of artificial baits? Traditionally, studies to improve the design of targets to control Palpalis-group of tsetse has focussed on responses to colour (Laveissière *et al.*, 1987a; Laveissière *et al.*, 1987b; Green, 1988, 1989; Laveissière & Grébaut, 1990). More recently, other studies explored the responses of these flies to targets of different sizes and shapes (Lindh *et al.*, 2009).

The effect of colour

Studies of the response of *G. m. morsitans* towards monochromatic light of different wavelengths suggested that UV wavelengths elicit phototactic behaviour in tsetse (Green & Cosens, 1983). Field studies of *G. p. palpalis* showed that, whereas highly UV reflective white targets caught less than half as many tsetse as phthalogen blue targets, they elicited higher landing response – *i.e.* 70% of tsetse that approached the UV reflective target landed on it compared with approximately 20% that landed on the blue targets (Green, 1988). The experiments showed that strongly reflective UV material enhanced the proportion of flies landing, whereas for most colours the majority of tsetse circled the targets without landing. Intriguingly, black targets elicited higher landing responses than any other colour, except white; this result seemed inconsistent with the hypothesis that tsetse land predominantly on highly UV reflective materials. Green (1993) argued that similar effects in landing responses obtained with black and white colours are due probably to different behavioural responses. He speculated that ultraviolet reflectivity functionally represents sky light, and tsetse do not intend to land on white targets, but instead, they collide 'accidentally' with them. Green (1988) also showed that phthalogen blue was the most attractive colour for *G. p. palpalis*, and caught approximately 1.2 times as many tsetse as with the black target, 1.7 times the red target, 1.9 times the yellow target, 2.2 times the violet target and 4.5 times the green target.

Results for *G. tachinoides* were slightly different: blue targets doubled the catch of tsetse compared to the catch obtained with highly reflective white targets, although no significant difference in the landing response was observed (Green, 1990).

The responses of Palpalis-group tsetse to blue targets, particularly females, contrasts with findings for *G. morsitans* (Mérot & Filledier, 1985) and *G. pallidipes* (Green & Flint, 1986). Morsitans-group tsetse seemed to be equally attracted to black and blue colours. The highest landing response for these species was observed with black screens, and the lowest with white ones, with blue being intermediate (Barrass, 1960; Vale, 1982; Green, 1986).

Most targets used currently in control campaigns combine two colours: (i) blue, which enhance high attraction; and (ii) black, which is highly attractive but also elicits a strong landing response (Mérot & Filledier, 1985; Laveissière *et al.*, 1987a; Green, 1989; Mérot & Filledier, 1989; Green, 1990).

The addition of a colour in the target that enhances the landing response maximises the proportion of tsetse exposed to the insecticide. However, these designs still miss the proportion of tsetse that circle the targets but do not alight. To solve the problem, the use of panels of netting, placed on the flanks of the screens (so-called flanking nets), has been suggested. Flanking nets are made of insecticide-impregnated fine black mesh, and are invisible for tsetse; in this way, tsetse circling the targets collide with the flanking net, picking up a lethal dose of insecticide, (Packer & Brady, 1990). Despite the potential of flanking nets to kill a proportion of the flies that do not land on the target, some authors argued that the relatively large size of the panels of netting – required to flank the standard targets used in control operation – will make this material extremely fragile in real operations (Laveissière *et al.*, 1987a).

The effect of size

Recent studies of *G. f. fuscipes* in Kenya showed that reducing the size of the target from 1 m^2 to 0.1 m^2 reduced material costs of targets by 90%, but only halved the number of tsetse that contacted the target, giving a five-fold improvement in the material cost (Lindh

et al., 2009). Lindh *et al.* (2009) reasoned that, in a hypothetical control operation, also logistic costs associated with transport, storage and deployment would be reduced with small targets.

These results contrast with findings obtained for Morsitans-tsetse, for which targets smaller than 1 m^2 are not recommended (Hargrove, 1980b; Vale, 1993b). The size of the target has a dramatic effect in the attraction and landing of savannah-tsetse. For example, Vale (1993b) showed that an increase in the target size from 0.25 m^2 to 2 m^2 improved the catches of *G. m. morsitans* approximately 50-fold. In another study *G. morsitans* and *G. pallidipes*, Hargrove (1980b) compared the catches obtained with two unbaited black cylinders of similar proportions: one with dimensions of about 0.6 m long and about 0.4 m in diameter, and a bigger one of about 1.7 m long and 1.1 m diameter. About half the *G. morsitans* visiting the smaller model alighted on it, and virtually none of the *G. pallidipes*. Conversely, nearly all the *G. morsitans* and about one-third of the *G. pallidipes* visiting the bigger model alighted on it.

The effect of shape

Various studies in Zimbabwe have shown that larger numbers of *G. morsitans* and *G. pallidipes* are attracted to and land on horizontal-oblongs compared to vertical ones (Vale, 1974e; Torr, 1989). For example, Vale (1974e) found that oblong models placed horizontally caught approximately 3 times more *G. morsitans* than the same models placed in upright position. This pattern is thought to enable tsetse to discriminate their hosts from the environment (Torr, 1989). Important hosts, such as warthog and buffalo, are horizontal oblongs living in a visual environment of vertical oblongs formed by savannah woodland. This attraction to horizontal shapes is also thought to explain, at least in part, why Morsitans-group tsetse are not attracted to humans (Vale, 1974e; Torr, 1989).

In contrast, Palpalis-group tsetse have a wider range of hosts, which includes humans (Sané *et al.*, 2000; Simo *et al.*, 2007), and they are not confined to savannah woodlands but rather, to the bushy riverine habitats. Hence, these species might be expected to display different behavioural responses to shape. Although such knowledge would contribute to the rational development of more cost-effective designs of target, studies of the effect of shape in the visual responses of Palpalis-tsetse are described in this work for the first time.

5.1.2. Aims of the study

Visual responses of *G. f. quanzensis* have not been studied since the implementation of the monoconical trap in the Republic of Congo (Lancien, 1981). Hence, studies described in this chapter assess, for the first time, the responses of *G. f. quanzensis* to targets of various shapes and sizes. One of the ultimate objectives of the study was to optimize targets for tsetse control. Improvements of the targets are expected to be done at different levels:

(i) The size of targets will be optimised.
(ii) Some of the targets used in control campaigns are not square, but rectangular (Kuzoe & Schofield, 2004). The study will assess responses of *G. f. quanzensis* to vertical and horizontal oblongs.
(iii) Currently, the tool used systematically in the DRC in control campaigns is the untreated pyramidal trap (Mansinsa, Programme National de Lutte contre la Trypanosomiase Humaine Africaine, DRC, personal communication). Targets are cheaper and easier to maintain than traps (Vale & Torr, 2004). Responses of tsetse to targets and traps will be compared.
(iv) The scientific literature consistently shows that the addition of an appropriate blue to the targets increases the catch (Green, 1986; Laveissière *et al.*, 1987a; Green, 1988; Mérot & Filledier, 1989; Steverding & Troscianko, 2004; Lindh *et al.*, 2009). Although not all the blues are equally effective (*i.e.* some reflectance spectra within the generic name of "blue" are more attractive than others)(Green & Flint, 1986; Laveissière *et al.*, 1987b; Green, 1989, 1990, 1993) during this study only Standard phthalogen blue was used. Experiments compared the catches when the blue cloth was incorporated in the target design.

When the experimental design included E-nets, the effect of the visual cues in the landing response was assessed.

Part of the result reported in this chapter were published in Tirados *et al.* (2011) (Annex III). Subsequently, additional experiments on *G. tachinoides*, *G. p. gambiensis* and *G. p. palpalis* were carried out in Burkina Faso and Côte d'Ivoire and reported in Rayaisse *et al.* (2011) and Esterhuizen *et al.* (2011) (Annexes IV and V).

5.2. Materials and methods

5.2.1. Study sites

Field studies of *G. f. quanzensis* were undertaken in the Democratic Republic of the Congo (DRC) in the valley of the river Lukaya as described in chapter two (see section 2.1.3). Experiments were conducted during the dry season between July and August in 2009 and 2010.

5.2.2. Collecting devices

Arrangements of electrocuting grids were used to assess the responses of tsetse to various visual baits as described in chapter two. In some experiments, catches of biconical and monopyramidal were compared to those obtained from with E-targets. Inert targets were used to compare the effect of size in the catch (see chapter two, sections 2.4.1. and 2.4.2.).

5.2.3. Experimental design

Different artificial baits were compared over 6-21 days in a series of replicated Latin squares of days × sites × treatments, as explained in chapter two. Experimental sites were at least 100 m apart. All experiments were carried out for 4 h, between 10:30 h and 14:30 h. Unless otherwise stated, to facilitate comparisons across species and experiments, all the setups included a standard treatment comprising an E-target (1 m×1 m) flanked by an E-net (1 m×1 m).

Vertical vs. horizontal oblongs (exp. A&B)

The responses of tsetse to vertical and horizontal oblongs was assessed by comparing the catches from oblong (0.5×1.0 m or 0.125×0.5 m) E-targets with (Figure 5-1, experiment A) or without (experiment B) accompanying E-nets (0.5 m wide×1.0 m high). E-targets were placed with their long axis arranged vertically or horizontally and the base on the ground.

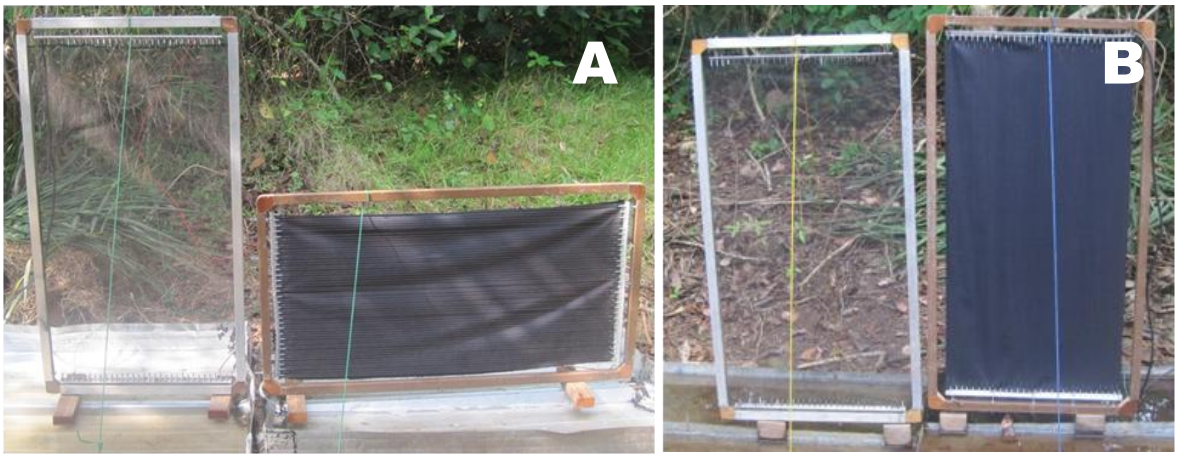


Figure 5-1: ***Example of E-targets to compared the responses of tsetse to 'shape':*** (A) 0.5×1.0 m horizontal E-target accompanied by a 0.5×1.0 m E-net. (B) 0.5×1.0 m vertical E-target accompanied by a 0.5×1.0 m E-net

Size (exp. C)

The effect of target size was studied by comparing the numbers of *G. f. quanzensis* attracted to 'inert square targets' of decreasing size: (i) 1.0×1.0 m, (ii) 0.75×0.75 m, (iii) 0.5×0.5 m (Figure 2-8), (iv) 0.25×0.25 m, (v) 0.1×0.1 m, and (vi) no target. An E-net was placed adjacent to each target to assess the numbers of tsetse attracted, but the targets themselves were not electrified.

Effect of the vegetation in host location (exp. D)

Palisades were used to mimic the effect of dense vegetation in the location of hosts (see section 2.6). The catches of 0.25×0.25 m E-targets, flanked by E-nets of the same dimensions, were compared when the catching devices were visible or concealed in the centre of the palisade, and when they were unbaited or baited with CO_2 (1 L/min), in a 4 × 4 Latin square. The standard target (1 m×1 m E-target + 1 m×1 m E-net) was not used in this experiment. The experiment was replicated for 16 days.

Assessment of different artificial-bait designs (exp. E-L)

A series of experiments was carried out to compare catches obtained with different artificial baits, in order to select the most cost-effective tools to control *G. f. quanzensis*. For simplification, the catches obtained with the Standard target for most of the experiments are not shown in the results. However, it was used to express the catches of the other treatments as a proportion of the catches obtained with the Standard target (*catch*

index). This provided a standardised method of comparing treatments in different experiments. Catches obtained with the Standard target for each experiment can be estimated by multiplying the catch of any treatment by the catch index of the same treatment.

In addition to the standard target, the features of the rest of the treatments in the experiments are detailed below.

Traps vs targets of different sizes (exp. E)

To study the effect of size, the catches of tsetse from the following treatments were compared with E-targets:

(a) 1.0 m × 1.0 m black target + 1.0 m × 1.0 m flanking net (Standard E-target)
(b) 1.0 m × 1.0 m black E-target
(c) 0.25 m × 0.25 m black E-target + 0.25 m × 0.25 m flanking net
(d) 0.25 m × 0.25 m black E-target
(e) biconical trap + 0.5 m wide × 1.0 m high flanking net
(f) biconical trap

The experiment was replicated for 12 days.

Effect of shape (exp. F)

To assess the effect of the shape in the catch, two experiments were carried out. In both experiments, four black E-targets of two different sizes and shapes were used, in addition to the Standard E-target. In the first experiment the four targets were accompanied by a flanking net of 0.5 m wide × 1.0 m high, whereas in the second experiment targets operated without flanking net. Each experiment was replicated 10 days. The dimensions of the targets were as follows:

(a) 1.0 m wide × 0.5 m high E-target
(b) 0.5 m wide × 1.0 m high E-target
(c) 0.5 m wide × 0.125 m high E-target
(d) 0.125 m wide × 0.5 m high E-target

Effect of colour

A series of experiments was carried out to compare responses of tsetse to black cloth, phthologen blue cloth, and the combination of both colours. Cotton fabric dyed

phathologen blue was provided courtesy of the National Program for Trypanosomiasis Control (Kinshasa) for these experiments.

Black and blue targets, and traps (exp. G)

This experiment had two objectives: (i) to assess the numbers of tsetse attracted to black and blue targets, and the landing responses elicited by both colours; and (ii) to assess the responses of *G. f. quanzensis* to two different traps, *i.e.* biconical and monopyramidal. Hence, the experiment comprised four treatments:

(a) 1.0 m wide × 0.5 m high E-black target + 0.5 m wide × 1.0 m high flanking net
(b) 1.0 m wide × 0.5 m high phthologen blue E-target + 0.5 m wide × 1.0 m high flanking net
(c) biconical trap
(d) monopyramidal trap

The experiment was replicated for 10 days.

Relationship between colours and size (exp. H)

The experiment compared the responses of tsetse to black/blue targets and black targets, and analysed differences with the size. The coloured strips in the bicolour targets were placed vertically. The targets employed in this experiment were as follows:

(a) 1.0 m wide × 0.5 m high black E-target
(b) 1.0 m wide × 0.5 m high E-target, formed of one piece of black cloth, and another piece of phthologen blue cloth, both 0.5 m × 0.5 m
(c) 0.5 m × 0.5 m black E-target
(d) 0.5 m × 0.5 m E-target, formed of one piece of black cloth, and another piece of phthologen blue cloth, both 0.25 m wide × 0.5 m high

All the E-targets were accompanied by a flanking net of 0.5 m × 0.5. The experiment was replicated 10 days.

Monochromatic target, vs. bicolour with vertical coloured strips (exp. I)

Three targets were used in this experiment:

(a) 1.0 m × 1.0 m black E-target
(b) 1.0 m × 1.0 m black/blue/black E-target, with a central black strip of 0.5 m wide × 1.0 m high, and two lateral phthologen blue bands of 0.25 m wide × 1.0 m high
(c) 1.0 m × 1.0 m black/blue E-target, formed with two pieces of cloth dyed black and phthologen blue respectively, both 0.5 m wide × 1.0 m high

All the E-targets operated with a flanking net of 1.0 m × 1.0 m. This experiment was replicated for six days

Double-striped bicolour vs. triple-striped bicolour (exp. J)

The experiment compared the responses to bi- or tri-coloured targets, with horizontal strips:

(a) 1.0 m wide × 0.5 m high black/blue/black E-target, with a central black strip of 1.0 m wide × 0.25 m high, and two pieces of phthologen blue bands of 1.0 m wide × 0.125 m high, one upper and one lower
(b) 1.0 m wide × 0.5 m high blue/black E-target, with an upper phthologen blue strip, and a lower black strip, both of 1.0 m wide × 0.25 m high
(c) 0.5 m × 0.5 m blue/black E-target, with an upper phthologen blue strip, and a lower black strip, both of 0.25 m wide × 0.125 m high

E-targets operated with flanking nets of 0.5 m × 0.5 m. The experiment was replicated for eight days.

Vertical coloured strips, vs horizontal coloured strips (exp. K)

The targets used in this experiment were as follows:

(a) 1.0 m × 1.0 m black/blue/black E-target, with a central black strip of 0.5 m wide × 1.0 m high, and two lateral phthologen blue bands of 0.25 m wide × 1.0 m high
(b) 1.0 m wide × 0.5 m high blue/black E-target, with an upper phthologen blue strip, and a lower black strip, both of 1.0 m wide × 0.25 m high. This target operated with a flanking net of 0.25 m wide × 0.5 m high

(c) 0.5 m wide × 0.25 m high blue/black E-target, with an upper phthologen blue strip, and a lower black strip, both of 0.5 m wide × 0.125 m high. This target operated with a flanking net of 0.25 m × 0.25 m

The experiment was replicated for eight days.

Correlation between visual and olfactory cues (exp. L)

To assess possible interactions between the olfactory and visual cues in host location, two different E-targets were exposed to pig odour, and compared with other two unbaited screens:

(a) 1.0 m × 1.0 m black E-target + 1.0 m × 1.0 m flanking net, unbaited
(b) 1.0 m × 1.0 m black E-target + 1.0 m × 1.0 m flanking net, baited with pig odour
(c) 1.0 m wide × 0.5 m high black E-target + 0.5 m wide × 1.0 m high flanking net, unbaited
(d) 1.0 m wide × 0.5 m high black E-target + 0.5 m wide × 1.0 m high flanking net, baited with pig odour

The odour was provided from three pigs (total weight approximately 60 kg) concealed in a tent as described in chapter two. The experiment was replicated for 12 days.

5.2.4. Statistical analyses

The data were treated and analysed as described in chapter two (see section 2.8). Values for the mean catches, as well as the catch density (*i.e.* mean catches/area of target) are provided. When there was no clear or consistent differences in the responses of male and female tsetse, catches of males and females were combined.

Catches

Detransformed means are reported accompanied by their respective transformed mean and standard error of the difference (SED) between means.

Catch density

The practical aim of the study was to provide a rational basis for designing cost-effective targets. For this purpose, it is useful to consider the numbers of tsetse killed per unit area of the target, henceforth termed the 'catch density' The catch density for each target was calculated by dividing the mean daily catch (x) by the area (m^2) of the target (E-target or inert target). For example, if E-nets (0.5 m^2) placed next to 'inert' targets of 0.1 m^2 and 1 m^2 caught respectively 20 and 100 tsetse/day, then the catch densities would be 20/0.1 =200 tsetse/m^2 and 100/1 = 100 tsetse/m^2, respectively. To allow comparisons across experiments, catch densities were expressed as a proportion of the mean daily catch of the Standard target and this value is termed the Catch Density Index. Hence, if in the above example, a Standard target caught 200 tsetse/day, then the above Catch Density Indices would be 200/200 =1 and 100/200 =0.5, respectively. Indices greater or less than unity imply that the catch density is more or less than the Standard.

Note that we do not include the area of the E-net in this calculation. The E-net would kill a proportion of the flies, preventing them from landing on the target, and resulting in an overestimation of the kill rate on the target. As all the E-nets are identical, the error in the catch index was balanced for all the treatments. We assume this error because size experiments are intended to assess the number of tsetse approaching the target, ignoring for the time being the proportion of flies that land on the target. The implications in the landing response were considered in other experiments.

Landing responses

Whenever E-nets were used with E-targets, landing responses were assessed. To assess whether target size and/or shape influenced landing response, the proportion of tsetse that landed on an E-target was quantified by expressing the catch from an E-target as a proportion of the total (E-target+E-net). These data were analysed by logistic regression as explained in chapter two (see section 2.8). The SE is asymmetric about the mean, and

thus, mean percentages are accompanied by the larger back-transformed SE. For all analyses, the level of significance was established at $P<0.05$.

5.3. Effects of size and shape

5.3.1. Vertical vs. horizontal (exp. A&B)

Horizontal oblongs were consistently more attractive than vertical ones for *G. f. quanzensis* (Figure 5-2A). For E-targets not accompanied by an E-net, shape ($F_{1,24}$ = 77.5, P<0.001) and size ($F_{1,24}$ = 54.4, P<0.001) had highly significant effects on catch but there was no interaction between these factors ($F_{1,23}$ = 0.4, *n.s.*).

Similarly, for the E-targets accompanied by a flanking E-net (Figure. 5-2B), shape ($F_{1,24}$ = 7.8, P<0.01) and size ($F_{1,24}$ = 21.6, P<0.001)(Figure 5-2B) were highly significant but there was no interaction between them ($F_{1,23}$ = 2.8, *n.s.*). Overall, the horizontal oblongs without or with accompanying E-nets caught 1.7-3.4 times more *G. f. quanzensis* than vertical oblongs, and the bigger targets (0.5 m^2) caught twice as many tsetse as small ones (0.03 m^2). No effects of the shape on the landing responses were observed (about 22% for the 0.5 m^2 target and 9% for the 0.03 m^2 target).

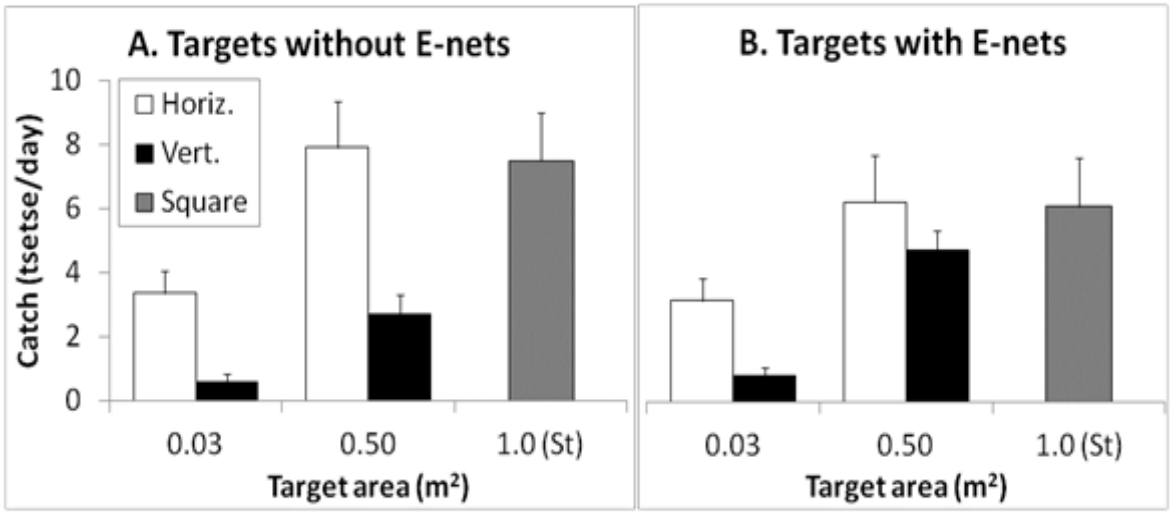


Figure 5-2: ***Attraction of* G. f. quanzensis *to different shaped targets.*** Detransformed mean catch of *G. f. quanzensis* (+SED) from horizontal (open bars), or vertical (solid bars) oblongs, or the Standard square (grey bars). E-targets operated (A.) alone or (B.) with flanking E-nets. Oblongs were 0.125×0.25 m (surface area = 0.03 m^2) or 1×0.5 m (0.5 m^2) and accompanying E-nets were 0.5 m wide×1.0 m high. Both experiments included a Standard target consisted of a square (1×1 m) black E-target accompanied by a 1×1 m E-net.

5.3.2. Effect of size (exp. C)

The effect of size was examined further by comparing the numbers of tsetse attracted to square targets ranging in size between 0.01 m^2 (0.1×0.1 m) to 1.0 m^2 (1×1 m)(experiment C). Despite the low catches of *G. f. quanzensis* (0.5-3 tsetse/day) a consistent pattern in the effect of size in the catches was observed: the catch increased with size up to 0.25 m^2 where it plateaus. No significant differences in the catches were observed among any of the treatments, ranging from 0.7 tsetse/day (0.23±0.080) for 'no-target' to 1.8 tsetse/day (0.75±0.080) for the 0.56 m^2 inert target. The mean catch obtained for the 0.01 m^2 inert target was 0.7 tsetse/day (0.23±0.080), almost identical to the mean catch obtained for 'no-target', suggesting that target of 0.10×0.10 m are too small to be detected by *G. f. quanzensis*.

The 1 m^2 inert target and the Standard E-target had the same size. However, the 1 m^2 inert target caught 1.0 tsetse/day (0.30±0.080), less than the Standard E-target, for which the mean catches were 2.4 tsetse/day (0.54±0.080). This may be because the Standard E-target had a larger flanking E-net (1 m^2) and the target was electrified; by contrast, the inert 1 m^2 target was not electrified and was accompanied by a 0.5 m^2 E-net.

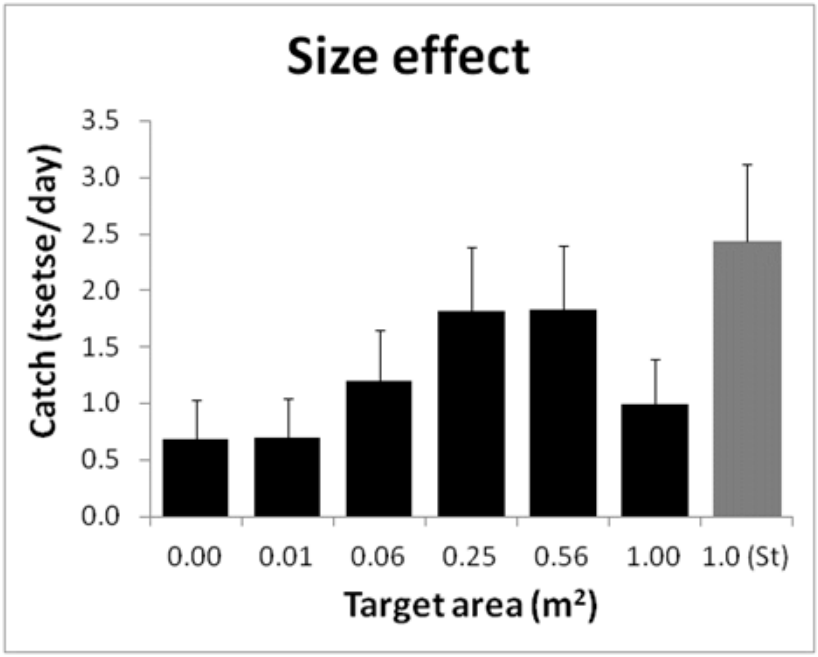


Figure 5-3: ***Attraction of* G. f. quanzensis *to different objects of different sizes.*** Detransformed mean catches (+SED) of *G. f. quanzensis* attracted to square inert targets of various size. Inert targets were accompanied by an E-net 0.5 m wide×1 m high. 'St' is the Standard, comprising an E-target (1×1 m) accompanied by an E-net (1×1 m).

5.3.3. Catch density (exp. A, B & C)

Figure 5-3 shows that for targets smaller than 0.25 m^2 the catch increased with the size of the target. However, the increase in the catches was relatively small in comparison with the size of the target. For example, in the experiment with the squares (experiment C) the catch density decreased rapidly as the area of the targets increased from 0.01 to 0.06 m^2; thereafter, the slope in the catch density was attenuated, fitting a 'power function' (Figure 5-4).

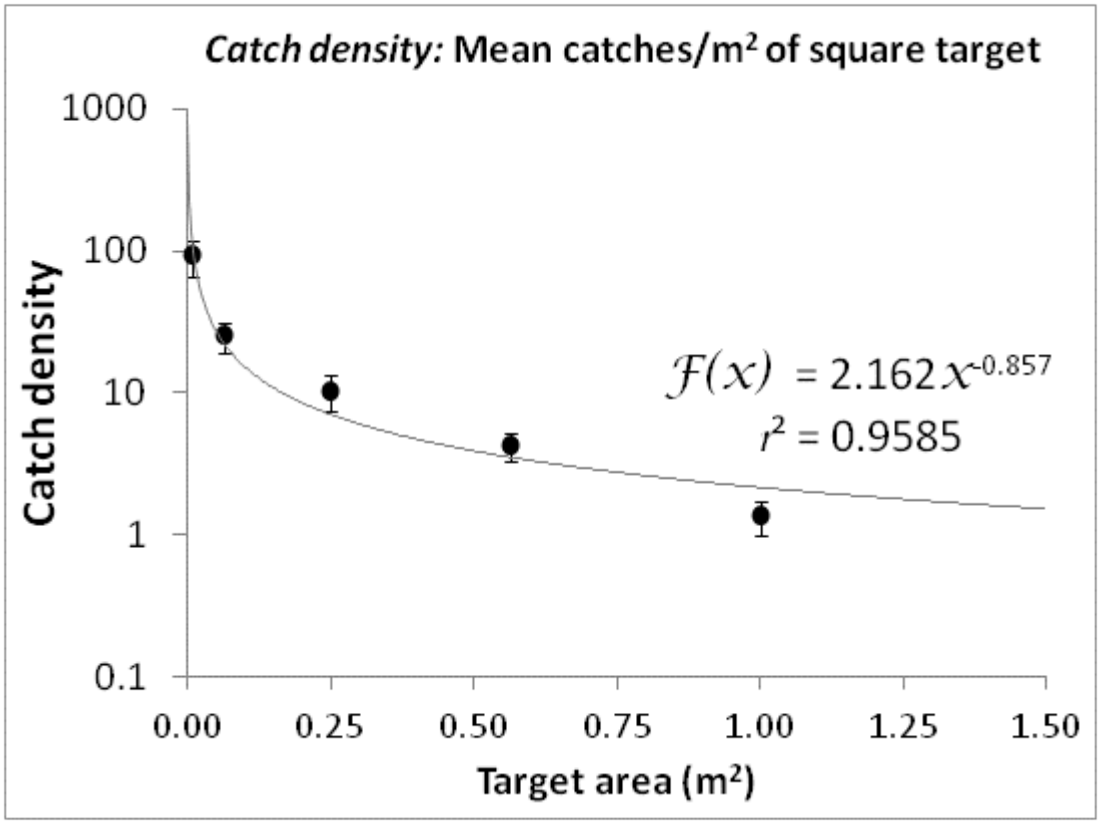


Figure 5-4: ***Extrapolation of the effect of target size in the catch density.*** Mean catch density (*G. f. quanzensis*/m^2) ±SE obtained for each square target. Inert square black targets of different sizes (0.01, 0.06, 0.25, 0.56 and 1 m2) were placed next to an E-net (0.5 m wide×1 m high).

Using the catch density index to compare different experiments, the results show that for all targets, irrespective of size (Figure 5-5) and shape (Figure 5-6), the catch density index decreases as the size of the target increases, suggesting that it is more cost effective for control programmes to produce large numbers of small targets from the material available.

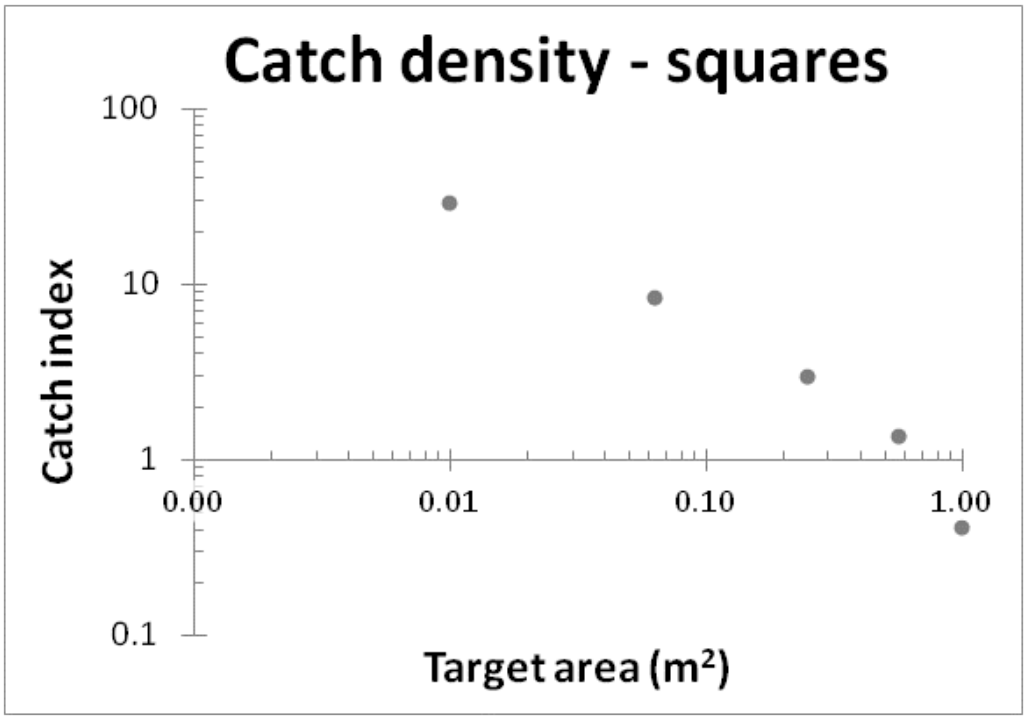


Figure 5-5: ***Proportional catch of G. f. quanzensis on square targets.*** Mean catch density (*G. f. quanzensis* /m^2) expressed as a proportion of that from a standard target for *G. f. quanzensis* attracted to squares. . Catches were obtained with the flanking E-nets; targets were not electrified. The horizontal line denotes the catch index of the Standard E-target (1 m^2)

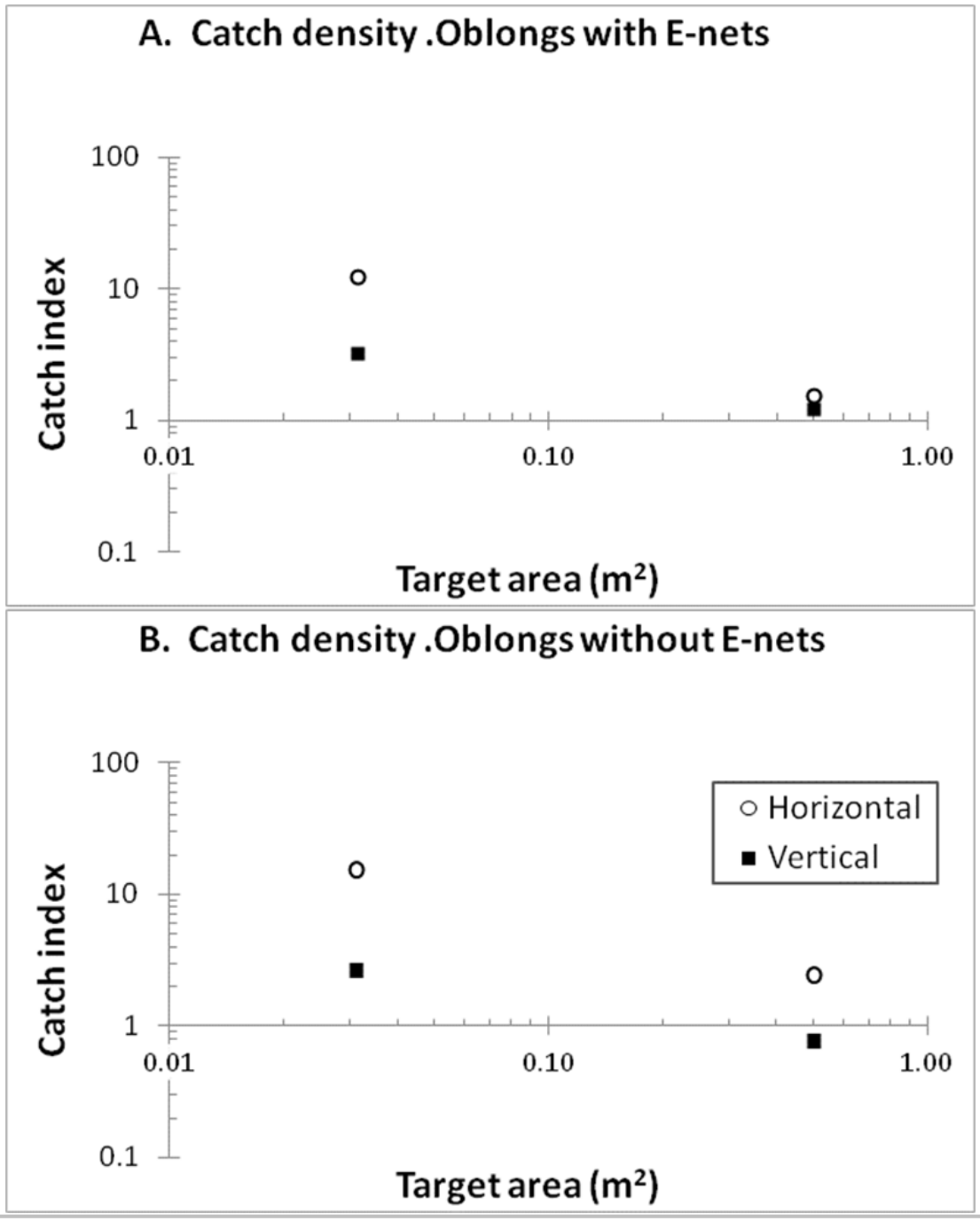


Figure 5-6: ***Proportional catch of G. f. quanzensis on rectangular targets.*** Mean catch density (*G. f. quanzensis* /m^2) expressed as a proportion of that from a standard target for *G. f. quanzensis* attracted to vertical and horizontal oblong targets. Targets were flanked by E-targets in A but not in B. The horizontal lines denotes the catch index of the Standard E-target (1 m^2)

5.4. Effect of the vegetation in host location (exp. D)

Both factors, the visibility of the targets and the CO_2 bait, had very significant effects in the catches of *G. f. quanzensis* ($F_{1,61}$ = 30.2, P <0.001; and $F_{1,62}$ = 18.3, P <0.001, respectively). However, the results did not show any significant interaction between both factors ($F_{1,60}$ = 0.8, *ns*) (Table 5-1). Significant differences in the landing response were not observed.

+/- CO_2	Hidden	Visible
+	6.1 (±1.37)	18.2 (±3.78)
-	1.7 (±0.47)	7.7 (±1.68)

Table 5-1: ***Effect of the visibility of visual baits in the catch of*** **G. f. quanzensis.** 'Hidden' targets were concealed with enclosures made with branches and leaves. One visible target and one hidden target were baited with CO_2 (1 L/min). Mean catches for each treatment are accompanied by the SE.

5.5. Assessment of different target designs

No significant differences in the landing response were observed among the experiments that included e-nets.

5.5.1 Traps vs targets of different sizes (exp. E)

The results showed that the bigger target caught consistently more tsetse than the small one, 5.6 times more when both targets were compared with their respective flanking net ((P<0.001), and 11.4 times when the targets without flanking net were compared (P<0.001) (Table 5-2).

Treatment	MALES		FEMALES	
	Mean	Inx	Mean	Inx
(A) 	1.9 (0.46± 0.117)	**1.0**	3.2 (0.62± 0.087)	**1.0**
(B) 	1.2 (0.34± 0.117)	**0.8**	0.8 (0.25± 0.087)	**0.2** **
(C) 	0.4 (0.16± 0.117)	**0.2**	0.5 (0.19± 0.087)	**0.2** ***
(D) 	0.1 (0.05± 0.117)	**0.1** *	0.1 (0.03± 0.087)	**0.0** ***
(E) 	1.8 (0.45± 0.117)	**1.0**	2.3 (0.52± 0.087)	**0.7**
(F) 	0.6 (0.20± 0.117)	**0.3**	0.5 (0.18± 0.087)	**0.2** ***

Table 5-2: ***Effect of size in the visual responses of*** **G.f. quanzensis.** Mean (detransformed mean±sed). Idx: Catch index. Asterisks indicate that the index is significantly different from unity at the P<0.05 (*), P<0.01 (**) or P<0.001 (***) levels of probability. Targets (E-targets) and flanking nets (E-nets) were electrified

5.5.2. Shape (exp. F)

Horizontal oblongs were consistently more attractive than vertical ones, although the differences were clearly significant when the targets were compared without E-nets, only. Without E-nets, the big and small horizontal oblongs caught 2.9 times (8.0 tsetse/day±0.16 horizontal big target) and 5.8 times (3.3 tsetse/day±0.16 small target), respectively, more tsetse than the vertical versions of the targets (2.7 tsetse/day±0.16 and 0.6±0.16 for the big and small targets, respectively). In the experiment with E-nets, the difference in the shape was significant for the small target only, the mean of male and female catches being 3.1

tsetse/day±0.27 for the horizontal small target and 0.8 tsetse/day±0.27 for the vertical small target (Table 5-3).

However, as the trend is consistent throughout, the data were pooled and reanalysed, considering 'shape' (*i.e.* vertical or horizontal) as a factor. This analysis showed that in both experiments, with and without E-nets, the horizontal targets attracted significantly more tsetse than the vertical oblongs (Table 5-4).

Treatment	MALES			FEMALES		
	Mean	Inx	Horz/Vert	Mean	Inx	Horz/Vert
 (A) H1.0×0.5m E-target +V1.0×0.5m E-net	3.7 (0.67± 0.102)	**1.5**	1.5	2.5 (0.54± 0.096)	**0.6**	1.0
 (B) H0.5×0.125m E-target +V 1.0×0.5m E-net	2.6 (0.55± 0.102)	**1.0**		2.4 (0.53± 0.096)	**0.6**	
 (C) V0.5×0.125m E-target +V 1.0×0.5m E-net	1.6 (0.41± 0.102)	**0.6**	3.5 ***	1.6 (0.42± 0.096)	**0.4**	4.0 ***
 (D) V0.5×0.125m E-target +V 1.0×0.5m E-net	0.4 (0.16± 0.102)	**0.2** ***		0.4 (0.15± 0.096)	**0.1** ***	
 (A') H1.0×0.5m E-target	4.3 (0.72± 0.084)	**2.2** *	3.4 **	3.7 (0.67± 0.086)	**0.7**	3.1 **
 (B') V1.0×0.5m E-target	1.3 (0.35± 0.084)	**0.6**		1.2 (0.34± 0.086)	**0.2** ***	
(C') H0.5×0.125m E-target	1.8 (0.44± 0.084)	**0.9**	6.3 **	1.4 (0.39± 0.086)	**0.3** **	4.5 *
(D') V0.5×0.125m E-target	0.3 (0.11± 0.084)	**0.2**		0.3 (0.12± 0.086)	**0.1** ***	

Table 5-3: ***Effect of shape in the visual responses of*** **G.f. quanzensis.** Mean (detransformed mean±sed). Idx: Catch index. Horz/Vertical: Mean catches of horizontal target divided by mean catches of vertical target. Asterisks indicate that the index is significantly different from unity at the P,0<05 (*), P<0.01 (**) or P<0.001 (***) levels of probability. Targets (E-targets) and flanking nets (E-nets) were electrified

	SHAPE	MEAN	Hori/Vert
With Flank Net	Horiz	4.4 (0.74± 0.092)	2.0 **
	Vert	2.2 (0.51± 0.092)	
Without Flank Net	Horiz	5.2 (0.80± 0.069)	3.7 ***
	Vert	1.4 (0.38± 0.069)	

Table 5-4: ***Pooled analysis of tsetse responses to oblongs.*** Mean (detransformed mean±sed). Horz/Vertical: Mean catches of horizontal target divided by mean catches of vertical target. Asterisks indicate that the index is significantly different from unity at the P<0.05 (*), P<0.01 (**) or P<0.001 (***) levels of probability

5.5.3. Colour

Black and blue targets, and traps (exp. G)

The results showed that the pyramidal trap caught significantly less tsetse than the Standard target and the black oblong, 33 (Table 5-5). The black oblong (A) was the device with the highest catch, although the differences in the catch were significant only when compared with the pyramidal trap (C) for females (Table 5-5).

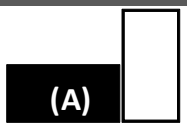
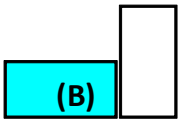
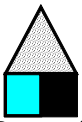
Treatment	MALES		FEMALES	
	Mean	Inx	Mean	Inx
 (A) H1.0×0.5m black E-target +V1.0×0.5m E-net	2.6 (0.56± 0.086)	**1.5**	3.4 (0.65± 0.094)	**1.5**
 (B) H1.0×0.5m blue E-target +V1.0×0.5m E-net	1.3 (0.37± 0.086)	**0.7**	1.3 (0.37± 0.094)	**0.6**
 **(C)** Pyramidal	1.0 (0.31± 0.086)	**0.6**	0.4 (0.14± 0.094)	**0.2 ****
 **(D)** Biconic	1.2 (0.34± 0.086)	**0.7**	1.5 (0.41± 0.094)	**0.7**

Table 5-5: ***Effect of shape in the visual responses of*** **G.f. quanzensis.** Mean (detransformed mean±sed). Idx: Catch index. Asterisks indicate that the index is significantly different from unity at the P<0.05 (*) or P<0.01 (**). Targets (E-targets) and flanking nets (E-nets) were electrified

Relationship between colours and size (exp. H)

Significant differences were observed for females only, and between the small black target and the Standard, with mean catches of 1.0 tsetse/day (0.31±0.101) and 3.5 tsetse/day (0.65±0.101) respectively (Table 5-6).

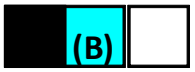
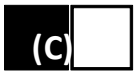
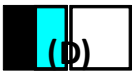
Treatment	MALES		FEMALES	
	Mean	Inx	Mean	Inx
 (A) H1.0×0.5m black E-target +V0.5×0.5m E-net	2.1 (0.49± 0.084)	**1.0**	3.0 (0.60± 0.101)	**0.8**
 (B) H1.0×0.5m black/blue E-target +V0.5×0.5m E-net	2.7 (0.57± 0.084)	**1.3**	2.1 (0.50± 0.101)	**0.6**
 (C) 0.5×0.5m black E-target +V0.5×0.5m E-net	2.0 (0.47± 0.084)	**0.9**	1.0 (0.31± 0.101)	**0.3 ***
 (D) 0.5×0.5m black/blue E-target +V0.5×0.5m E-net	1.3 (0.37± 0.084)	**0.6**	2.1 (0.49± 0.101)	**0.6**

Table 5-6: ***Relationship between colours and size.*** Mean (detransformed mean±sed). Asterisks indicate that the index is significantly different from unity at the P<0.05 (*), P<0.01 (**) or P<0.001 (***) levels of probability. Targets (E-targets) and flanking nets (E-nets) were electrified

Monochromatic target, vs. bicolour with vertical coloured strips (exp. I)

The results showed that the black/blue target and the black/blue/black target caught significantly more tsetse than the Standard, although the differences were significant for males, only (Table 5-7).

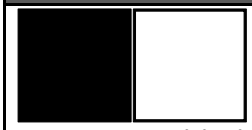
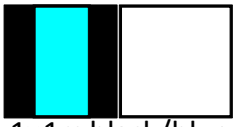
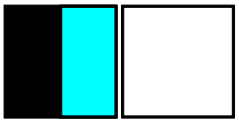
Treatment	MALES		FEMALES	
	Mean	Inx	Mean	Inx
 **(A)** 1×1m black E-target +1×1m E-net	3.2 (0.63± 0.056)	**1.0**	3.6 (0.66± 0.144)	**1.0**
 **(B)** 1×1m black/blue/black E-target +1×1m E-net	5.6 (0.82± 0.056)	**1.7 ***	3.3 (0.63± 0.144)	**0.9**
 **(C)** 1×1m black/blue E-target +1×1m E-net	5.8 (0.82± 0.056)	**1.8 ***	5.3 (0.80± 0.144)	**1.5**

Table 5-7: ***Monochromatic target, vs. bicolour, vs. tricolour with vertical coloured strips.*** Mean (detransformed mean±SED). Asterisks indicate that the index is significantly different from unity at the P<0.05 (*). Targets (E-targets) and flanking nets (E-nets) were electrified

Double-striped bicolour, vs. triple-striped bicolour (exp. J)

No significant differences were observed in this experiment after eight replicates (*n*=232). Mean catches of male and female *G. f. quanzensis* were identical for treatments (A) and (B), being 5.9 tsetse/day (0.84±0.089), and slightly lower for treatment (C), with 4.3 tsetse/day (0.72±0.089) (Table 5-8).

Treatment	MALES			FEMALES		
	Mean		Index	Mean		Index
 **(A)** H1×0.5m black/blue/black E-target +0.5×0.5m E-net	3.0	(0.60± 0.099)	**2.1**	2.7	(0.57± 0.095)	**0.9**
 **(B)** H1×0.5m black/blue E-target +0.5×0.5m E-net	2.8	(0.58± 0.099)	**2.0**	3.3	(0.63± 0.095)	**1.1**
 **(C)** 0.5×0.5m black/blue E-target +0.5×0.5m E-net	1.9	(0.46± 0.099)	**1.3**	2.4	(0.53± 0.095)	**0.8**

Table 5-8: ***Bicolour, vs. tricolour with horizontal coloured strips.*** Mean (detransformed mean±SED). Targets (E-targets) and flanking nets (E-nets) were electrified

Vertical coloured strips, vs horizontal coloured strips (exp. K)

The experiment was replicated eight days (*n*=226). No significant differences were observed in this experiment; thus, the mean catches of the combined male and female *G. f. quanzensis* were 4.3 tsetse/day (0.73±0.123) for treatment (A), 4.6 tsetse/day (0.75±0.123) for treatment (B) and 4.0 (0.70±0.123) for treatment (C) (Table 5-9).

Treatment	MALES		FEMALES	
	Mean	Index	Mean	Index
 (A) 1×1m black/blue/black E-target	2.7 (0.57± 0.118)	**1.5**	1.8 (0.45± 0.115)	**0.5**
 (B) H1×0.5m black/blue E-target +H0.5×0.25 E-net	2.3 (0.52± 0.118)	**1.2**	2.5 (0.55± 0.115)	**0.8**
 (C) H0.5×0.25m black/blue E-target +0.25×0.25 E-net	2.1 (0.50± 0.118)	**1.1**	2.0 (0.47± 0.115)	**0.6**

Table 5-9: Vertical coloured strips, vs horizontal coloured strips. Mean (detransformed mean±SED). Targets (E-targets) and flanking nets (E-nets) were electrified

5.5.4. Correlation between visual and olfactory cues (exp. L)

The experiment was replicated 12 days (*n*=337). For both target sizes, odour-baited targets obtained greater catches (males+females) than unbaited targets. Thus, mean catches of the combined male and female *G. f. quanzensis* were 3.8 tsetse/day (0.67±0.079) for the big unbaited target, compared to 7.9 tsetse/day (0.95±0.079) for the big baited target, and 3.6 tsetse/day (0.67±0.079) for the small unbaited target, compared to 6.6 tsetse/day (0.88±0.079) for the same target baited with pig odour. By sex, only the difference in the female catches between the baited and unbaited targets were significant (Table 5-10), The ratios of baited/unbaited targets for both target size were similar, *i.e.* 2.1 for the big targets and 1.8 for the small targets. Therefore, no correlation was observed between the size of the target and the addition of olfactory attractants (Table 5-10).

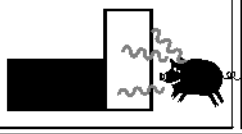
Treatment	MALES		FEMALES	
	Mean	Index	Mean	Index
(A) 	2.2 (0.50± 0.094)	**1.0**	1.3 (0.36± 0.088)	**1.0**
(B) 	4.2 (0.71± 0.094)	**1.9**	3.5 (0.66± 0.088)	**2.7** *
(C) 	2.0 (0.48± 0.094)	**0.9**	1.3 (0.37± 0.088)	**1.0**
(D) 	3.4 (0.64± 0.094)	**1.6**	3.4 (0.65± 0.088)	**2.6** *

Table 5-10: ***Effect of pig odour and target size..*** Mean (detransformed mean±sed). Asterisks indicate that the index is significantly different from unity at the P<0.05 (*). Targets (E-targets) and flanking nets (E-nets) were electrified

5.6. Discussion

The results described in this chapter showed that:

i. **Size:** The numbers of *G. f. quanzensis* attracted to a bait is influenced by the bait size and shape. Thus, as the target increases from 0.06 m^2 to 0.25 m^2, the catch doubles. Further increases up to 1 m^2 in size do not appear to increase the catch significantly. Targets of about 0.06 m^2 are likely to provide the best ratio of number of tsetse killed per square meter of target (about 20 tsetse/m^2, compared with 1.0 tsetse/m^2 for the 1 m^2 target, 3.5 tsetse/m^2 for 0.56 m^2 target and 6.1 tsetse/m^2 for 0.25 m^2 target).

ii. **Shape:** Catches of *G. f. quanzensis* with horizontal target were 3-6 times greater than with vertical targets

iii. **Effects of vegetation if host location:** CO_2 increased the catch of *G. f. quanzensis* 3.6-fold for hidden targets, and 2.4-fold for visible targets, although the difference was not statistically significant.

iv. **Targets *vs.* traps:** Targets of 0.5 × 0.25 m with a flanking net of 0.25 × 0.25 caught about 18 times more *G. f. quanzensis* per square metre than pyramidal or biconical traps.

v. **Target design suggested for control operations:** Targets of 0.06 m^2 were the most cost-efficient per unit of material. The addition of a flanking net of the same size would offset the relatively low landing response of small targets.

5.6.1. Effect of size and shape

The results showed that for *G. f. quanzensis*, the numbers of tsetse attracted to a bait is influenced by the bait's size and shape. For example, E-nets placed next to very small objects (*e.g.* 0.01 m^2) obtained the same catch as E-nets without a target, suggesting that *G. f. quanzensis* do not detect objects of that size. As the object increases from 0.06 m^2 to 0.25 m^2, the catch doubles but further increases up to 1 m^2 in size do not appear to increase the catch significantly. Therefore, discarding the target size that is too small to show significant differences in the catches with no-target (*i.e.* about 0.01 m^2), relatively small targets of about 0.06 m^2 are likely to provide the best ratio of number of tsetse killed per square meter of target. This result is in agreement with previous studies of size responses for *G. f. fuscipes*, in which a similar target size was suggested as the most cost-effective (Lindh *et al.*, 2009). Using similar arrangements of E-targets, Lindh *et al.* (2009) showed that targets 16 times greater than 0.25 × 0.25 m targets (*i.e.* 1 × 1 m) merely doubled the catches of *G. f. fuscipes*.

A possible criticism about the size experiment using non-electrified targets, is that results were based on the number of flies killed on the E-nets. In this way, the experiment did not show the proportion of tsetse that would alight on the target, but catches just gave an indication of the effect in the attraction elicited by visual stimulus of different sizes. This experiment was completed with others, in which the landing responses, in addition to the attraction, were considered.

The relatively small effect of the size was confirmed in other experiments carried out with E-targets, where the numbers of tsetse landing on the target were also taken into account. For example, targets of 0.1 m^2 caught about five times more tsetse per square metre of

cloth than targets of 1 m^2 (exp. E), and targets of 0.06 m^2 caught about 16 times more tsetse per square metre of cloth than targets of 0.5 m^2 in exp. F and about five times more in exp J.

The experiments showed that catches of horizontal oblongs were 3-6 times greater than catches of upright targets. This was consistent with studies on Morsitans-group of tsetse (Vale, 1974e; Torr, 1989). For example, Vale (1974e) found that horizontal targets were about three times more effective catching *G. morsitans* than vertical oblongs. Torr (1989) suggested that this behavioural pattern might help tsetse to discriminate hosts (*i.e.* warthog, buffalo, etc.) from the environment. The same hypothesis could apply to *G. fuscipes*, which feeds largely on monitor lizard (Mohamed-Ahmed & Odulaja, 1997; Clausen *et al.*, 1998), a 'horizontal-shaped' host.

5.6.2. Effect of the vegetation in host location

We have shown in this chapter the effect of size in the catches on *G. f. quanzensis*, and in chapter 3 the responses of the same species to carbon dioxide. As other species of the Palpalis-group, *G. f. quanzensis* occupies riverine habitats, where dense vegetation imposes a limitation to locate hosts. In experiment D (Table 5-1) we aimed to assess whether or not in conditions where visibility is limited, host olfactory cues played a more important role in the host location. The hypothesis was tested trying to mimic artificial visual baits, with and without odour (carbon dioxide) in two conditions: (i) when the target was visible, and (ii) when the model was partially covered. As expected, the visibility had a large effect in the catches, being 3-5 times greater when targets were visible compared to the catches of concealed targets. Consistent with previous results (see chapter three), the addition of CO_2 increased significantly the catch 2-4-fold. Although the increase in the catch with CO_2 was 3.6-fold for hidden targets and only 2.4-fold for visible targets, the difference was not statistically significant. That is, there is not statistical evidence to support the hypothesis that *G. f. quanzensis* rely more on olfactory cues when the visual bait is partially hidden in the vegetation.

5.6.3. Assessment of different target designs

These series of experiments aimed to test different target designs that could be used in a control campaign against tsetse. The results showed:

Horizontal oblongs were significantly more attractive for *G. f. quanzensis* than vertical targets. This is of great importance in the design of control devices for this species, as targets currently used in west Africa are vertical oblongs or squares (Laveissière *et al.*, 1987a). The horizontal shape can be further improved by combining blue and black cloth, and with the addition of flanking nets. For example, the blue-black 0.5 × 0.25 m with a flanking net of 0.25 × 0.25 m caught almost as many tsetse as the Standard (Catch Index=0.8). The area of this small target with the flanking net is 11 times smaller than the Standard, and therefore, it caught about eight times more tsetse per square metre of cloth than the Standard did.

Similarly, the Standard target caught 5.6 times more tsetse than the tiny 0.25×0.25 m black screen, when both targets operated with flanking nets, despite having 16× more surface. The effect of size in the catch efficiency for *G. f. quanzensis* were consistent with results obtained in Kenya for *G. f. fuscipes*, where the catches obtained with small targets (*i.e.* 0.25×0.25 m) were half of the catches obtained with big ones (*i.e.* 1×1 m), whereas the target surface was about $1/16^{th}$ (Lindh *et al.*, 2009). Consistently with the 'size experiments' explained above, these results suggest that the correlation between the size and the numbers of tsetse attracted to the target is not linear, and small screens attract comparatively more flies per area. When the catches of both targets were compared without flanking nets, the 1.0×1.0 m target collected 11.4× more flies than the 0.25×0.25 m, suggesting that the landing response increases with the area of the target. However, the differences in the landing responses observed among the treatments were marginal. Perhaps the relatively low catches gave insufficient statistical power to detect the differences. In control operations, the relatively poorer landing response of '*tiny targets*', compared to big ones, could be offset by placing an insecticide-impregnated flanking net of the same size next to the target. The flanking net would kill a proportion of the flies than are attracted by the visual cue (*i.e.* target) but do not alight on it.

Conversely, for Morsitans-species (*e.g. G. morsitans* and *G. pallidipes*) targets smaller than 1.7 m wide × 1.0 m high were much less efficient and were not recommended (Vale,

1993b). Vale (1993b) suggested that two flanking nets of 0.5 m wide × 1.0 m high, placed on the sides of a target of 0.7 m wide × 1.0 high – for a total size target+flanking nets of 1.7 m wide × 1.0 m high – should be as effective for control as an all-black cloth target 1.7 m wide. For *G. morsitans* and *G. pallidipes* the added panels of net are hardly better than added cloth panels of about the same size, since the extra visual stimulus improves significantly the landing responses. Laveissière *et al.* (1987a) working with *G. p. palpalis* indicated that large panels of net used to flank targets of about one metre high are prone to damage and suggested to substitute the nets by solid black cloth. However, small flanking nets to operate with *tiny targets* are likely to be more resistant to the field conditions.

In our experiments, the differences between the landing response of the Standard and the entry response in the biconical trap were not significant, it is remarkable that the trap caught 9.5% of the total catch, whereas the target efficiency was 32.2%.

5.6.4. Traps vs targets

Treatments in different experiments can be compared expressing the catch as a proportion of the catch obtained with the Standard (known as catch index). Comparing the catch index of two treatments that were tested in two different experiments cannot be statistically conclusive, as factors such as site or days cannot be included in the model. However, using the catch index to compare two treatments that were tested in two different experiments is useful to provide an indication of their relative performance. For example, the catch index obtained with a small target (0.25 × 0.25 m with a flanking net of 0.25 × 0.25 m; Table 5-6, treatment C) was 0.6, compared to 0.4 catch index for a pyramidal trap (Table 5-5, treatment C) . As pyramidal traps use about 1.5 m^2 of fabric, the small target was 18 times more efficient. Biconical traps required about 3 m^2 for their manufacture. Using the catch index in Table 5-6 for the target (treatment B) and the catch index of Table 5-5 (treatment D) for the biconical trap, the former was approximately 20 times more cost-effective than the latter. Standard targets (1 × 1 m) with the flanking nets (1 × 1 m) were about twice as cost-effective as pyramidal and biconical traps. Studies in Kenya with *G. f. fuscipes* compared the efficiency of different control devices, defining "efficiency" as the relative catch per unit of area of the material used to manufacture the target or trap (Lindh

et al., 2009). These studies reported that targets of 1 × 1 m were about 2.7 times more efficient than biconical traps.

The low efficiency of the traps can be offset in control campaigns by treating them with insecticide, which transforms a retaining device into a killing tool. This practice improves the performance of the trap, when the collection of the flies is not required (Laveissière *et al.*, 1980; Laveissière & Grébaut, 1990; Lancien, 1991a).

CHAPTER SIX

VISUAL RESPONSES OF *GLOSSINA PALPALIS*

6.1. Introduction

6.1.1. Landing or colliding

The introduction in chapter five covered the literature review about visual responses of the Palpalis-group of tsetse, and included the species studied in the present chapter: *G. palpalis*. As mentioned in chapter five, during the 1970s and 1980s, profuse literature was published describing the visual responses of riverine tsetse, including *G. palpalis* (Challier & Laveissière, 1973; Challier *et al.*, 1977; Gouteux *et al.*, 1981; Gouteux & Noireau, 1986; Laveissière *et al.*, 1987a; Laveissière *et al.*, 1987b; Green, 1988, 1989) and *G. fuscipes* (Lancien, 1981; Dagnogo & Gouteux, 1985; Gouteux & Lancien, 1986). These were the decades when most of the targets (Laveissière *et al.*, 1987a) and traps (Challier & Laveissière, 1973; Challier *et al.*, 1977; Gouteux *et al.*, 1981; Lancien, 1981; Dagnogo & Gouteux, 1985; Gouteux & Lancien, 1986; Gouteux & Noireau, 1986), used currently to control riverine tsetse, were developed. Studies in the francophone countries of West and Central Africa referred above were 'technological research' – trying to develop the most effective tool to control tsetse – rather than 'biological studies' to describe the tsetse behaviour behind the observations. As a result of these works, the experiments carried out by Laveissière *et al.* (1987a) led to the development of the 1 m^2 black/blue/black target, used nowadays in areas of West Africa, where *G. palpalis* is abundant. The authors compared different target sizes, combination of colours, shape and fabric quality in a large

series of experiments. The studies were complemented by Green (1988, 1989). Results from both, Laveissière *et al.* and Green were consistent, finding that: (a) pthalogen blue was the most attractive colour; (b) when blue and black were combined, about 70% of the flies landed on the black; and (c) catches were improved when fine black net flancks the sides of a blue target.

Laveissière *et al.* (1987a) assumed that tsetse cannot see the black colour, and tsetse collide with black targets when they are attracted by the blue colour. Accordingly, they designed an experiment with black/blue/black targets (Laveissière *et al.*, 1987a). In this experiment, they changed the fabric of the black section, turning from opaque (cotton/polyester), into semi-transparent polyamide fabric, and finally a fine transparent mosquito net. They found that the target flanked by mosquito net caught significantly more tsetse than the others did, suggesting this design as the most efficient. For the authors, tsetse caught in the opaque black targets did not try to land on it, but rather, they collided with the cloth trying to fly through it. Laveissière *et al.* (1987a) supposed that black colour is perceived by tsetse as open space.

Were Laveissière and his colleagues correct in their assumption? Can tsetse not see black objects? If they had included a black/blue/black target flanked with fine netting in their experiment, would they have found it more efficient than the other treatments? Do tsetse 'land' or 'accidentally collide' with black fabric? The authors compared several targets and suggested the one that killed more tsetse as the standard design to control *G. palpalis*. However, if contrary to their hypothesis, solid black targets were visible for tsetse, they would have combined too many factors to be able to assess their effects. For instance, by changing the external black strips to make targets more transparent, Laveissière *et al.* might have changed several factors: (i) first, they changed the transparency, which was the only factor to be tested; (ii) if the netting was invisible for the flies, they had changed the size of the nets, as flies would not be able see the external net flanking the blue target; (iii) similarly, they might have changed the shape of the target from a square to a vertical rectangle; (iv) for the same reason, they might have changed the combination of colours (as the black netting is supposed to be invisible for tsetse); (v) they might have compared catches from flanked targets (*i.e.* target + flanking net) with catches from a single target (*i.e.* target alone). They concluded that the differences in the catches were explained by the difference in the quality of the black fabric (*i.e.* from opaque to transparent). The conclusion was based on the hypothesis that black targets are invisible for tsetse;

conversely, if tsetse can see opaque black targets, Laveissière and his colleagues did not change one factor but five.

6.1.2. Inter-specific variation

Colour

The effect of the colour in the attraction of tsetse is probably the visual cue that has been studied the most extensively across different species. Studies of the response of Morsitans- (Green & Cosens, 1983; Mérot & Filledier, 1985; Green, 1993) and Palpalis-groups of tsetse (Green, 1986, 1988, 1990) shared similar experimental designs, which allowed to establish differences and similarities between different groups. The results of these works are reviewed in section 5.1.1.

Size and shape

As discussed above, most of the studies in western and central Africa to assess the visual responses of tsetse were essentially practical, looking at the development and improvement of control tools rather than at the factors affecting the behavioural responses. For example, experiments carried out by Laveissière *et al.* (1987a) in Côte d'Ivoire aimed to study the responses of *G. palpalis* to targets of different shapes and sizes. However, shape and size were combined in one factor; *i.e.*, they compared the catches of a 1 × 1 m with targets with the same height but reduced width. Behavioural responses of *G. morsitans* and *G. pallidipes* to size and shape, as independent factors, have been studied since the 1970s (Vale, 1974e; Hargrove, 1980b; Torr, 1989; Vale, 1993b), whereas similar works on *G. fuscipes* are more recent (Lindh *et al.*, 2009), and before the current study, and with the exception of the work done by Rayaisse *et al*. (2011), nothing has been published on *G. palpalis* (see section 5.1.1.).

6.1.3. Aims of the study

The aim of this chapter was to assess the responses of *G. palpalis* to visual stimuli, and compare the results with the response of other species to similar cues. Results will be used

to improve cost-efficiency of targets to control tsetse in western Africa. Accordingly, a series of experiment were designed to explore the response of *G. palpalis* to objects of different sizes and shape. Additionally, the role of the vegetation in the location of hosts and the effect of fine nets in the catches of *G. palpalis* were investigated. In order to compare the results with those obtained for *G. fuscipes*, the design of most of the experiments described in this chapter and chapter five are similar.

Part of the result reported in this chapter were published in Tirados *et al.* (2011) (Annex III). Subsequently, additional experiments on *G. f. quanzensis* , *G. f. fuscipes* and *G. f. martini* were carried out in DRC, Kenya and Tanzania, and reported in Esterhuizen *et al.* (2011) (Annex V).

6.2. Materials and methods

6.2.1. Study sites

Studies of *G. p. palpalis* were carried out between January and March 2009 at selected sites near Azaguié (Côte d'Ivoire) (see chapter two, section 2.1.2). One experiment to assess the 'site effect' was performed during April 2011 in Orodara (Burkina Faso), where *G. p. gambiensis* is abundant (see chapter two, section 2.1.1.).

6.2.2. Collecting devices

Combinations of E-target operating with E-nets, and E-nets baited with inert targets were used to assess the responses of tsetse to various visual baits as described in chapter two (see sections 2.4.1. and 2.4.2.).

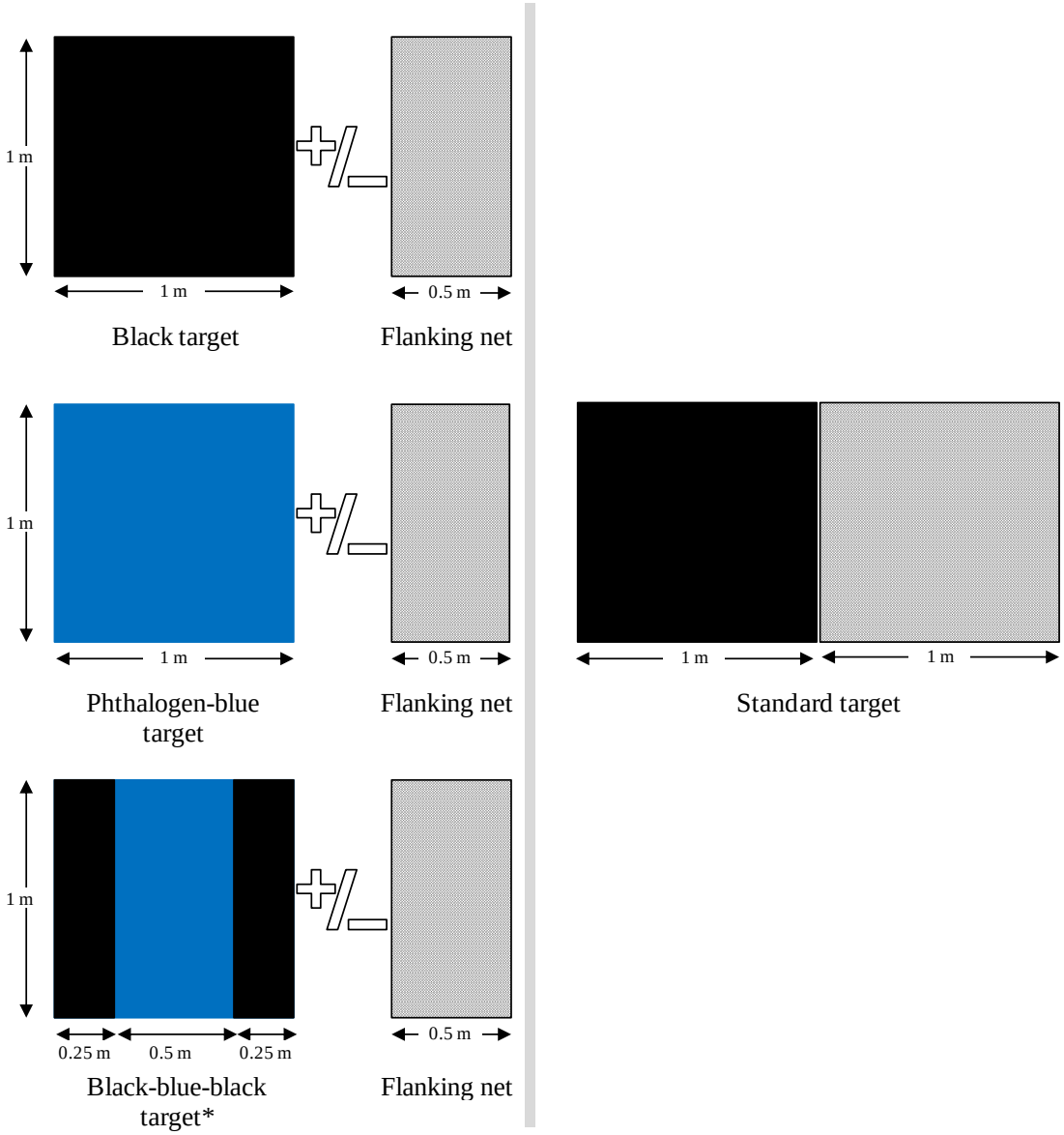
6.2.3. Experimental design

Different artificial baits were compared over 6-21 days in a series of replicated Latin squares of days × sites × treatments, as explained in chapter two. Experimental sites were at least 100 m apart. All experiments were carried out for 4 h, between 10:30 h and 14:30

h. Unless otherwise stated and to facilitate comparisons across species and experiments, all the setups included a Standard target, comprising a black E-target (1 m×1 m) flanked by an E-net (1 m×1 m).

Effect of flanking nets in the catch (exp. A)

As a follow-up experiment from Laveissière *et al.*'s (1987a) studies (see section 6.1.1.) we aimed to assess the effect of fine nettings, placed adjacent to targets, in the catches of *G. p. palpalis*. Targets with flanking nets could be an option in control operations to kill a proportion of the tsetse that are attracted to the visual cue (*i.e.* target) but do not land on it. The purpose of experiment A was to assess the landing responses of *G. p. palpalis* for three targets of the same size but different colour patterns. Additionally, as discussed in the introduction of this chapter, we tried to assess in the field whether or not tsetse is able to see black colour. Hence, an experiment was carried out to compare the catches of three different targets (Figure 6-1), with or without a netting panel of 0.5 m wide × 1 m high, placed adjacent to the E-targets. A Standard target (1 × 1 m black target + 1 × 1 m flanking net) was added in the experiment to complete a 7 × 7 Latin square. The experiment was replicated for 14 days.



*Similar to *Laveissière*'s target

Figure 6-1: ***Experiment A: Potential role of flanking nets in the catches:*** The experiment compared the catches of three E-targets (i.e. black, blue and black/blue/black, each of them with or without flanking net) and the standard target (1 × 1 m target + 1 × 1 m flanking net) in a 7×7 Latin-square design

Vertical vs. horizontal oblongs (exp. B)

The responses of *G. p. palpalis* to vertical and horizontal oblongs was assessed in Côte d'Ivoire by comparing the catches from E-targets that were: (i) 0.5×1.0 m (Figure 5-1A and 5-1B), (ii) 0.25×0.50 m or (iii) 0.125×0.25 m with their long axis arranged vertically or horizontally and the base on the ground. All E-targets were accompanied by an upright E-net of 0.5×1.0 m (Figure 5-1).

Vertical oblong vs. squares (exp. C)

The numbers of *G. p. palpalis* attracted to four black inert target of various size and shape were compared. The targets were: (i) 0.35 m wide×0.71 m high, (ii) 0.5×0.5 m (Figure 2-8), (iii.) 0.5 m wide×1.0 m high, and (iv) 0.71×71 m. Catches were obtained with an accompanying E-net (0.5 m wide×1.0 m high), placed adjacent to the inert targets.

Size (exp. D)

Similar to the experiment described in chapter five, the effect of target size was studied further by comparing the numbers of *G. p. palpalis* attracted to 'inert square targets' of decreasing size: (i) 1.0×1.0 m, (ii) 0.75×0.75 m, (iii) 0.5×0.5 m (Figure 2-8), (iv) 0.25×0.25 m, (v) 0.1×0.1 m, and (vi) no target. An E-net was placed adjacent to each target to assess the numbers of tsetse attracted to the inert targets.

Effect of the vegetation in host location (exp. E&F)

The ability of *G. p. palpalis* to locate hidden hosts was studied in Côte d'Ivoire following the same experimental design described in section 5.2.3 (see also section 2.6; experiment E). For *G. p. gambiensis* in Burkina Faso, the design was modified slightly: (i) a blend of octenol and 4-methylphenol was used as the olfactory bait, instead of CO_2; (ii) catches of two different E-targets were compared, when they were visible or hidden in a palisade, and when they were baited or unbaited with the mix of octenol and 4-methylphenol (see section 2.3 for dispensing methods). The designs of the targets are described in Figure 6-2. Therefore, the treatments were: (i) odour-baited and hidden 50NB[2] target; (ii) odour-baited and visible 50NB target; (iii) unbaited and hidden 50NB target; (iv) unbaited and visible 50NB target; (v) odour-baited and hidden 75NBN[3] target; (vi) odour-baited and visible

[2] **50NB:** 50 cm wide, net and blue target
[3] **75NBN:** 75 cm wide, net-blue-net target

75NBN target; (vii) unbaited and hidden 75NBN target; and (viii) unbaited and visible 75NBN target (experiment F). These two experiment did not include the Standard target.

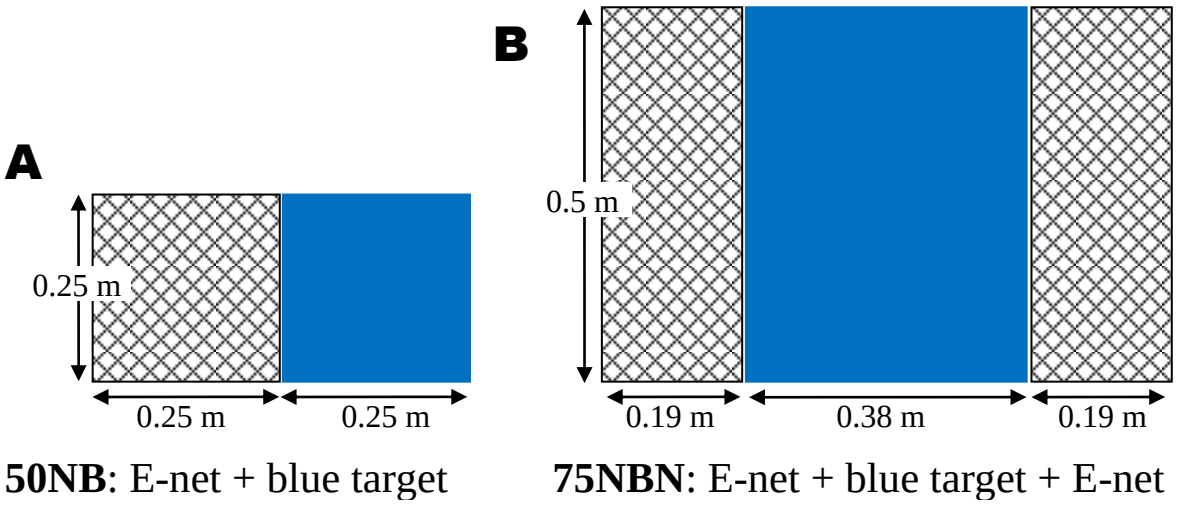


Figure 6-2: ***Experiment E:*** Diagram of E-targets used in Burkina Faso to explore the responses of *G. p. gambiensis* to hidden/odour-baited objects

6.2.4. Statistical analysis

The data were treated and analysed as described in chapter two (see section 2.8). Values for the mean catches, as well as the catch density (*i.e.* mean catches/area of target) are provided. No clear differences in the responses of male and female tsetse were observed, and therefore, catches of males and females were combined.

Catches

Detransformed means are reported accompanied by their respective transformed mean and standard error of the difference (SED) between means.

Catch density

To estimate the cost-effectiveness of targets of different sizes, catches obtained per unit area were provided (see section 5.2.4). To allow comparisons across experiments, catch densities were expressed as a proportion of the mean daily catch of the Standard target and this value is termed the Catch Density Index (see section 5.2.4).

Landing responses

Whenever E-nets were used with E-targets, landing responses were assessed. To assess whether target size and/or shape influenced landing response, the proportion of tsetse that landed on an E-target was quantified by expressing the catch from an E-target as a proportion of the total (E-target+E-net). These data were analysed by logistic regression as explained in chapter two (see section 2.8). The SE is asymmetric about the mean, and thus mean percentages are accompanied by the larger back-transformed SE. For all analyses, the level of significance was established at $P<0.05$.

In this chapter, two different approaches were used to estimate the landing response:

a) In experiments where E-targets operated with E-nets, the landing response (L) was defined as the proportion of tsetse killed in the E-target, compared with the total number of tsetse killed in the arrangement of electrified grids (*i.e.* E-target + E-net), *i.e.* L= A/(A+B) (Figure 6-3) This is the standard method used in this thesis. For example, in experiment A, this method was used as follows (Figure 6-3):
   - Black target: L = a/(a+b)
   - Blue target: L = d/(d+c)
   - Black/Blue/Black target: L= g/(g+h)

b) The approach described above assumes that all the tsetse killed on the E-net are flies that avoid the target. An argument against this approach is that this method does not give the chance for the flies to land on the target after circulating it, since they would be already killed on the E-net. In experiment A, treatments included E-targets operating alone, and in different treatments, the same E-targets accompanied by E-nets. This experiment design allowed the landing response to be expresed, also, as a proportion of the tsetse killed on each E-target operating without E-net, compared with the number of tsetse killed in the arrangement of E-grids (*i.e.* E-target + E-net) running as a different treatment, *i.e.* L= C/(A+B) (Figure 6-3). For example, in experiment A, this method was used as follows (Figure 6-3):
   - Black target: L = c/(a+b)
   - Blue target: L = f/(d+c)
   - Black/Blue/Black target: L= i/(g+h)

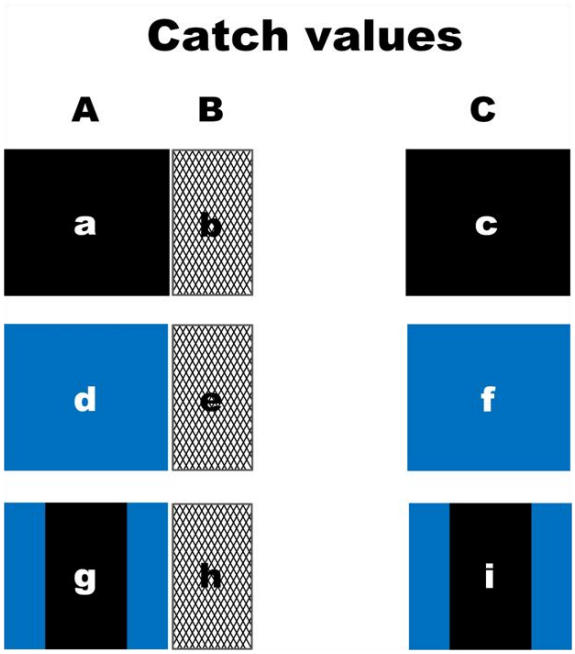


Figure 6-3: ***Data arrangement to estimate landing responses for* G. p. palpalis *in experiment A.***

(i) **Same arrangement of E-target+E-net:** Landing response (*L*) was defined as:
L = (A)/(A+B) expressed as a %, i.e.:
   a. $L_{black} = (a)/(a+b)$
   b. $L_{blue} = (d)/(d+e)$
   c. $L_{black\&blue} = (g)/(g+h)$

(ii) **E-target operating along *vs.* arrangement of E-target+E-net:**
L = (C)/(A+B) expressed as a %, i.e.:
   a. $L_{black} = (c)/(a+b)$
   b. $L_{blue} = (f)/(d+e)$
   c. $L_{black\&blue} = (i)/(g+h)$

6.3. Effect of flanking nets in the catch (exp. A)

In the absence of flanking nets, the black/blue/black target caught twice as many *G. p. palpalis* as the black target ($P<0.01$), and the latter almost four times more tsetse than the blue target ($P<0.001$, Figure 6-4). The addition of the flanking nets increased very significantly all the catches ($P<0.001$), although this increase differed between targets ($P<0.001$). The deployment of flanking nets increased the catches of the blue target about 20-fold, but only 3.5- and 4-fold the catches of the black and black/blue/black targets, respectively (Figure 6-4). The difference in the catches observed for the three targets in the absence of flanking nets disappeared when flanking nets were added, *i.e.* no significant difference was observed in the catches of the three targets operating with flanking nets (Figure 6-4). Similarly, the catch obtained with the Standard target did not differ significantly with any of the other three targets operating with flanking nets, but was significantly greater than the catches of the targets operating alone (Figure 6-4).

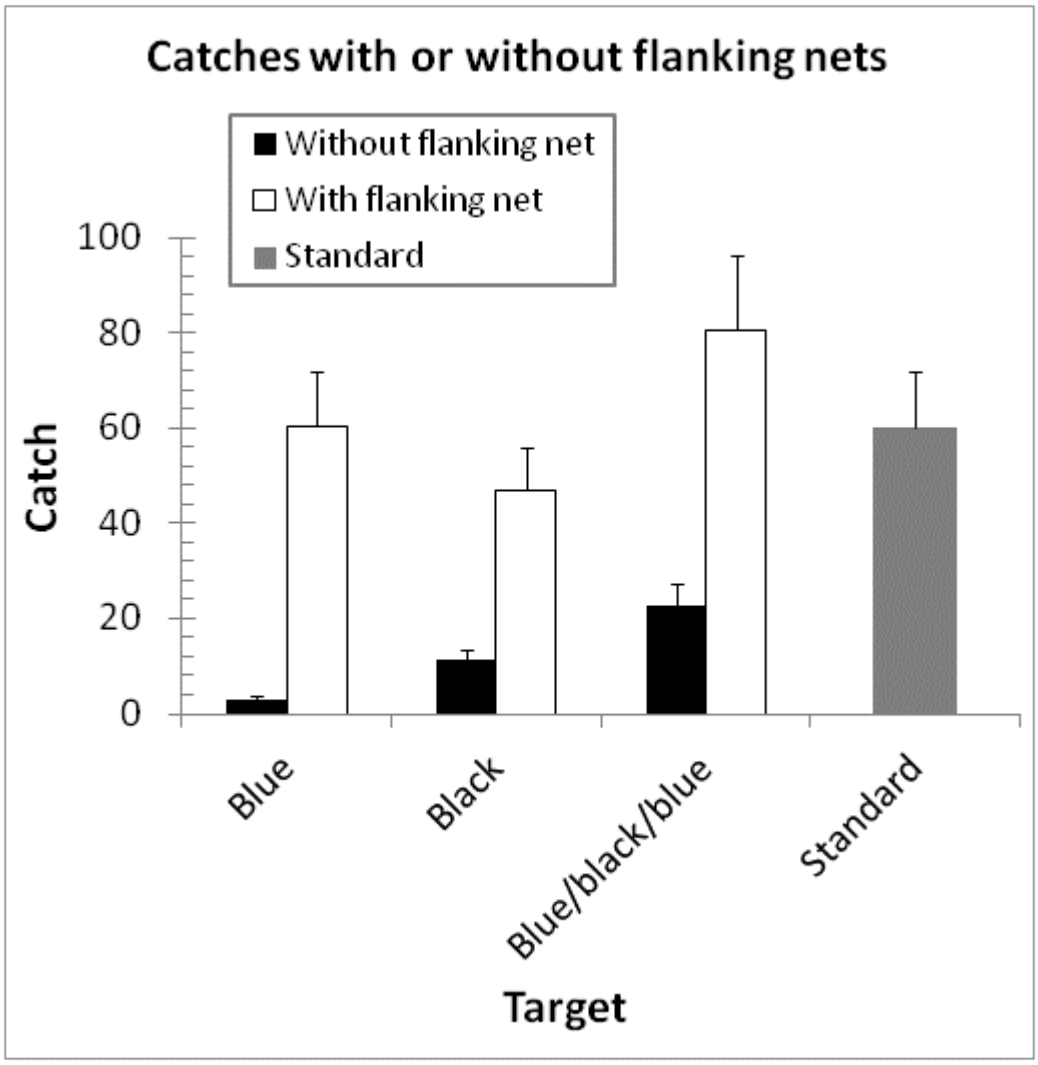


Figure 6-4: ***Effect of flanking nets in the catches:*** Detransformed mean catch of *G. p. palpalis* (+SED) from square targets (1 m^2) in absence (solid black bars) or presence (open bars) of flanking nets (0.5 m wide × 1 m high). The mean catch of the Standard target (1×1 m target + 1×1 m flanking net) is indicated in grey.

The results suggest that whereas all the targets attracted similar number of tsetse, they elicited different landing response. Thus, 'landing response' was defined in a first analysis as the proportion of tsetse obtained with a target operating with an E-net, compared to the catches obtained with the same target and its flanking net. The data showed that 21 and 25% of the tsetse were caught on the black and the black/blue/black target respectively, and the difference for both targets was not significant (Figure 6-5). This result contrasts with the landing responses obtained for the blue target, which was only 5% (P<0.001, Figure 6-5). The landing response obtained for the Standard target did not differ

significantly from the landing response obtained for the black and the black/blue/black targets, despite having a flanking net that doubled the area (Figure 6-5).

The definition of 'landing response' used above may underestimate the proportion of tsetse landing on the target, as explained in section 6.2.4. Consequently, 'landing response' was redefined as the proportion of tsetse obtained with a given target operating alone, compared to the catches obtained with the arrangement of a similar target and a flanking net (Figure 6-3).

The results showed that both approaches are consistent and give comparable estimations of the landing response (Figure 6-5).

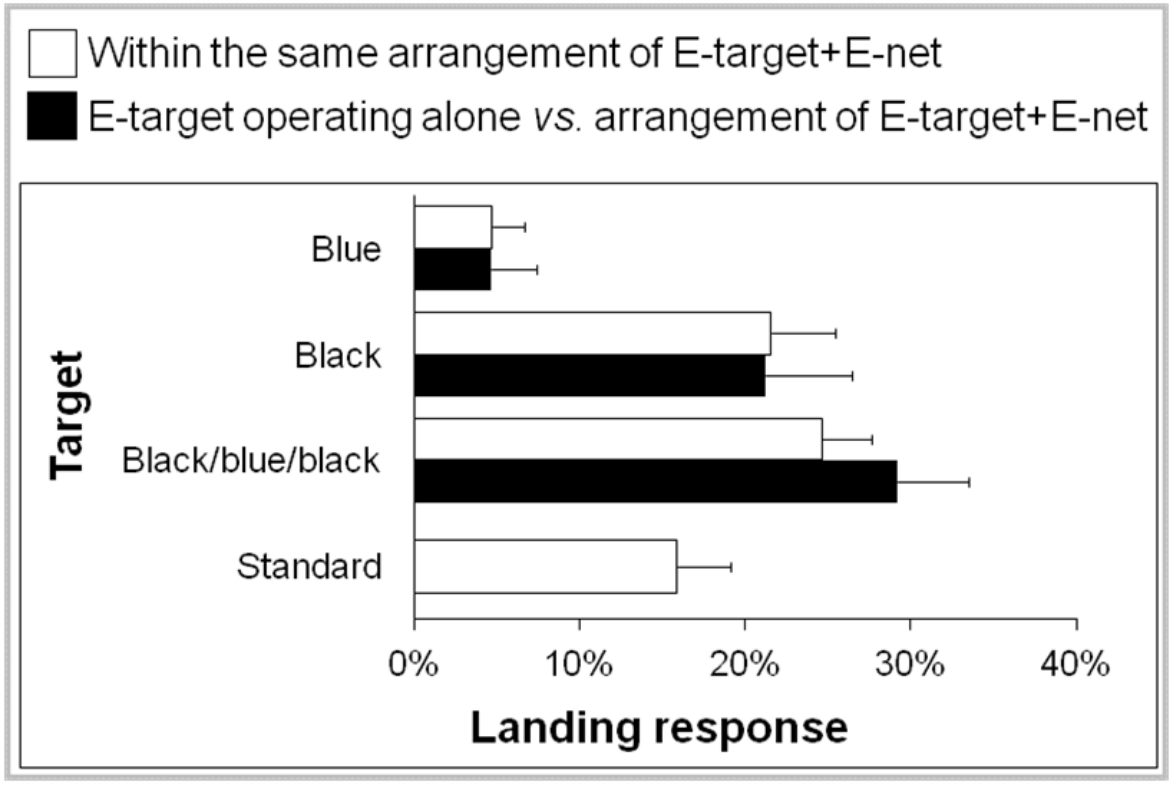


Figure 6-5: ***Landing response of*** **G. p. palpalis** ***for different targets:*** Mean of the proportion of *G. p. palpalis* that landed on the targets +SED. The landing response was calculated using two different approaches: (a) In open bars: as the proportion of *G. p. palpalis* obtained with a target operating with an E-net, compared to the catches obtained with the same target and its flanking net; (b) In solid bars: as the proportion of tsetse obtained with targets operating alone compared to the catches obtained with the arrangement of targets+flanking nets. All the targets were 1×1 m. E-nets used with black and black/blue/black were 0.5 m wide×1 m high. E-net in the Standard target was 1×1 m

6.4. Effects of size and shape (exp. B, C & D)

6.4.1. Vertical vs. horizontal (exp. B)

Vertical-oblong targets caught consistently more (1.4-1.8-fold) *G. p. palpalis* than horizontal ones of the same surface area (Figure 6-6); this contrast with results obtained for *G. f. quanzensis*, where horizontal oblongs were more attractive than vertical ones (see section 5.3.1.). The data were subjected to analysis of variance with shape and size specified as factors. Both factors had a highly significant effect on the catch (Shape: $F_{1,61}$ = 23.6, P<0.001; Size: $F_{2,61}$ =45.1, P<0.001) but there was no significant interaction between them ($F_{2,59}$ = 0.5, *n.s*). All oblongs caught significantly fewer tsetse than the Standard target, with the largest vertical oblong (area = 0.5 m^2) catching about half (64 tsetse/day) that of the Standard square target (121 tsetse/day).

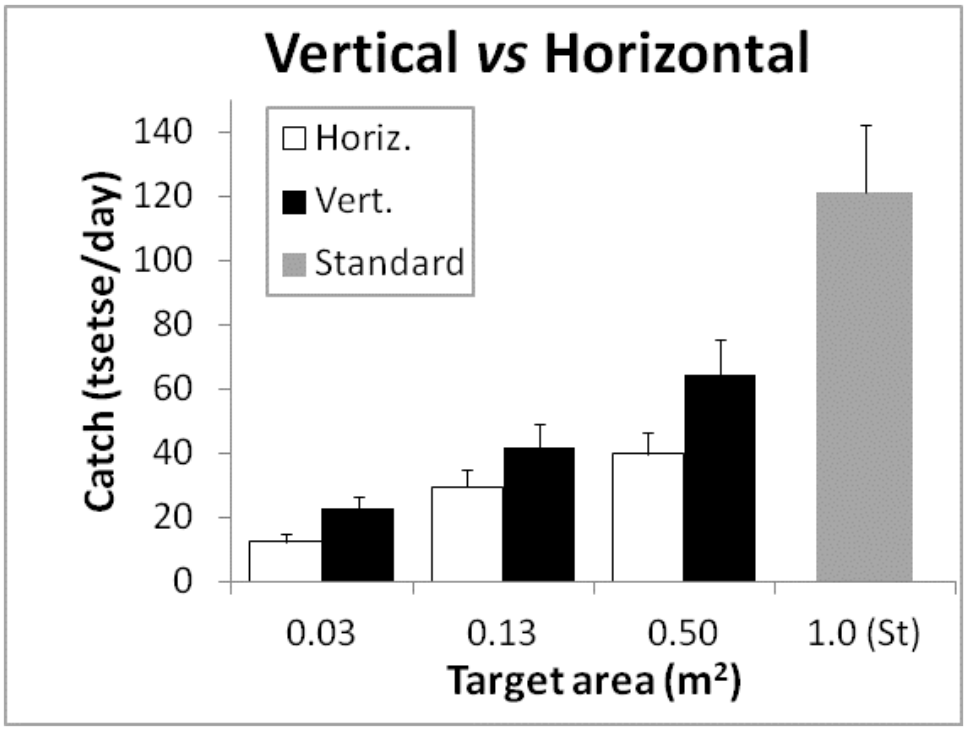


Figure 6-6: ***Comparative attraction of*** **G. p. palpalis** ***to vertical and horizontal oblongs.*** Detransformed mean catch of *G. p. palpalis* (+SED) from horizontal (open bars), or vertical (solid bars) oblongs or the Standard square (grey bars). Oblongs were 0.125×0.25 m (surface area = 0.03 m^2), 0.25×0.50 cm (0.13 m^2), or 1×0.5 m (0.5 m^2). All oblong targets were adjacent to an E-net, 0.5 m wide×1.0 m high. The Standard comprised a 1×1 m black E-target accompanied by a 1×1 m E-net.

The percentage of tsetse caught on the target also increased with target size but, for each size, the landing response was greater on the horizontal-oblong (Figure 6-7). Shape ($F_{1,61}$ = 18.7, P<0.001) and size ($F_{2,61}$ = 32.7, P<0.001) had a highly significant effect on the landing response but there was no interaction between them ($F_{2,59}$ = 0.9, *n.s*).

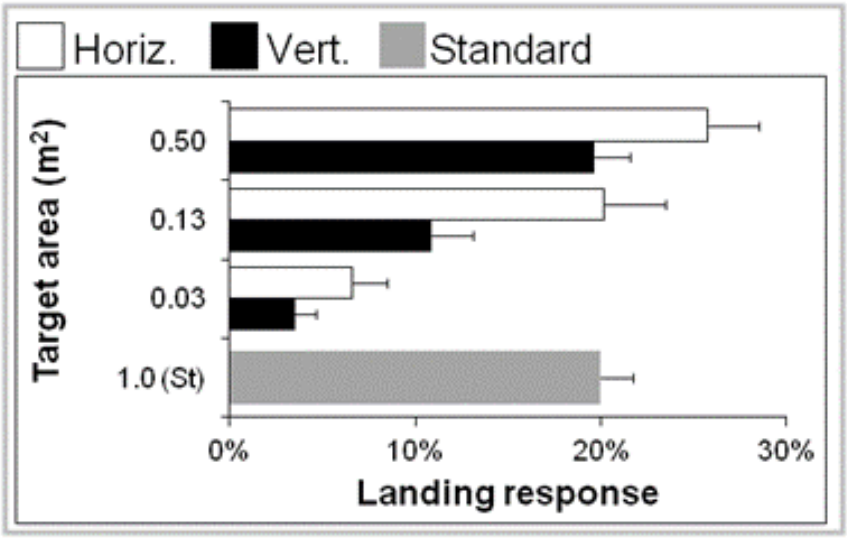


Figure 6-7: ***Landing response of*** **G. p. palpalis** ***to different shaped targets*** (+SE) from horizontal (open bars), or vertical (solid bars) oblongs or the Standard square (grey bars). Oblongs were 0.125×0.25 m (surface area = 0.03 m^2), 0.25×0.50 cm (0.13 m^2), or 1×0.5 m (0.5 m^2). All oblong targets were adjacent to an E-net, 0.5 m wide×1.0 m high. The Standard comprised a 1×1 m black E-target accompanied by a 1×1 m E-net.

6.4.2. Vertical vs. square (exp. C)

To test the influence of the shape, we compared the catches from vertical oblongs and squares of equivalent area. The results (Figure 6-8) show that there was no significant difference in the numbers attracted to squares and vertical oblongs of equal surface area ($F_{1,39}$ = 0.2, *n.s.*). Thus, square and vertical oblong shapes are equally attractive. The standard (1×1 m) target caught 67 tsetse/day compared to 47 tsetse/day for the 0.5 m^2 square target (*i.e.* 0.71×0.71 m) and 55 tsetse/day for the 0.25 m^2 one (*i.e.*, 0.5×0.5 m). Thus, while smaller targets caught fewer tsetse, the reduction was relatively slight (about 25%). In addition, one has to keep into consideration that in the Standard target, both, the target and the flanking net were electrified; conversely, in the other treatments only the flanking net was electrified.

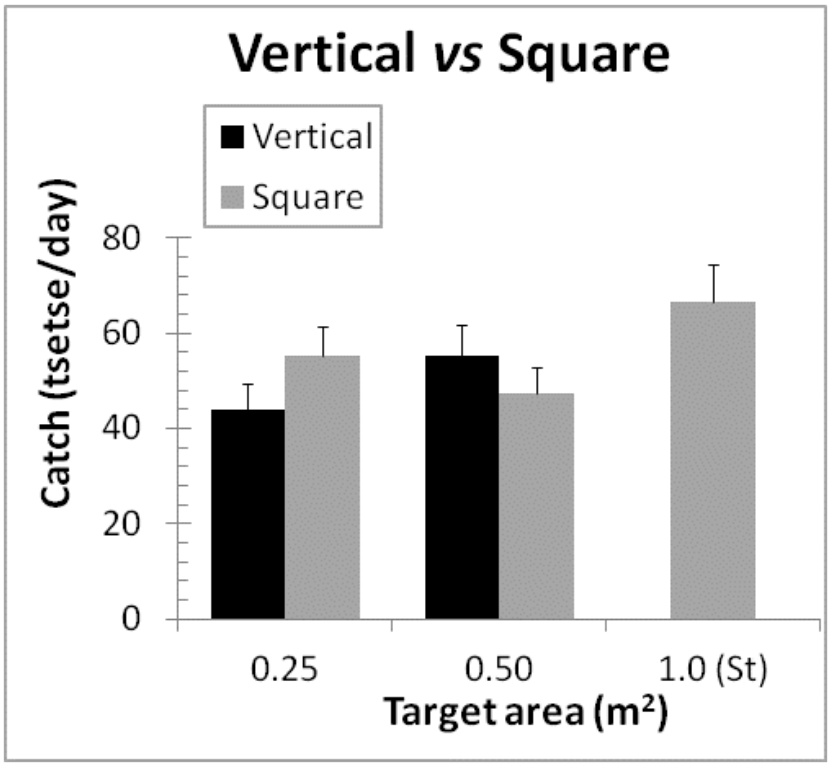


Figure 6-8: ***Comparative attraction of*** **G. p. palpalis** ***to vertical oblongs and squares.*** Detransformed mean catches (+SED) of *G. p. palpalis* attracted to the vicinity of vertical oblong (solid bars) or square (grey bars). Oblongs were 0.71×0.35 m (surface area = 0.25 m^2) or 1×0.5 m (0.5 m^2) and the matching square targets had dimensions of 0.5×0.5 m or 0.71×0.71 m, respectively. Vertical and horizontal objects were not electrified (inert targets); catches were obtained from an adjacent E-net (0.5 m wide×1 m high). The Standard target comprised one E-target (1×1m) and one E-net (1×1 m)

6.4.3. Effect of Size (exp. D)

As with *G. f. quanzensis* (see section 5.3.2) the effect of size was further examined by comparing the numbers of tsetse attracted to the vicinity of square targets of various size, ranging between 0.01 m^2 (0.1×0.1 m) to 1.0 m^2 (1×1 m).

The results (Figure 6-9) show that the effect of size for *G. p. palpalis* is very similar to that for *G. f. quanzensis* (see section 5.3.2), despite the large difference in the absolute size of catches: the catch increased with size but plateaued for targets with a surface area between 0.5 m^2 and 1 m^2. The difference in the absolute numbers observed for *G. f. quanzensis* (section 5.3.2.) and *G. p. palpalis* is merely a reflection of the total number of flies at each site (0.5-3 *G. f. quanzensis*/day vs. 8-59 *G. p. palpalis*/day). No significant difference

between the catches with the smallest target (0.01 m^2) and no target (*i.e.*, an E-net without any adjacent target) was observed.

As in chapter five, the 1 m^2 inert target caught fewer *G. p. palpalis* than the Standard, which also had a 1 m^2 E-target. This may be because the Standard target had a larger flanking E-net (1 m^2) and the target was electrocuted; by contrast, the inert 1 m^2 target was not electrified and was accompanied by a 0.5 m^2 E-net.

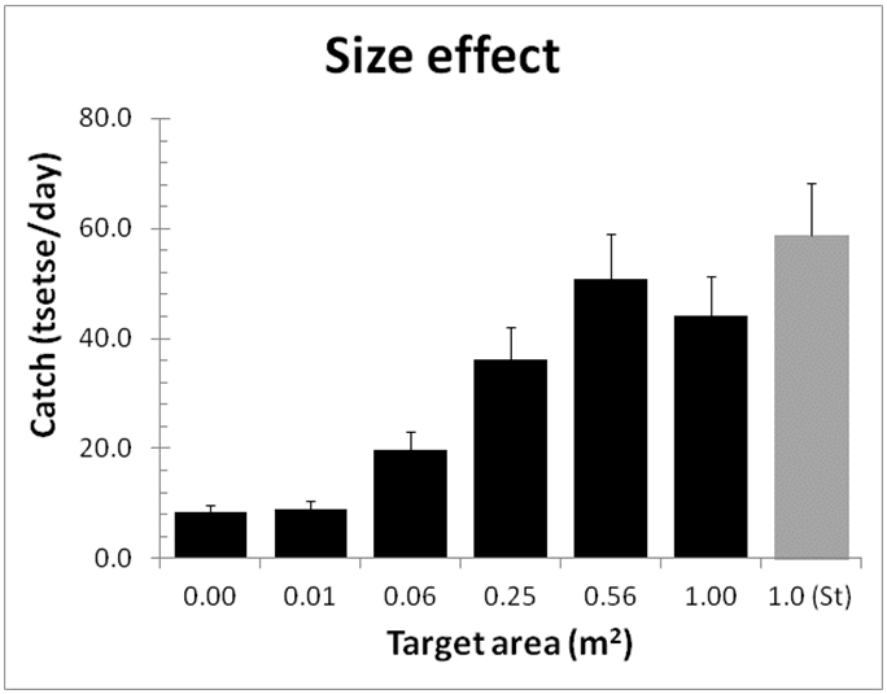


Figure 6-9: ***Attraction of* G. p. palpalis *to objects of different sizes.*** Detransformed mean catches (+SED) of *G. p. palpalis* attracted to square inert targets of various size. Inert targets were accompanied by an E-net 0.5 m wide×1 m high. 'St' is the Standard, comprising an E-target (1×1 m) accompanied by an E-net (1×1 m)

6.4.4. Catch density (exp. B, C & D)

As for *G. f. quanzensis* (see section 5.3.3), larger targets caught more *G. p. palpalis* but the increase was relatively small. For instance, increasing from a 0.06 m^2 to a 1 m^2 target only doubled the catch of *G. p. palpalis*. Consistent with *G. f. quanzensis*, in the experiment with squares (experiment D) the catch density decreased as the area of the targets increased; this effect was more visible for target areas smaller than about 0.5 m^2 (Figure 6-10). Likewise, for all targets, irrespective of size and shape, the catch density index decreases as the size of the target increases (Figure 6-11).

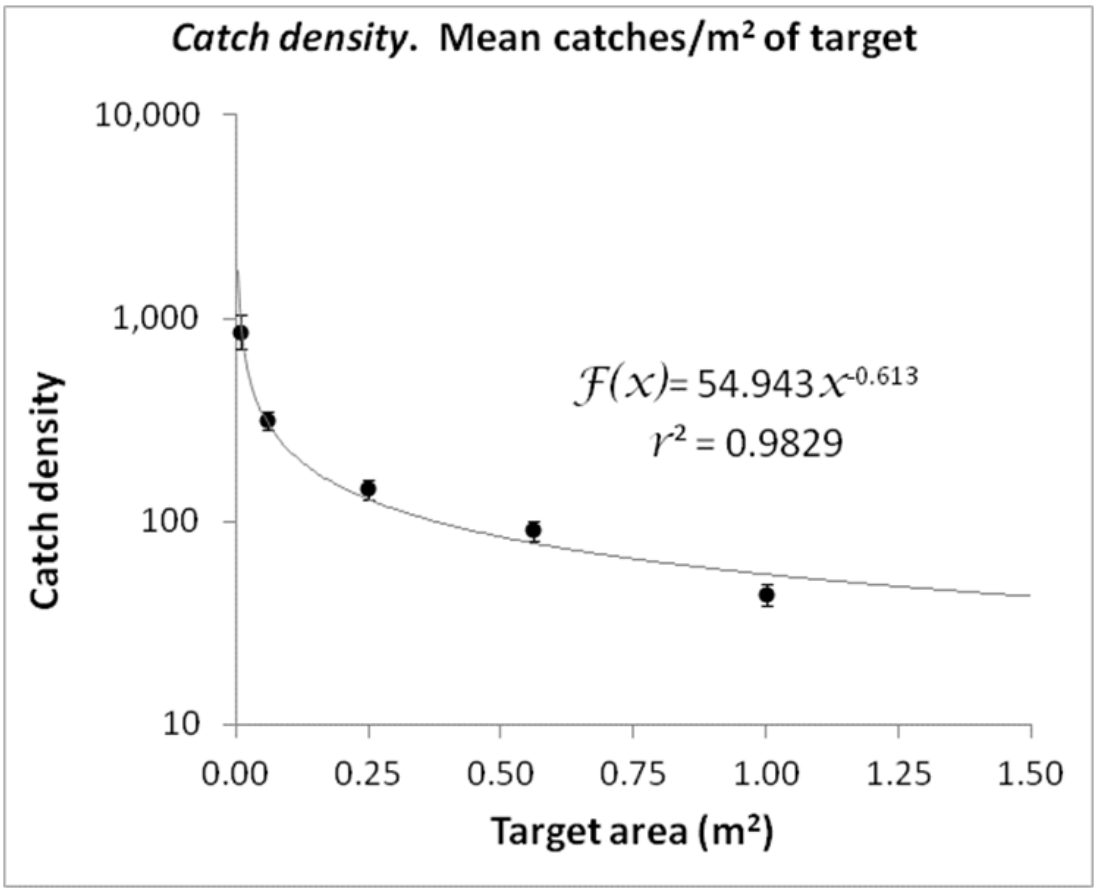


Figure 6-10: ***Extrapolation of the effect of target size in the catch density.*** Mean catch density (*G. p. palpalis* /m^2) ±SE obtained for each square target. Inert square black targets of different sizes (0.01, 0.06, 0.25, 0.56 and 1 m2) were placed next to an E-net (0.5 m wide×1 m high).

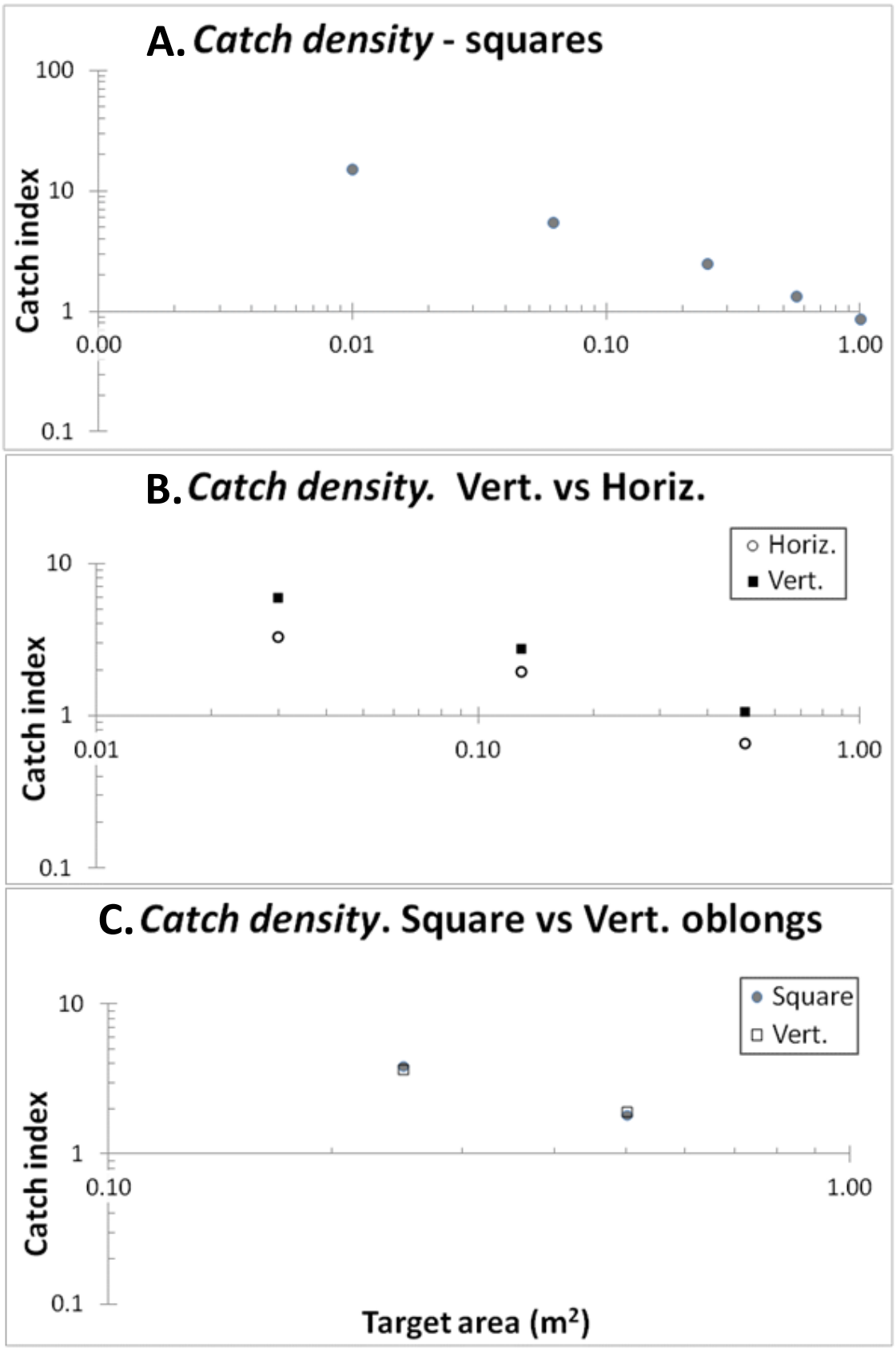


Figure 6-11: ***Proportional catch of G. f. quanzensis on rectangular targets.*** Mean catch density (*G. f. quanzensis* /m^2) expressed as a proportion of that from a standard target for *G. f. quanzensis* attracted to vertical and horizontal oblong targets. Targets were flanked by E-targets in A but not in B. The horizontal lines denotes the catch index of the Standard

6.5. Effects of the vegetation in host location (exp. E&F)

Consistent with the results obtained for *G. f. quanzensis* (see section 5.4.), the visibility of the target and the addition of CO_2 had very significant effect in the catches of *G. p. palpalis* ($F_{1,46}$ = 102.3, P<0.001; and $F_{1,45}$ = 50.7, P<0.001 respectively), but the interaction between both factors was not significant ($F_{1,44}$ = 48.7, *n/s*) (Table 6-1A). Carbon dioxide increased the catch of *G. p. palpalis* 3.4-fold for the concealed targets, and only 2.4-fold when the targets were in the open. However, similar to *G. fuscipes* in chapter 5 the difference was not significant

For *G. p. gambiensis*, visibility was the only factor that had an effect in the catches ($F_{1,125}$ = 146.9, P<0.001. Tabla 6-1B). The blend of octenol and 4-methylphenol and the target type had no significant effect in the catches ($F_{1,124}$ = 145.9, *n/s*; $F_{1,126}$ = 248.0, *n/s*, respectively. Tabla 6-1B). No significant interaction between factors was observed (target type *vs.* visibility: $F_{1,123}$ = 145.5, *n/s*; target type *vs.* +/- odour: $F_{1,122}$ = 145.5, *n/s*; visibility *vs.* odour: $F_{1,121}$ = 142.7, *n/s*; target type *vs.* visibility *vs.* +/- odour: $F_{1,120}$ = 143.2, *n/s*. Tabla 6-1B). No significant difference in the landing response was observed in any of the experiments.

A

G. p. palpalis		
+/- CO_2	Hidden	Visible
+	8.5 (±1.28)	116.2 (±13.49)
-	2.5 (±0.50)	48.2 (±5.80)

B

G. p. gambiensis				
	Target: 50NB		Target: 75NBN	
+/- OC	Hidden	Visible	Hidden	Visible
+	3.3 (±0.69)	11.3 (±2.00)	3.8 (±0.79)	13.1 (±2.31)
-	2.6 (±0.59)	10.9 (±1.94)	2.3 (±0.52)	13.8 (±2.41)

Table 6-1: ***Effect of the visibility of visual baits in the catch of* G. palpalis.** 'Hidden' targets were concealed with enclosures made with branches and leaves. Experiments were carried out in Azaguié (Côte d'Ivoire) for *G. p. palpalis* (A), and in Orodara (Burkina Faso) for *G. p. gambiensis* (B). In 'A', blue targets (0.25×0.25 m) operated with a flanking net (0.25×0.25 m) placed on one of the sides of the target. CO_2 was dispensed at 1 L/min. In 'B', the a blend of octenol and 4-methylphenol (OC, 'O' stands for 'octenol' and 'C' for 'cresol') was used as the olfactory bait. For this experiment, two different targets were used: (a) 50NB: similar to targets used in 'A'; and (b) 75NBN: 0.38 m wide × 0.5 m high blue target, operating with 0.19 m wide × 0.5 m high flanking nets, placed on both sides of the target. Mean catches for each treatment are accompanied by the SE.

6.6. Discussion

Main findings in this chapter are summarised bellow:

iv. **Flanking nets:** Without flanking nets the black/blue/black target caught twice as many *G. p. palpalis* as the black target, and the latter almost four times more tsetse than the blue target. Flanking nets increased the catches 20-fold for blue targets and 3.5-4-fold for black and black/blue/black targets. In presence of flanking nets, the catches of the three targets were similar.

v. **Size:** As for *G. f. quanzensis* in chapter 5, the numbers of *G. p. palpalis* attracted to an object is affected by the size of the bait: (i) very small objects of about 0.01 m^2 were not detected, (ii) catches increased with the object size, for objects from 0.06 m^2 to 0.5 m^2, but then (iii) they plateaued for objects bigger than to 0.5 m^2.

vi. **Shape:** Upright oblongs caught about 1.4-1.8 times more *G. p. palpalis* than horizontal ones, and about the same number with square targets.

vii. **Effect of the vegetation in host location:** The catches of *G. p. palpalis* for targets CO_2-baited targets of 0.13 m^2 and 0.38 m^2 was 1.2 and 1.7 times greater, respectively, for hidden targets. However, the differences were not significant.

viii. **Target design suggested for control operations:** Targets of 0.06 m^2 were the most cost-efficient per unit of material. The same model could be used to control *G. p. palpalis* and *G. fuscipes*.

6.6.1. Effect of flanking nets

The results showed that all blue, all black and black/blue/black targets attracted similar numbers of *G. p. palpalis*. In the absence of flanking nets, the blue target collected significantly fewer numbers of tsetse than the other two. The difference in the catches of the blue target in the absence or presence of flanking nets, compared to the other two targets, was explained by the poor landing response that this colour elicits in *G. p. palpalis*. For example, only about 4% of the tsetse that approached the blue target landed on it, compared with the 20% obtained for the black target or 25% for the black/blue/black target. This is consistent with previous studies, where blue targets showed the lowest

landing response among the colours tested, although it was the most attractive (Green, 1989).

For all targets, the addition of flanking nets increased greatly the catch of *G. p. palpalis*. The results suggest that the performance of the black/blue/black target, used as the standard device to control tsetse in West Africa (Laveissière *et al.*, 1987a), could be further improved by adding this type of material. Insecticide impregnated flanking nets would kill a proportion of the tsetse that circulate the target but do not land on it. Laveissière *et al.* (1987a) argued that the short-lived netting panels make them unsuitable to be used in control campaigns in the field. The statement is probably true when the netting panels are meant to flank large targets, as the ones currently used. As stated in chapter 5, flanking nets of 0.25 × 0.25 m are expected to resist field conditions easier than bigger nets..

6.6.2. Effect of size and shape

Consistent with the results shown in chapter five for *G. f. quanzensis*, the numbers of *G. p. palpalis* attracted to a visual bait is affected by the size of the bait. For example, according to the data, we interpret that very small objects, *i.e.* 0.01 m^2, were not detectable by *G. p. palpalis*, as the catches were similar to those obtained in the absence of a visual bait. With objects from 0.06 m^2 to 0.5 m^2 the catches increased with the size, but the catches plateaued thereafter, suggesting that targets bigger than 0.5 m^2 are inefficient. Therefore, an ideal target in a control operation should be big enough to attract and kill sufficient number of tsetse, but sufficiently small to reduce costs. The similarity in the results obtained for *G. p. palpalis* (see chapter five) and *G. f. quanzensis* suggest that a target with dimensions of about 0.25×0.25 m would meet both requirements and be the most cost-effective option to control the two species. Targets used in West Africa to control *G. p. palpalis* are about 1 m^2. According to the results, objects of about 0.06 m^2 (0.25×0.25 m) attracts about half as many *G. p. palpalis* as objects 1 m^2 (0.25×0.25 m), but use 16 times less material.

In contrast to size responses, *G. f. quanzensis* and *G. p. palpalis* differ in the responses to shape. Whereas *G. f. quanzensis* are attracted more to horizontal oblongs than vertical ones, *G. p. palpalis* exhibited a higher attraction to vertical oblongs; conversely, a higher

proportion of *G. p. palpalis* land on horizontal oblongs, and no significant difference in the landing response of *G. f. quanzensis* were observed for the two target shapes. Similar experiments had been carried out with Morsitans-tsetse in the past, although there are no recent publications (Vale, 1974e; Torr, 1989). To our knowledge, this is the first tsetse species known to exhibit this behaviour. In previous studies, *G. morsitans* and *G. palpalis* exhibit a preference for horizontal oblongs (Vale, 1974e; Torr, 1989), and in chapter 5 we described a similar behaviour in *G. f. quanzensis*. However, square shapes were as effective in attracting *G. p. palpalis* as vertical oblongs (see chapter 5), and therefore, square targets are likely to be effective to control both species.

6.6.3. Effect of the vegetation in host location

Impairing the visibility of targets had a large effect on the catches. For example, visible targets caught about 15 times as many *G. p. palpalis* as concealed targets, and about 4 times as many *G. p. gambiensis*. There is no evidence suggesting that any of the two subspecies of *G. palpalis* rely more on odours to locate hosts; *i.e.* the interactions between the visibility of the targets and the presence or absence of an olfactory bait were not significant. Although the CO_2 was a powerful attractant for *G. p. palpalis*, baiting the targets with a blend of octenol and 4-methylphenol did not have a significant effect in the catches of *G. p. gambiensis*. As shown by Cheke & Garms (1988) and in chapter four, the effect of octenol and 4-methylphenol in *G. palpalis* is relatively small, increasing the catch in about 1.5-fold. To confirm statistically the effect of this blend, probably the experiment would have required a larger sample size.

The target types used to collect *G. p. gambiensis* did not affect the catches, neither an interaction between the target type and the visibility of the baits, or target type and the olfactory bait was observed. This confirms previous results suggesting that, in general, the increase in the size of the visual bait has a relatively small effect in the catches, even when the visibility of the object is limited.

CHAPTER SEVEN

GENERAL DISCUSSION

7.1. Introduction

During the 1970-1980s, a large number of published works resulted from several research programmes which aimed to develop bait technologies to control tsetse. These studies were concerned particularly with analysing the responses of tsetse olfactory and visual cues involved in the location natural and artificial baits. For example, biconical and monoconical traps were designed to control Palpalis-group tsetse (Challier & Laveissière, 1973; Gouteux & Lancien, 1986) while Ngu traps were designed to control Morsitans-group tsetse (Brightwell *et al.*, 1987). Traps were made simpler, and insecticide-treated targets were developed to control the Palpalis- (Laveissière *et al.*, 1987a) and Morsitans-group of tsetse (Vale *et al.*, 1985; Vale *et al.*, 1986b). Simultaneously, the principal kairomones for *G. morsitans* and *G. pallidipes*, (*i.e.* octenol, acetone, butanone, 4-methylphenol, 3-*n*-propylphenol), present in cattle odour, were identified (Hall *et al.*, 1984; Vale & Hall, 1985; Hassanali *et al.*, 1986; Bursell *et al.*, 1988). Consequently, synthetic kairomones are used in eastern and southern Africa to lure tsetse to visual baits, increasing not only the numbers of tsetse attracted to traps and targets, but also the proportion of attracted tsetse that are caught or killed (the device's efficiency). In central and western Africa, however, the results of the responses of species of the Palpalis-group to synthetic odours were not conclusive.

Most of the technology currently in use, particularly that used to control species of the Palpalis-group, were developed during those two decades. Subsequently, studies of the visual and olfactory attractants in central and western Africa stopped in the 1990s. Perhaps, this may be explained partially by the fact that control of HAT had been based,

which it still is, largely on the detection and treatment of disease in humans (Simarro *et al.*, 2008). Due to the lack of olfactory attractants available for tsetse of the Palpalis-group, artificial baits have to be deployed at high densities (*i.e.* approximately 10 times higher than those to control Morsitans-group of tsetse, which are highly responsive to odours), making the cost unaffordable for the communities and donors (Laveissière & Grébaut, 1990; Shaw *et al.*, 2006).

The body of work presented in this thesis was designed to test the hypothesis that more cost-effective baits can be developed to control vectors of HAT. Two different approaches were adopted to address this overall objective. First, the responses of tsetse of the Palpalis-group, namely *G. f. fuscipes*, *G. f. quanzensis*, *G. p. palpalis*, *G. p. gambiensis* and *G. tachinoides*, to natural and synthetic olfactory attractants that tsetse use to locate their hosts were analysed. Second, studies were made of the responses of *G. f. quanzensis* and *G. p. palpalis* to visual cues.

Present findings suggest that the use of artificial attractants and changes to the design of targets could improve the performance of baits and reduce the cost of control operations. These changes could encourage intergovernmental and national agencies, and communities to use vector control synergistically with the current campaigns of case-detection and treatment.

7.2. Responses to odours

The study of responses of blood-sucking insects and other pests to olfactory stimuli is a major topic in entomological research, and it has led to novel tools and methods successfully applied in control campaigns. For example, the implementation of the "push-pull" strategy to control stem borers (*Ostrinia* spp) and other agricultural pests has been regarded as highly successful (Khan *et al.*, 1997a; Khan *et al.*, 1997b). The push-pull approach relies on using attractants to trap or kill efficiently the stem borers ("pull"), while driving them away from the main crop by using repellents ("push").

A similar concept has been implemented to control vector-borne diseases of human importance, such as malaria. For example, the use of insecticide-treated bednets ("push")

increases the effectiveness of the mosquito magnet traps ("pull") to catch *An. gambiae s.s.* (Kitau *et al.*, 2009). Additionally, the use of artificial repellents to drive the mosquitoes away from human hosts, and direct them to the traps, has been also suggested (Jawara *et al.*, 2009).

Thompson (1976), working with the onchocerciasis vectors *Simulium damnosum s.l.* in southern Cameroon, demonstrated that the catches of sticky traps baited with the odour of a man were significantly greater than those obtained with a CO_2-baited trap, and the later, greater to an unbaited trap. The author stated that "forest" *S. damnosum* s.l. (presumably *S. squamosum*) females rely heavily on smell to locate their hosts. Thompson (1977) suggested that the attractant chemicals in the human odour may be contained in the sweat. Other studies with blackflies in Sanaga Valley (southern Cameroon) showed that the odour from a cow attracted more *S. squamosum* than the odour from three men, the latter more than CO_2 (1 L/min), and the CO_2 more than an unbaited sticky trap (Tirados, *unpublished*).

Parasitic Diptera agents of myiasis also require host cues for the host location, although depending of their mechanism of host invasion, different families of these flies are attracted to different chemicals (Hall, 1995). For example, while botflies (Diptera: Oestridae) – obligate parasites that infect healthy mammals – respond to odours that are produced by healthy hosts, blowflies (Calliphoridae) and fleshflies (Sarcophagidae) – agents of traumatic myiasis – respond to odours associated with host wounding and necrosis (Hall, 1995). Furthermore, while botflies require visual and olfactory stimuli to locate their host (Hall, 1995), odours are more important in host location for blowflies and fleshflies (Green *et al.*, 1993; Wall & Warnes, 1994; Hall *et al.*, 1995). For example, activation of *Lucillia* spp (Diptera: Calliphoridae), upwind orientation and landing was in response to putrefactive sulphurous volatiles from bacterial decomposition products (Emmens & Murray, 1983; Sutcliffe, 1987; Ashworth & Wall, 1994). Studies of responses of Calliphoridae to host odours led to the development of suphur-based blends, such as swormlure-4 (Mackley & Brown, 1984) that have been used to monitor populations of blowflies (Torr & Hall, 1992; Warnes & Green, 1992).

Studies of the responses of blowflies to host odours were based on previous works to assess olfactory cues used by tsetse of the Morsitans-group (Hall, 1995). For example, studies of the response of *G. morsitans* and *G. pallidipes* to host odours in Zimbabwe (Vale, 1974a, e, d; Vale, 1977b) led to the development of a synthetic attractant made as a

blend of octenol, acetone, 4-methylphenol and 3-*n*-propylphenol (Hall *et al.*, 1984; Vale & Hall, 1985; Vale *et al.*, 1988a). This blend was subsequently used to control populations of *G. morsitans* and *G. pallidipes* in Zimbabwe (Vale *et al.*, 1986c).

Although important advances have been achieved in about forty years of research, many questions remain unanswered regarding the mechanisms used by vectors, and more specifically, by tsetse of the Palpalis-group, to locate, land and feed on a host (Torr & Solano, 2010). Despite the importance of *G. palpalis* and *G. fuscipes* as vectors of sleeping sickness, relatively few scientific articles have been published on the responses of *G. palpalis* and *G. fuscipes* to odours (Torr & Solano, 2010). The most noteworthy published work for these species were probably a short communication showing the effect of octenol and acetone doubling the catches of *G. palpalis* (Cheke & Garms, 1988), and the responses of *G. fuscipes* to carbon dioxide and lizard odour (Frézil & Carnevale, 1976; Gouteux *et al.*, 1995; Mohamed-Ahmed, 1998; Mohamed-Ahmed & Mihok, 1999). Hitherto, this is the most comprehensive study of responses of *G. palpalis* and *G. fuscipes* subspecies to natural and artificial odours in field conditions.

7.2.1. Responses to natural host odours

Torr & Solano (2010) reported in their review that several studies did not find significant effects of odours in the catches of Palpalis-group species, and remained unpublished. This might explain why the literature in the topic is so scant. In our studies, we found that with the addition of monitor lizard odour the catch of *G. f. fuscipes* increased 1.5-2-fold, pig odour doubled the catches of *G. f. quanzensis* and *G. p. palpalis* and cattle odour doubled the catches of *G. p. gambiensis* and *G. tachinoides*. However, the results confirmed a marked difference in the response to natural host odours between Morsitans- and Palpalis-groups of tsetse. Tsetse of the Morsitans-group, are much more responsive to cattle odour, which increased the catches about 10-fold (Vale, 1974e; Makumi *et al.*, 1996).

The results in the thesis also suggested that particular tsetse species show a preference for certain hosts, and that this preference is modulated by odours. For example, *G. f. fuscipes* showed a preference for lizard odour but they did not respond to cattle, human or pig odour; *G. f. quanzensis* responded to pig odour, but not to cattle or human odour; *G. p.*

palpalis responded to pig and human odour, but not to cattle; *G. p. gamiensis* responded to human and cattle odour, but not to pig; and *G. tachinoides* only to cattle odour. Inter-specific variation in host preference has been demonstrated already for species of the Fusca-, Morsitans- and Palpalis-groups (Späth, 1995; Brightwell & Dransfield, 1997; Clausen *et al.*, 1998).

7.2.2. Responses to CO_2

Carbon dioxide released inside a tent at 1 L/min (similar to the CO_2 contained in the natural host odour), and at 12-15 metres from the trap, doubled the catches of *G. p. palpalis*, *G. p. gambiensis*, *G. f. quanzensis* and *G. tachinoides*. In general, catches obtained with CO_2-baited targets were comparable to those obtained with pig (for *G. f. quanzensis* and *G. p. palpalis*) and cattle odours (for *G. f. quanzensis* and *G. tachinoides*). This suggests that the responses observed for these natural odours could be attributable to the CO_2 released with the animal respiration. In contrast, *G. f. quanzensis* and *G. p. palpalis* did not respond to cattle or human odour, *G. p. gambiensis* did not respond to pig odour and *G. tachinoides* did not respond to either pig or human odour, despite the concentration of CO_2 being similar in all cases, and comparable with the concentration of the artificial CO_2. The results suggest that, for some tsetse species, odours produced by particular hosts may contain repellents, and confirms an inter-specific variation in tsetse species of the Palpalis-group.

Intriguingly, in experiments carried out along the shores of Lake Victoria, *G. f. fuscipes* did not respond to either CO_2 (released inside the tent) or to any of the mammalian natural host odours. In the only experiment that took place away from the vicinity of the lake, pig odour increased significantly the catches of *G. f. fuscipes*. The variable responses to carbon dioxide obtained by Mohamed-Ahmed & Mihok (1999) along the Lake Victoria were attributed to the linear nature of the habitat. They argued that CO_2 was ineffective in the 'linear forest' because the odour plume extended into areas outside the forest, where tsetse were absent. However, we carried out the experiments in a variety of habitats, where the distribution did not appear to be markedly linear, and yet mammalian odours were always ineffective for *G. f. fuscipes*. The locations of the sites, along the shores of Lake Victoria, might provide another explanation. It has been observed that large water bodies

may alter the atmospheric concentration of CO_2 (Berry & Colls, 1990; Reid & Steyn, 1997). The variability of CO_2 in the habitat might impair the capacity of tsetse to detect hosts above the background noise of atmospheric carbon dioxide (Zollner *et al.*, 2004). High-resolution measurements to test the variability of the atmospheric carbon dioxide would be required to test this hypothesis.

7.2.3. Responses to artificial blends

Responses to blends containing different combinations of octenol, acetone, 4-methylphenol, 3-*n*-propylphenol (POCA blend) were tested for *G. p. palpalis*, *G. p. gambiensis* and *G. tachinoides*. These chemicals were identified as attractants for the Morsitans-group of tsetse (Vale *et al.*, 1988a), and have been used to enhance the performance of visual baits. *G. tachinoides* was the most responsive species, and the combination of the four chemicals increased the catches about five-fold. The same blend increased the catches of *G. p. gambiensis* and *G. p. palpalis* 2.2-fold and 1.5-fold respectively, and did not have any effect for *G. f. quanzensis*. Removing acetone from the full blend (POC) showed similar increases in the catches.

Octenol, acetone, 4-methylphenol and 3-*n*-propylphenol are chemicals contained in mammalian natural odours. Octenol, contained in the breath and sweat, is known for being an attractant for other haematophagous Diptera, for example, horseflies (French & Kline, 1989; Foil & Hribar, 1995), stable flies (Holloway & Phelps, 1991) and mosquitoes (Takken & Kline, 1989). 4-Methylphenol and 3-*n*-propylphenol result from the bacterial-mediated fermentation of proteins contained in the urine and sweet (Okech & Hassanali, 1990), and they are known for being attractants of mosquitoes (Hallem *et al.*, 2004). Acetone has also been used in different blends to attract mosquitoes (Merdić *et al.*, 2010; Kline *et al.*, 2012).

7.3. Effect of size and shape of artificial visual baits

The results showed that the numbers of *G. p. palpalis* and *G. f. quanzensis* attracted to a bait is influenced by the bait's size and shape. They also showed that big objects (*e.g.* 1 m^2 of area) do not attract necessarily more tsetse than medium-sized objects (*e.g.* 0.5 m^2). More importantly, the catch density (*i.e.*, number of tsetse killed per square meter of material) decreases dramatically with size of the target.

G. f. quanzensis and *G. p. palpalis* differed in the responses to shape, *G. f. quanzensis* being attracted more to horizontal oblongs and *G. p. palpalis* more attracted to vertical oblongs. Square shapes were as attractive as vertical oblongs for *G. p. palpalis*.

Apart from *Glossina* spp, artificial visual baits have been used to control or monitor the population of other biting Diptera. The development of these visual baits were based on host- or oviposition-seeking behaviour. For example, tabanids lay their eggs onto marsh plants near water bodies. Tabanidae are able to detect polarised water reflected from the water to locate breeding sites (Schwind, 1991; Schwind, 1995). This behaviour served to build traps, which used an electromotor to collect horse flies. The electromotor was powered with a solar panel, with additionally reflected polarised light that attracted the flies (Blahó *et al.*, 2012).

Host seeking behaviour has been used to design 'silhouettes', that mimic a natural host (Mason, 1986; Ballard, 1989; McCall & Trees, 1989) and biconical traps designed for the control of tsetse (Ham & Sachs, 1986). The use of silhouettes and traps were originally not intended for control operations, but rather, as a monitoring tool to replace human-landing catches.

How can we tell that silhouettes and traps explore the host-seeking behaviour of tsetse, and no other, such as larviposition or swarming behaviour? The similar numbers of males and females that were caught in all the experiments suggest that they were caught while trying to feed on a host. In the experiments, the numbers of the male and female tsetse caught are not only an indication of the responses of each sex to each treatment, but also they are a representation of their abundance in the experimental sites. Male and female tsetse increased in similar proportion when the targets were baited with odours (see chapters 3 & 4). In addition, mathematical models predict a similar impact in the density of both sexes

when either insecticide-treated cattle or artificial visual baits are used as control techniques (Torr & Vale, 2005).

7.4. Host-seeking behaviour

7.4.1. Are Palpalis-tsetse relatively unresponsive to odours?

As seen in section 7.2., all the species in the study responded to odours to a greater or lesser extent. Catches of *G. f. fuscipes* were significantly increased when targets were baited with lizard odour, cattle odour increased the catches of *G. p. gambiensis* and *G. tachinoides*, and pig odour did the same with *G. f. quanzensis* and *G. p. palpalis*. Feeding preferences are influenced by olfactory and visual attraction to the host, but also by the defensive response of the host and their availability in the tsetse habitat (Vale, 1977a; Clausen *et al.*, 1998). Not surprisingly, natural hosts of *G. tachinoides* in La Comoé National Park in Northern Côte d'Ivoire were wild animals (mostly hippopotamus, bushbuck and monitor lizards) (Küpper *et al.*, 1990), whereas depending on the host availability, the same species in peridomestic habitats have a preference for pigs and cattle (Clausen *et al.*, 1998). Similarly, almost all the *G. f. fuscipes* bloodmeals collected in the shores of the Lake Victoria were from monitor lizards (Clausen *et al.*, 1998). A large study of feeding preferences with samples from different parts of Africa showed that cattle, domestic pigs and primates (including humans) are part of the diet of *G. tachinoides*, *G. palpalis* and *G. fuscipes* (Table 7-1) (Clausen *et al.*, 1998). The results of this thesis suggest that the responses to odours exhibited by *G. tachinoides*, *G. palpalis* and *G. fuscipes* contribute to the host location, as they are attracted by the odour of hosts that form part of their diet.

Bloodmeal	*G. p. palpalis*	*G. f. fuscipes*	*G. tachinoides*
Cattle (%)	0.70%	7.92%	0.15%
Domestic pigs (%)	18.11%	15.30%	0.60%
Monitor lizards (%)	9.66%	40.51%	12.57%
Primates, including humans (%)	18.17%	8.92%	2.01%
Total bloodmeals	**1,563**	**1,301**	**2,680**

Table 7-1*:* Feeding preferences of *G. p. palpalis*¸*G. f. fuscipes* and *G. tachinoides* (Clausen *et al.*, 1998)

Particularly intriguingly is the response of tsetse to acetone. Ketone bodies are produced by ketogenesis in the mitochondrial matrix of the liver cells when glucose is scarce and energy for the brain and heart has to be provided from breaking down fatty acids. Then, ketone bodies break down into acetone by spontaneous decarboxylation of acetoacetate. Increased concentration of ketone bodies in blood leads to ketosis; at this stage smell of acetone in breath is a common feature (Stipanuk & Caudill, 2006). Ketosis can be caused by diabetes, low-carbohydrate diet or metabolic disorders, including those caused by certain infections. Studies with birds and mosquitoes demonstrated that house flinches (*Carpodacus mexicanus*) infected with *Mycoplasma gallisepticum* showed a reduced defensive response against *Culex pipiens pipiens*, and concluded that the infection may have a role in the transmission of vector-borne diseases, such as West Nile Virus (Darbro *et al.*, 2007). In a similar way, ketosis might be a symptom of another condition, and therefore, animals with a high concentration of acetone in their breath are more likely to have impaired their ability to defend themselves from tsetse. In addition, Wang *et al.*, (2008) demonstrated in mice that ketosis can be a consequence of *Trypanosoma* infection. Consequently, trypanosomiasis could: (i) increase the number of bites by impairing the availability of the host to prevent bites and by increasing the attraction of tsetse to the host, and (ii) increase the chances of infecting tsetse by making sick animals more likely to be bitten.

Despite the responses of tsetse to odours demonstrated in chapters 3 & 4, the effect of odours for the Palpalis-group of tsetse is far from those observed for Morsitans-group. The difference between both groups suggests that the host-finding strategy of the riverine species must be different. Studies showed that odour plumes from a cow can trigger anemotactic responses in tsetse of the Morsitans-group, about 100 m from the source of the odour (Torr, 1988c, 1990; Brady *et al.*, 1995; Zollner *et al.*, 2004), and that the flies can

see a stationary cow from about 10 m (Vale, 1974c; Vale, 1974e, 1983). Female tsetse of the Morsitans-group can displace at least 1 Km/day, moving in a sequence of hops: tsetse fly few meters before landing (and 'sitting' briefly), to take-off again in a different direction (Vale *et al.*, 1984). While 'sitting' they can ambush a host that enters the 'detectable range'. This behaviour allows the fly to discover hosts at densities of about ten animals per square Km in the savannah habitats (Vale *et al.*, 1984).

Like *G. fuscipes* with monitor lizards (Mohamed-Ahmed & Odulaja, 1997), riverine tsetse often rely on small and relatively abundant hosts. In the riverine habitats, odour plumes are probably disturbed by the vegetation. In this circumstances, where the range of odour plumes and visual cues are reduced, perhaps tsetse use sites where hosts are likely to pass by, like animal tracks or near the water, to ambush, or use those animal tracks or the waterbed to patrol. Recent models have suggested the importance of the daily tsetse displacement in the host-selection, for savannah and riverine tsetse (Vale *et al.*, 2014). For example, restricted and bushy 'band-shaped' riverine habitats can reduce tsetse displacement by up to 70% (Vale *et al.*, 2014), from about 1 Km/day displacement observed on Morsitans-tsetse occupying large homogeneous areas of the savannah (Vale *et al.*, 1984), to few hundred metres per day in the case of the Palpalis-tsetse in the riverine habitats (Rogers, 1977). The model suggested that the differences in the daily displacement are mostly due to the habitat geometry, tsetse mobility being reduced in restricted habitats (Vale *et al.*, 2014). The authors stated that the reduction in the daily displacement reduces the relative advantage of using odours to locate hosts, but conversely, in restricted band-shaped habitats the host numbers required to allow tsetse to find a host were much lower. For example, the model showed that whereas in large blocks (*e.g.* savannah) about 15-30 lizards would lead to the same feeding success as one elephant, in band-shaped habitats (*e.g.* riverine habitats) only 2-3 lizards would be required (Vale *et al.*, 2014). In riverine habitats, the full benefit of stimuli from large baits is lost because some of these stimuli cover places with no tsetse. In the riverine habitats, odour plumes might not lead to the host, and therefore anemotactic activation and long range olfactory responses are unlikely to be the strategy: tsetse of the Palpalis-group probably requires visual cues to activate, even in the presence of odours.

According to Vale *et al.* (2014) the distinction between riverine and savannah tsetse are due largely to the habitat geometry, rather than genetic differences. Nevertheless, different species are likely to have evolved some innate behaviour patterns suiting the distinctive

demands of finding food in their particular habitats. These genetic differences might relate not to host-location, but rather, to the response of tsetse to particular host species (Vale *et al.*, 2014).

7.4.2. Shape and host-seeking behaviour

The present results are the first demonstration of a tsetse species (*G. p. palpalis*) being attracted to a vertical oblong in preference to a horizontal one. For all other species, vertical and horizontal oblongs are either equally attractive [*G. m. morsitans* and *G. pallidipes*, (Vale, 1974e); *G. f. fuscipes* (Lindh *et al.*, 2009)] or horizontal oblongs are more attractive [*G. m. morsitans* and *G. pallidipes* (Torr, 1994b); *G. f. quanzensis*, present study]. Previously, the preference for horizontal oblongs has been assumed to be related to the general shape of the mammalian hosts of tsetse (Clausen *et al.*, 1998). It is therefore remarkable that just one species should not display this response. It is tempting to speculate that this is related to its anthropophilic feeding habit (Torr, 1989); responding to an upright form may be an adaptation of day-active Diptera that feed on humans.

The present study found that while *G. p. palpalis* was attracted to vertical oblongs, horizontal oblongs elicited a stronger landing response. Studies of the responses of Morsitans-group tsetse have also found marked differences in the orientation and landing responses of tsetse to shape: for *G. m. morsitans* and *G. pallidipes*, vertical and horizontal oblongs are either equally attractive or horizontal oblongs are more attractive; in both cases horizontal targets elicit stronger landing responses. For *G. f. quanzensis*, the catch with the horizontal oblong E-targets was 7 times greater than with the vertical ones when they were not accompanied by flanking E-nets, compared to a two-fold difference when the E-nets were present. This suggests that the horizontal targets are more attractive and elicit a stronger landing response.

7.4.3. Relying on visual or olfactory cues to detect concealed hosts

Some of the differences observed in the responses of Morsitans- and Palpalis-groups of tsetse to odours may be determined by their ecosystems. For example, Morsitans-group of

tsetse inhabits open savannah habitats and feed on large hosts (*e.g.*, buffalo, antelope, warthog, etc.). In this type of habitat the odour plume can travel uninterrupted and be detectable by tsetse at distances up to 100 m (Zollner *et al.*, 2004). In addition, large hosts in the open woodlands are also visible from long distance. On the contrary, tsetse of the Palpalis-group inhabits bushy habitats, where the odour plumes can easily be disrupted and their relatively small hosts (*e.g.*, monitor lizards) hidden in the vegetation. We explored the interactions between the visibility of an artificial bait and the presence or absence of an olfactory bait. These interactions were not found, suggesting that tsetse do not rely on olfactory or visual cues depending of the visibility of the hosts; rather, in habitats where the odour plume is disrupted and the visibility impaired, tsetse make use of both olfactory and visual cues simultaneously. Even if tsetse in riverine habitats make use of both olfactory and visual stimuli to locate the hosts, Vale *et al.* (2014), based on deterministic models, suggested that activation of Palpalis-species is probably triggered by visual stimuli.

One important difference in the host-orientated behaviour between tsetse and other biting Diptera concerns the interactions of visual and olfactory stimuli in the location of the host. Vale (1974e) observed that tsetse do not locate precisely the host odour source without a visual stimulus. Conversely, studies carried out with the screwworm *Cochliomyia hominivorax* showed that these flies do not require visual stimuli to locate the host, but visual stimuli (*i.e.* colour) was important to enhance the landing response (Torr & Hall, 1992).

7.5. Practical implications

7.5.1. Use of flanking nets

Tsetse are not killed just by being attracted to insecticide-impregnated targets. Flies circling the target do not contact the insecticide, and hence would remain alive, eventually, to transmit the disease to the next host (Laveissière *et al.*, 1980; Laveissière *et al.*, 1987a). Landing responses differ depending on the target type; for example, for targets of 1 m^2 Green (1988) found that the landing responses for a pthalogen blue and a black target for *G. p. palpalis* were ~7% and ~11% respectively. The efficiency of insecticide-

impregnated targets can be improved by flanking the targets with fine black nets, also impregnated with insecticide (Packer & Brady, 1990). Thus, highly attractive colours with low landing rates, such as pthalogen blue, would be more efficient with the addition of these flanking nets: the blue colour attract the flies, which are killed, mostly by the insecticide impregnated net placed next to the target.

In chapter 6 (experiment A) we demonstrated that the addition of flanking nets to black, blue and black/blue/black targets increased greatly the catch of tsetse for all the targets. Without flanking nets the blue/black/blue target caught more tsetse than the black target, and the later more than the blue target; conversely, when the flanking nets were operating, there was no significant difference in the catches between any of the targets. Experiment E in chapter 5 showed that a small target of 0.25×0.25 m (treatment D) caught five times less tsetse than the same target with a net of the same dimension (treatment C). Experiment H in chapter 5 showed that a small target of 0.25×0.25 m (treatment C) caught the same number of tsetse than a targets that doubled the size, when all the targets were operating with flanking nets of 0.25×0.25 m.

7.5.2. Cost-effectiveness of targets

This study demonstrated that target catch increases with the target size, but the increase is not in proportion to the increase in target surface area. Hence, the numbers of tsetse killed per area of cloth, and by implication tsetse killed per dollar, decreases as target size increases. The response to size shown here is similar to that of other Palpalis group species (Lindh *et al.*, 2009; Rayaisse *et al.*, 2010; Esterhuizen *et al.*, 2011). In particular, there is only a relatively modest doubling in the number of tsetse attracted to large (1 m^2) targets versus small (*e.g.*, 0.25×0.25 m) ones. Given that *tiny targets* plus flanking nets (0.25×0.5 m) use $1/8^{th}$ (1 m^2 target) and $1/24^{th}$ (3 m^2 biconical trap) the amount of materials required for the large 1 m^2 targets or biconical traps, which are currently used in control programmes, it is clear that considerable savings in costs could be gained by using tiny targets in control operations. As the size of a target is decreased, the number of tsetse attracted per unit area of target increases for Palpalis-group species (Figures 5-4 and 6-10) but decreases for Morsitans-group tsetse (Torr *et al.*, 2011).

7.5.3. Optimal size and shape of a target

Morsitans-group of tsetse rely on relatively big hosts, such as elephants, hippopotamus and buffalo, whereas smaller animals, such as pigs or monitor lizards, can form the main diet of Palpalis species (Clausen *et al.*, 1998). In line with the feeding preferences, recent models suggested that the size of targets, in the restricted bushy riverine habitat, would have little effect in efficacy of control operations (Vale *et al.*, 2014).

As seen above, in general, the smaller the target the more cost-effective. Beyond the general principle, the present results should be used with caution in identifying the optimal size of target. Taking the results at face value, a very small target (0.01 m^2) had the highest catch density index, and since an E-net without any target caught some tsetse it has an *infinitely* high catch density, which obviously does not make any sense in biology. It is likely that since Palpalis-group tsetse are very sensitive to small targets, the structures associated with electric grids (*i.e.*, transformer, 12 V battery, supporting frame of the grid,etc.) attract some tsetse, despite our efforts to make these items as inconspicuous as possible. The 0.01 m^2 target did not catch significantly more tsetse than no target, and hence it seems that tsetse are not responding to targets of 0.1×0.1 m or smaller. The 0.25×0.25 m target did catch significantly more tsetse than no target and this probably represents the smallest target that might be considered. The catch density declines steadily as size increases and there is no evidence that more tsetse were attracted to a 1 m^2 target than a 0.5 m^2 one. Hence, a target in the region of 0.25×0.25 to 0.5×0.5 m seems likely to be optimal. Big targets enhanced a larger landing response than small ones, but this difference was marginal. The experiments showed that the addition of a flanking net increased the catches more than increasing the size of the target, and therefore, a 0.25×0.25 m target with a flanking net of the same size would be expected to perform better than a target of double size without flanking net.

The performance of these small targets is crucially dependent on the presence of a flanking net: while Palpalis-group tsetse are attracted to small objects, few land on them, and hence, a flanking net treated with insecticide is essential for killing flies that visit but do not land. Previous studies (Lindh *et al.*, 2009), together with the results of this thesis, suggest that a flanking net equal in size to the target is optimal.

The present results suggest that while there are marked differences in the responses of *G. f. quanzensis* and *G. p. palpalis* to oblongs, squares were as attractive as oblongs, providing each had an equivalent surface area. Hence, square targets are likely to be effective to a wider range of species rather than, for example, visual vertical oblong targets for *G. p. palpalis* and horizontal ones for *G. f. quanzensis*.

The results showed that lager targets attract more tsetse than smaller ones, and therefore, in control operations *tiny targets* would be required at higher densities per unit area to achieve similar results. However, in restricted riverine habitats these high densities are offset by the fact that such habitats cover a small proportion of the land surface. For example, trials with *tiny targets* in West Nile (Uganda) to control *G. f. fuscipes* showed that deploying 20 targets per Km of river implied a density of about 7 targets/Km^2 of land surface (Tirados, *unpublished data*). This is less than twice as many as the about 4 targets/Km^2 required to control Morsitans-tsetse (Vale *et al.*, 1988b; Dransfield *et al.*, 1990; Willemse, 1991). Moreover, further saving would be expected from material costs, transport, man-power, etc., which would reduce the operational price of about \$300/Km^2 (Shaw *et al.*, 2006) with the Standard targets to about \$62/Km^2 when *tiny targets* are used (Shaw, *personal communication*).

7.5.4. Baited or unbaited targets

Chapters 5 & 6 showed a difference in the response of *G. fuscipes*, and *G. palpalis* and *G. tachinoides* to synthetic odours. The results suggest that targets baited with synthetic kairomones (*e.g.* POCA) would increase their performance in 1.5-2-fold for *G. palpalis* and about four-fold for *G. tachinoides*, resulting in reduced target density to control tsetse. Acetone is the most volatile chemical in the POCA blend, and therefore, the most difficult to use in control operations as it has to be replaced more often than octenol, 4-methylphenol or 3-*n*-propylphenol. The catches obtained with traps baited with octenol, 4-methylphenol and 3-*n*-propylphenol (*i.e.*, without acetone) were similar to those obtained with the full blend. The use of POC in control operations would avoid the use of large volumes of acetone, with the consequent logistic and economical benefits.

Conversely, the synthetic odours used in the experiments did not have any effect in the catches of *G. fuscipes*, although responses of this species to monitor lizard odour suggest the presence of unidentified kairomones in this reptilian odour. To our knowledge, there are not studies being carried out to identify the kairomones present in monitor lizard odour. Identification and synthesis of these kairomones could used to bait targets or traps to control *G. fuscipes*.

This study, as well as previous work with other tsetse species (Hargrove *et al.*, 1995), suggest that larger doses of host kairomones produce larger catches of tsetse. Accordingly, we might reasonably expect that super-normal doses of synthetic attractants will produce greater improvements in the efficacy of baits for controlling vectors of HAT. According to deterministic models, targets baited with artificial baits are likely to be more useful when deployed in relatively broad habitats, in the case of Palpalis-group of tsetse, places like the mangrove ecosystem of, for example, Guinea, Equatorial Guinea, Gabon, etc., or the broad forests of Central and southern East Africa (Vale *et al.*, 2014).

Further studies about dosage of kairomones would be required to maximise the blend. These studies should be complemented with economical analyses to assess whether or not it is economically sound to bait targets with odours that might double the catches, or to increase the number of unbaited targets in control operations, considering that the price of an insecticide-impregnated *tiny target* is about US$1 (Vestergaard Frandsen, *personal communication*).

7.6. Future work

Despite the answers provided in this thesis as discussed above, further questions arose from the study. To answer these questions, further research is advised with the studies suggested below:

Studies of host-seeking behaviour

Host-seeking behaviour in rivernine tsetse appeared markedly different to those observed for the savannah species. We know that Morsitans-tsetse are activated by host odours and exhibit long-range olfactory responses. In the bushy riverine habitat, the odour plume is

unlikely to travel as far as in the savannah, and therefore, tsetse of the Palpalis-group might require different strategies to locate the host. For example, may Palpalis-group tsetse rely on a 'sit and wait' strategy to ambush their hosts and maximise their energy consumption? If they 'sit and wait', do they land in 'preferred' sites, where they can ambush their hosts? On the contrary, do riverine tsetse patrol actively to find their hosts? If so, do they use animal tracks, riverbeds, or other natural paths? Or, as Morsitans-tsetse, do they move in hops, sitting to wait for potential hosts between two flights? Do riverine tsetse enter into the densest parts of their habitats while looking for hosts, like monitor lizards? Are these tsetse species more attracted to mobile hosts than stationary ones?

Video recording (Gibson *et al.*, 1991), radar tracking (Riley *et al.*, 1996) or mobile baited traps (Vale, 1974e) are technologies that could help to answer some of these questions. Studies of dispersion using mark-release-recapture techniques could help to assess the mobility of riverine tsetse (Bouyer *et al.*, 2009).

If Vale *et al.* (2014) were right in their assumption that intra-specific differences in host-seeking behaviour are due mainly to the habitat geometry, same species are expected to behave differently in different habitats. Experiments designed to answer the questions above should be carried out for same species in different habitats. For example, *G. f. fuscipes* in the shores of Lake Victoria in Kenya and the same species in the riverine habitats of Teso (Kenya), Tororo (southern Uganda) or West Nile (northern Uganda), the Congolese forest and the swamps of Mandoul (southern Chad); or *G. p. gambiensis* in the riverine habitats of Burkina Faso and in the mangroves of Guinea.

The role of sick hosts in the transmission of trypanosomiasis

Torr & Hargrove (1998) demonstrated that the feeding success of tsetse is dependent upon the defensive response of the host. For example, impalas and cattle attracted similar number of tsetse per unit of biomass; however, the higher defensive response exhibited by impalas results in a lower overall feeding rate. Sick hosts are likely to have a restricted capacity to elude bites from tsetse and other haematophagus insects. For example, Darbro *et al.*, (2007) showed that house flinches infected with *M. gallisepticum* showed a reduced defensive response against *Cx. p. pipiens.* In addition, trypanosomiasis might produce

ketosis in hosts, increasing the concentration of acetone in their breath. Therefore, sick hosts might influence the epidemiology of trypanosomiasis in two different ways:

- Sick hosts are likely to have their capacity to elude haematophagus insects compromised, and therefore the disease might be a determinant factor to increase the biting rates.
- Hosts affected with trypanosomiasis might produce more acetone than healthy ones. The increased production of acetone might make sick animals more attractive to tsetse. Increased biting rates on infected animals would increase the infection rates in tsetse, and therefore, the transmission of the disease.

Studies of the role of sick animals in the transmission of trypanosomiasis might provide new clues in the epidemiology of the disease.

Genetic studies in relation with host-seeking behaviour

According to Vale *et al.* (2014), differences in the host-seeking behaviour might not be explained by genetic differences, but rather the habitat geometry. That might explain why some electrophysiologically active chemicals do not elicit behavioural responses in the field. For example, Gikonyo *et al*. (2000), studying the behaviour of *G. m. morsitans*, observed that tsetse stayed longer on oxen than on waterbucks. The authors stated that unique chemicals present in waterbuck odour should explain the difference in the tsetse behaviour. Several electrophysiologically active chemicals were found in waterbuck natural odour, such as δ-octalactone, 2-methoxyphenol, series of methyl ketones, and 3-isopropyl-6-methylphenol (Gikonyo *et al.*, 2002), but only 2-methoxyphenol showed moderate repellent effects for *G. m. morsitans* in the field (Torr *et al.*, 1996). Therefore, the presence of active receptors in the tsetse antenna for particular semiochemicals might not result in a behavioural response in the field.

Behavioural studies, as those suggested above, should be completed with genetic studies. Genetic studies in relation with host-seeking behaviour should establish whether or not behavioural difference might be attributed to genetic adaptation.

Optimization of synthetic blends

After over a decade without published works in the research aimed at developing attractants to control tsetse, the study presented in this thesis represents a revival in the topic. The lack of published data during this period perhaps was due the general perception that all the important attractants had been identified for savannah species and there was none to fiend for riverine flies (Torr *et al.*, 1995; Torr & Solano, 2010). However, there are reasons to think otherwise:

(i) There are still unidentified attractants for savannah species: studies in Zimbabwe showed that E-targets baited with natural cattle odour caught twice as many *G. pallidipes* and 1.5 times as many *G. morsitans* as targets baited with the known kairomones found in the cattle odour (Torr *et al.*, 1995).

(ii) Species of the Palpalis-group responded to host kairomones other than CO_2. The best example is perhaps *G. f. fuscipes*, which responded to lizard odour but not to the synthetic odours used to bait Morsitans-tsetse or mammalian odours (chapter 3).

The identification of new attractants (*e.g.* kairomones contained in lizard odour) and the optimization of the dosage in the blend would improve the effectiveness of targets in control campaigns, reducing the density of targets deployed thereby making the operation more affordable.

Economical assessment of tiny targets in control operation, with and without odours

Currently, the synthetic blend used to control tsetse of the Morsitans-group improve the performance of traps in about 1.5-2.5 times for *G. palpalis* and *G. tachinoides*. To know whether it would be financially sound to use the bait to control theses species, or increase the number of targets, economical assessments of control operations using either one or the other approach would be required.

Field trials with tiny targets

Recently, the *Bill and Melinda Gates Foundation* have funded two large-field trials in Uganda (*G. f. fuscipes*) and Guinea (*G. p. gambiensis*) to test the performance of *tiny targets* in natural conditions (Figure 7-1). During the trials, the following parameters are

monitored: (i) impact on the tsetse population, (ii) impact in the transmission of AAT in Uganda and HAT in Guinea, (iii) insecticide activity after exposure, (iv) social acceptability, and (v) cost of the operation. In due time, the results of the trials will be published in relevant peer-reviewed journals.

Figure 7-1: ***Field trials.*** *Tiny targets* deployed in West Nile (Uganda) near rivers. The pictures show two different ways of deploying *tiny targets*: (A) driven in the ground, or (B) hanging from the branches in the riverine bushy habitat

ANNEX I

OMOLO, MO, HASSANALI, A, MPIANA, S, ESTERHUIZEN, J, LINDH, J, LEHANE, MJ, SOLANO, P, RAYAISSE, J-B, VALE, GA, TORR, SJ & **TIRADOS**, I (2009). "Prospects for developing odour baits to control *Glossina fuscipes* spp., the major vector of human African trypanosomiasis." *PLoS Neglt Trop D* **3**(5): e435.

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PLoS NEGLECTED TROPICAL DISEASES

Prospects for Developing Odour Baits To Control *Glossina fuscipes* spp., the Major Vector of Human African Trypanosomiasis

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Abstract

We are attempting to develop cost-effective control methods for the important vector of sleeping sickness, *Glossina fuscipes* spp. Responses of the tsetse flies *Glossina fuscipes fuscipes* (in Kenya) and *G. f. quanzensis* (in Democratic Republic of Congo) to natural host odours are reported. Arrangements of electric nets were used to assess the effect of cattle-, human- and pig-odour on (1) the numbers of tsetse attracted to the odour source and (2) the proportion of flies that landed on a black target (1×1 m). In addition responses to monitor lizard (*Varanus niloticus*) were assessed in Kenya. The effects of all four odours on the proportion of tsetse that entered a biconical trap were also determined. Sources of natural host odour were produced by placing live hosts in a tent or metal hut (volumes<16 m^3) from which the air was exhausted at ~2000 L/min. Odours from cattle, pigs and humans had no significant effect on attraction of *G. f. fuscipes* but lizard odour doubled the catch ($P<0.05$). Similarly, mammalian odours had no significant effect on landing or trap entry whereas lizard odour increased these responses significantly: landing responses increased significantly by 22% for males and 10% for females; the increase in trap efficiency was relatively slight (5–10%) and not always significant. For *G. f. quanzensis*, only pig odour had a consistent effect, doubling the catch of females attracted to the source and increasing the landing response for females by ~15%. Dispensing CO_2 at doses equivalent to natural hosts suggested that the response of *G. f. fuscipes* to lizard odour was not due to CO_2. For *G. f. quanzensis*, pig odour and CO_2 attracted similar numbers of tsetse, but CO_2 had no material effect on the landing response. The results suggest that identifying kairomones present in lizard odour for *G. f. fuscipes* and pig odour for *G. f. quanzensis* may improve the performance of targets for controlling these species.

Citation: Omolo MO, Hassanali A, Mpiana S, Esterhuizen J, Lindh J, et al. (2009) Prospects for Developing Odour Baits To Control Glossina fuscipes spp., the Major Vector of Human African Trypanosomiasis. PLoS Negl Trop Dis 3(5): e435. doi:10.1371/journal.pntd.0000435

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Introduction

Between 1931 and 1961, the annual number of recorded Human African Trypanosomiasis (HAT) cases was reduced by >90%, from >60,000 reported cases/year to >5000 cases/year, through the systematic screening and treatment of millions of individuals across sub-Saharan Africa [1]. When the incidence of HAT across the continent dropped to such low numbers, the newly-independent nations of sub-Saharan Africa reduced their efforts to monitor and control the disease. This reduction, combined with political and economic turbulence in some of the countries most affected by the disease (e.g., Uganda, Sudan, Angola, Democratic Republic of Congo) led to a resurgence in HAT across the continent, such that by the late 1990s there were >30,000 recorded cases/year. Consequently, the World Health Organization (WHO) revived a major programme of disease surveillance and treatment which has now reduced the annual number of reported cases to <5,000/year [1]. Thus, over the past 80 years, programmes against HAT have been based largely on the detection and treatment of disease in humans and this continues to be the case [1]. Interventions against tsetse flies (*Glossina* spp.) [2], the vector of the *Trypanosoma* spp which cause HAT, have, with some exceptions normally based on the rodesiense form of the disease [3], played a minor role. This emphasis on tackling the trypanosome rather than the tsetse is due to a variety of humanitarian, socio-economic [4,5,6] and epidemiological [7,8] factors. By contrast, tsetse control has played a major role in the control of animal trypanosomiasis [4]. Should vector control play a greater role in tackling HAT?

More than 90% of HAT cases are caused by *T. brucei gambiense* transmitted by Palpalis-group species of tsetse found in Central and West Africa [1]. Moreover, modern methods of tsetse control, based on the use of natural (insecticide-treated cattle) or artificial (traps or insecticide-treated targets) baits to lure and kill tsetse, have the particular advantage that they can be applied and afforded by local people. Such interventions could overcome the

ANNEX II

RAYAISSE, JB, **TIRADOS**, I, KABA, D, DEWHIRST, SY, LOGAN, JG, DIARRASSOUBA, A, SALOU, E, OMOLO, MO, SOLANO, P, LEHANE, MJ, PICKETT, JA, VALE, GA, TORR, SJ & ESTERHUIZEN, J (2010). "Prospects for the development of odour baits to control the tsetse flies Glossina tachinoides and G. palpalis s.l." PLoS Neglt Trop D 4(3): e632.

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PLoS NEGLECTED TROPICAL DISEASES

Prospects for the Development of Odour Baits to Control the Tsetse Flies *Glossina tachinoides* and *G. palpalis* s.l.

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Abstract

Field studies were done of the responses of *Glossina palpalis palpalis* in Côte d'Ivoire, and *G. p. gambiensis* and *G. tachinoides* in Burkina Faso, to odours from humans, cattle and pigs. Responses were measured either by baiting (1.) biconical traps or (2.) electrocuting black targets with natural host odours. The catch of *G. tachinoides* from traps was significantly enhanced (~5×) by odour from cattle but not humans. In contrast, catches from electric targets showed inconsistent results. For *G. p. gambiensis* both human and cattle odour increased (>2×) the trap catch significantly but not the catch from electric targets. For *G. p. palpalis*, odours from pigs and humans increased (~5×) the numbers of tsetse attracted to the vicinity of the odour source but had little effect on landing or trap-entry. *For G. tachinoides* a blend of POCA (P = 3-n-propylphenol; O = 1-octen-3-ol; C = 4-methylphenol; A = acetone) alone or synthetic cattle odour (acetone, 1-octen-3-ol, 4-methylphenol and 3-*n*-propylphenol with carbon dioxide) consistently caught more tsetse than natural cattle odour. For *G. p. gambiensis*, POCA consistently increased catches from both traps and targets. For *G. p. palpalis*, doses of carbon dioxide similar to those produced by a host resulted in similar increases in attraction. Baiting traps with super-normal (~500 mg/h) doses of acetone also consistently produced significant but slight (~1.6×) increases in catches of male flies. The results suggest that odour-baited traps and insecticide-treated targets could assist the AU-Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) in its current efforts to monitor and control Palpalis group tsetse in West Africa. For all three species, only ~50% of the flies attracted to the vicinity of the trap were actually caught by it, suggesting that better traps might be developed by an analysis of the visual responses and identification of any semiochemicals involved in short-range interaction.

Citation: Rayaisse JB, Tirados I, Kaba D, Dewhirst SY, Logan JG, et al. (2010) Prospects for the Development of Odour Baits to Control the Tsetse Flies Glossina tachinoides and G. palpalis s.l.. PLoS Negl Trop Dis 4(3): e632. doi:10.1371/journal.pntd.0000632

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Introduction

Tsetse flies (Diptera: Glossinidae) infest ,10 million km^2 of sub-Saharan Africa where they transmit trypanosomes which cause Human African Trypanosomiasis (HAT; also known as sleeping sickness) and African Animal Trypanosomiasis (AAT; also known as Nagana). This complex of diseases has an important impact on health and productivity in sub-Saharan Africa [1,2]. HAT occurs in two forms; ''rhodesiense'' which is caused by *Trypanosoma brucei rhodesiense* and occurs in eastern and southern Africa; ''gambiense'' which is caused by *T. b. gambiense* and occurs in western and central Africa. Currently the latter causes ,97% of the total number of reported cases of HAT [1] and is transmitted in West Africa by tsetse of the Palpalis group where the most dangerous species are G. palpalis s.l. and G. tachinoides.

Means of tackling HAT and AAT differ fundamentally. Control of AAT transmitted by riverine flies is funded and implemented largely by livestock keepers [3] who treat their livestock with trypanocides and insecticides and/or deploy odour-baited traps or targets to control tsetse. Control of HAT is managed and funded by intergovernmental and national agencies and, in the case of the gambiense form, relies mainly on systematic screening, treatment and follow-up of millions of human individuals across the affected region [1]. With a few local exceptions [4] vector control has generally played little role in the management of HAT over the past 80 years. Paradoxically, vector control could contribute significantly to the management of HAT. The relatively low infection rates (<0.1%) and long incubation period (~25 days) of *T. brucei* spp. in the vector [5], compared to the *Trypanosoma* spp. of veterinary importance, means that comparable reductions in the density and life-expectancy of tsetse populations would have a relatively greater effect on HAT than AAT. A cost-effective method of tsetse control that could be implemented by local people would complement the efforts of agencies that support mass screening and treatment and hence improve sustainability. Analyses of the history of efforts against sleeping sickness reveal that sustainable solutions have proved elusive [6,7]. An integrated approach, based on a combination of interventions directed at both tsetse and trypanosomes, may provide a better way forward.

ANNEX III

TIRADOS, I, ESTERHUIZEN, J, RAYAISSE, JB, DIARRASSOUBA, A, KABA, D, MPIANA, S, VALE, GA, SOLANO, P, LEHANE, MJ & TORR, SJ (2011). "How do tsetse recognise their hosts? The role of shape in the responses of tsetse (Glossina fuscipes and G. palpalis) to artificial hosts." PLoS Neglt Trop D 5(8): e1226.

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PLoS NEGLECTED TROPICAL DISEASES

How Do Tsetse Recognise Their Hosts? The Role of Shape in the Responses of Tsetse (Glossina fuscipes and G. palpalis) to Artificial Hosts

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Abstract

Palpalis-group tsetse, particularly the subspecies of *Glossina palpalis* and *G. fuscipes*, are the most important transmitters of human African trypanomiasis (HAT), transmitting >95% of cases. Traps and insecticide-treated targets are used to control tsetse but more cost-effective baits might be developed through a better understanding of the fly's host-seeking behaviour. Electrocuting grids were used to assess the numbers of *G. palpalis palpalis* and *G. fuscipes quanzensis* attracted to and landing on square or oblong targets of black cloth varying in size from 0.01 m^2 to 1.0 m^2. For both species, increasing the size of a square target from 0.01 m^2 (dimensions = 0.1×0.1 m) to 1.0 m^2 (1.0×1.0 m) increased the catch ~4x however the numbers of tsetse killed per unit area of target declined with target size suggesting that the most cost efficient targets are not the largest. For *G. f. quanzensis*, horizontal oblongs, (1 m wide×0.5 m high) caught ~1.8x more tsetse than vertical ones (0.5 m wide×1.0 m high) but the opposite applied for *G. p. palpalis*. Shape preference was consistent over the range of target sizes. For *G. p. palpalis* square targets caught as many tsetse as the oblong; while the evidence is less strong the same appears to apply to *G. f. quanzensis*. The results suggest that targets used to control *G. p. palpalis* and *G. f. quanzensis* should be square, and that the most cost-effective designs, as judged by the numbers of tsetse caught per area of target, are likely to be in the region of 0.2560.25 m^2. The preference of G. p. palpalis for vertical oblongs is unique amongst tsetse species, and it is suggested that this response might be related to its anthropophagic behaviour and hence importance as a vector of HAT.

Citation: Tirados I, Esterhuizen J, Rayaisse JB, Diarrassouba A, Kaba D, et al. (2011) How Do Tsetse Recognise Their Hosts? The Role of Shape in the Responses of Tsetse (Glossina fuscipes and G. palpalis) to Artificial Hosts. PLoS Negl Trop Dis 5(8): e1226. doi:10.1371/journal.pntd.0001226

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Introduction

Between 1997 and 2006, about 250,000 new cases of Human African Trypanosomiasis (HAT, or sleeping sickness) were reported [1]. For >95%% of these cases, the disease started with a bite from one of four subspecies of tsetse: *Glossina palpalis gambiensis* (in Guinea and Côte d'Ivoire), *G. p. palpalis* (in Benin, Nigeria, western Cameroon, Equatorial Guinea, Gabon, south- western Republic of Congo, south-western Democratic Republic of Congo and western Angola), *G. fuscipes fuscipes* (in eastern Cameroon, Central African Republic, western Republic of Congo, northern DRC, Sudan, Uganda), and *G. f. quanzensis* (in southern DRC and northern Angola) [2].

Efforts to tackle HAT have been based largely on case-detection and treatment in humans [1] rather than vector control, largely because methods for controlling tsetse are too expensive and logistically demanding [3]. The use of natural (insecticide treated cattle) or artificial (traps and insecticide-treated targets, sometimes baited with attractants) baits are the only techniques that might be applied by local communities [3–7]. However, their wider use is constrained by the low densities of livestock in HAT-affected areas [8] and/or the poor performance of artificial baits for Palpalis-group tsetse. In contrast to Morsitans-group tsetse, Palpalis-group species are less responsive to host odours [9] and hence artificial baits must be deployed at densities that are not affordable or sustainable for poor people. However, recent results have revived the prospects for the use of cost-effective baits against HAT.

The performance of artificial baits can be enhanced by the use of attractants which double the capture rates [10,11]. Second, several studies [12–14] suggest that significant improvements in cost-effectiveness of baits for vectors of HAT might be achieved through the exploitation of the visual responses to hosts. For instance, studies of *G. f. fuscipes* in Kenya showed that reducing the size of the target from 1 m^2 to 0.125 m^2 only halved the number of tsetse that contacted the target thereby giving a four-fold improvement in the tsetse killed per dollar spent on cloth [12]. Of course, the material cost of targets is only part of the total cost of deploying them and we would expect that the logistical costs of

ANNEX IV

RAYAISSE, JB, ESTERHUIZEN, J, **TIRADOS**, I, KABA, D, SALOU, E, DIARRASSOUBA, A, VALE, GA, LEHANE, MJ, TORR, SJ & SOLANO, P (2011). "Towards an optimal design of target for tsetse control: comparisons of novel targets for the control of palpalis group tsetse in West Africa." PLoS Negl Trop Dis 5(9): e1332.

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PLoS NEGLECTED TROPICAL DISEASES

Towards an Optimal Design of Target for Tsetse Control: Comparisons of Novel Targets for the Control of Palpalis Group Tsetse in West Africa

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Abstract

Background: Tsetse flies of the Palpalis group are the main vectors of sleeping sickness in Africa. Insecticide impregnated targets are one of the most effective tools for control. However, the cost of these devices still represents a constraint to their wider use. The objective was therefore to improve the cost effectiveness of currently used devices.

Methodology/Principal Findings: Experiments were performed on three tsetse species, namely *Glossina palpalis gambiensis* and *G. tachinoides* in Burkina Faso and *G. p. palpalis* in Côte d'Ivoire. The 1×1 m^2 black blue black target commonly used in W. Africa was used as the standard, and effects of changes in target size, shape, and the use of netting instead of black cloth were measured. Regarding overall target shape, we observed that horizontal targets (i.e. wider than they were high) killed 1.6-5x more *G. p. gambiensis* and G. tachinoides than vertical ones (i.e. higher than they were wide) ($P<0.001$). For the three tsetse species including *G. p. palpalis*, catches were highly correlated with the size of the target. However, beyond the size of 0.75 m, there was no increase in catches. Replacing the black cloth of the target by netting was the most cost efficient for all three species.

Conclusion/Significance: Reducing the size of the current 1*1 m black-blue-black target to horizontal designs of around 50 cm and replacing black cloth by netting will improve cost effectiveness six-fold for both *G. p. gambiensis* and *G. tachinoides*. Studying the visual responses of tsetse to different designs of target has allowed us to design more cost-effective devices for the effective control of sleeping sickness and animal trypanosomiasis in Africa.

Citation: Rayaisse JB, Esterhuizen J, Tirados I, Kaba D, Salou E, et al. (2011) Towards an Optimal Design of Target for Tsetse Control: Comparisons of Novel Targets for the Control of Palpalis Group Tsetse in West Africa. PLoS Negl Trop Dis 5(9): e1332. doi:10.1371/journal.pntd.0001332

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Introduction

Tsetse flies (Diptera: Glossinidae) infest about10 million km^2 of sub-Saharan Africa where they transmit trypanosomes which cause Human African Trypanosomiasis (HAT; also known as sleeping sickness) and African Animal Trypanosomiasis (AAT; also known as Nagana). This complex of diseases has an important impact on health and economic development in sub-Saharan Africa [1,2]. Tsetse are commonly divided into three, ecologically distinct groups: savannah tsetse (=Morsitans group) which are largely responsible for transmitting the trypanosomes that cause nagana; riverine tsetse (=Palpalis group) which play a major role the transmission of Trypanosoma brucei spp., the causative agents of sleeping sickness; and forest tsetse (=Fusca group) which, generally speaking, do not play an important epidemiological role.

Tsetse traps or their simplified two-dimensional derivative targets, when impregnated with insecticides, have constituted a central component of tsetse control campaigns in many countries in Africa [3–6], albeit such baits have been more used against AAT than HAT, except for a few notable exceptions [7,8]. The reasons it has not been used more widely against HAT are several, but one of the most important is the financial and logistical cost of using baits [9]. Hence, if the method is to be more widely used, especially by communities directly afflicted by HAT, then these costs must be reduced [10].

The type of target used to control tsetse varies according to the geographical location of the operation and the target species of tsetse. However, in general targets are coloured blue and/or black [11,12]. The use of blue in combination with contrasting colours such as white or black significantly improves landing behaviour of tsetse on targets [11,13,14,15].

The shape of the target is also important for both the overall shape (horizontal versus vertical) and the patterns (e.g. banding) on the target. For example, vertical banding seems to be more

ANNEX V

ESTERHUIZEN, J, RAYAISSE, JB, **TIRADOS**, I, MPIANA, S, SOLANO, P, VALE, GA, LEHANE, MJ & TORR, SJ (2011). "Improving the cost-effectiveness of visual devices for the control of riverine tsetse flies, the major vectors of human african trypanosomiasis." PLoS Neglt Trop D 5(8): e1257.

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PLoS NEGLECTED TROPICAL DISEASES

Improving the Cost-Effectiveness of Visual Devices for the Control of Riverine Tsetse Flies, the Major Vectors of Human African Trypanosomiasis

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Abstract

Control of the Riverine (Palpalis) group of tsetse flies is normally achieved with stationary artificial devices such as traps or insecticide-treated targets. The efficiency of biconical traps (the standard control device), 1m^2 black targets and small 25×25 cm targets with flanking nets was compared using electrocuting sampling methods. The work was done on *Glossina tachinoides* and *G. palpalis gambiensis* (Burkina Faso), *G. fuscipes quanzensis* (Democratic Republic of Congo), *G. f. martinii* (Tanzania) and *G. f. fuscipes* (Kenya). The killing effectiveness (measured as the catch per m^2 of cloth) for small targets plus flanking nets is 5.5–15X greater than for 1 m^2 targets and 8.6–37.5X greater than for biconical traps. This has important implications for the costs of control of the Riverine group of tsetse vectors of sleeping sickness.

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Introduction

African sleeping sickness or Human African Trypanosomiasis (HAT) is endemic to 36 countries in sub-Saharan Africa covering 9 million km^2 with 60 million of the 400 million inhabitants at risk of the disease. Africa has emerged from a recent sleeping sickness epidemic. In 1997 about 450,000 people were afflicted [1] which has now been reduced to about 70,000 cases per year [2,3]. Two forms of the disease exists, the Rhodesian (or East African) form being more acute and the Gambian form more chronic. Both these forms of the disease are fatal if left untreated and has an impact of 1.59M DALYs (disability adjusted life years). The related disease (nagana) in domesticated animals causes estimated losses to African agriculture of US$4.5bn per year [4]. In 2000 the African Union recognized trypanosomiasis as ''one of Africa's' greatest constraints to socio-economic development'' [5]. The trypanosomes causing HAT are transmitted by tsetse flies, particularly those of the Riverine (Palpalis) group. Antigenic variation in the trypanosome makes it unlikely that an effective vaccine will be produced in the foreseeable future. The available drugs are too toxic for prophylactic use. Consequently the only means of preventing the disease is vector control although this is not routinely practiced largely because of the cost.

Drug treatment of HAT is in a parlous state. The drugs available were developed many years ago and their toxicity and consequent human mortality allied to the increasing resistance to the drugs is a great worry [6]. Recent introduction of Nifurtimox Eflornithine Combination Therapy (NECT) has improved the situation but there is serious concern that no other drug for stage II treatment is in reserve should this fail. Vector control is essential for control of the Rhodesiense form of the disease [7] and can play a valuable role in support of case detection and treatment programmes for the Gambiense form of the disease especially in areas of high tsetse challenge when case detection and treatment alone is insufficient for control to be achieved [8,9]. Given worries about the sustainability of case detection and treatment it is essential that effective vector control measures are available.

A major obstacle in control programmes against Riverine tsetse is cost. Consequently, for the reasons given above, cheaper control techniques are needed. A standard method for control of Riverine tsetse is to use biconical traps, treated or untreated with insecticide or large insecticide-treated targets [9,10,11,12]. Because of their size both are expensive to make and deploy at the high densities required (10–30+/km^2). Our aim is to develop a more cost-efficient device than the standard biconical trap or 1 m^2 targets. Work is underway on developing artificial odour attractants to improve device efficiency [13]. Other studies have looked for improvements in the colour and shape of targets and traps [14,15,16]. However, few studies have focused on reduction of size of targets as a way to achieve better cost efficiency. Recent work on *G. f. fuscipes* [17] has shown the potential for a dramatic reduction in target size promising a considerable cost saving in control programmes against Riverine tsetse.

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