Tsetse flies are attracted to the invasive plant *Lantana camara*

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Abstract

In tsetse both sexes feed exclusively on the blood of vertebrates for a few minutes every 2–3 days. Tsetse flies seek cover from high temperatures to conserve energy and plants provide shelter for tsetse in all the biotopes they occupy. Recently, tsetse have taken cover in plantations and under the invasive bush *Lantana camara* that has invaded large areas of the tsetse fly belt of Africa. Flies from such refugia are implicated in sleeping sickness epidemics. In a wind tunnel we show that both foliage and an extract of volatiles from foliage of *L. camara* attract three tsetse spp. from different habitats: *Glossina fuscipes fuscipes* (riverine), *G. brevipalpis* (sylvatic) and *G. pallidipes* (savannah).

Gas chromatography analysis of volatiles extracted from leaves and flowers of *L. camara* coupled to electroantennogramme recordings show that 1-octen-3-ol and β-caryophyllene are the major chemostimuli for the antennal receptor cells of the three tsetse spp. studied. A binary mixture of these products attracted these flies in the wind tunnel. The gas chromatography linked electroantennogramme analysis of the *L. camara* extracts also show that the antennal receptor cells of the three tsetse spp. respond similarly to groups of volatiles derived from the major biosynthetic and catabolic pathways of plants, i.e. to mono- and sesquiterpenes, to lipoxidation products and to aromatics. Mixtures of these plant volatiles also attracted tsetse in the wind tunnel. These findings show that tsetse flies have conserved a strong sensitivity to volatile secondary products of plants, underlining the fundamental role of vegetation in tsetse survival.

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1. Introduction

The nature of vegetation cover across sub-Saharan Africa dictates species distributions in tsetse flies (*Glossinidae*). There are three tsetse subgenera: *fusca* flies inhabit dense forests, *palpalis* flies occupy gallery forests along water courses and *morsitans* flies thrive in savannah habitats, comprising some 23 spp. in all. The present-day distribution of tsetse flies across Africa most probably arose via species radiation from the tropical rain forests to drier habitats (Bursell, 1958), a lineage recently supported by work on molecular phylogeny of the genus *Glossina* (Gooding et al., 1991; Chen et al., 1999). Tsetse flies are daytime active, with both sexes hazardously seeking a blood meal at regular 2–3 day intervals. They must seek cover at temperatures over 40 °C (Hargrove, 1994). The role played by shelters for tsetse has long been recognised, for without the use of refugia the flies’ fat reserves are quickly depleted and any surviving generation is characterised by individuals of lower size (Dransfield et al., 1989). Indeed, the degree of vegetation cover as obtained from satellite images is shown to affect survival and population densities of different tsetse species in East and West Africa (Rogers and Randolph, 1991). The female tsetse fly is larviparous, i.e. nurturing a single larva in its abdomen for two weeks at a time. Plants provide shelter for tsetse in all the biotopes they occupy, permitting flies to mature, digest and gestate under equitable conditions, and also provide females with places protected from direct sunlight for larval deposition. Although the well-known ability of tsetse to respond to visual and olfactory stimuli from vertebrates has been exploited to develop odour-baited traps (Dransfield and Brightwell, 1992), little attention has
been paid to their propensity to find suitable cover under vegetation.

Tsetse flies have been found to take cover in plantations (Turner, 1981; Fournet et al., 1999), and under the bush Lantana camara L. (Verbenaceae) (Okoth and Kapaata, 1987). L. camara, commonly known as wild or red sage of Central and South American origin, is a highly invasive weed in many parts of the world (Ghisalberti, 2000). This bush has already colonised vast areas of sub-Saharan Africa where it has been documented to afford shelter to tsetse (Nash, 1969) and flies from such refugia have been implicated in disease epidemics (Okoth, 1999).

We tested the sensory and behavioural responses to L. camara of three tsetse species that serve as vectors of African trypanosomiasis: G. fuscipes fuscipes (palpalis group) is riverine, G. brevipalpis Newstead (fuscata group) is sylvatic, and G. pallidipes Austen (mor-sitans group) inhabits open savannah from Ethiopia to Namibia. Here we show that tsetse flies are attracted to L. camara: electrophysiological recordings from antennal chemoreceptor cells and behavioural responses of three tsetse species from different African habitats indicate perception of products from major biosynthetic and catabolic pathways leading to volatiles in this plant.

2. Materials and methods

2.1. Insects

Pupae and imagos of G. brevipaplis, G. pallidipes and G. f. fuscipes were held in an environmental chamber at 28 °C, 65 ± 5% relative humidity (RH), 8 h light and 22 °C, 90 ± 5% RH, 10 h dark, with 3 h light ramps at dawn and dusk to facilitate the behavioural experiments. Mature, unmated 5–10 day-old flies were used for electrophysiological and behavioural experiments. Flies were fed on bovine blood through a silicone membrane every second day starting from day two of emergence and starved for 2-days before behavioural tests. Flies were maintained in a cage (30 x 30 cm) prior to flying in the wind tunnel.

2.2. Plant volatile extracts

The essential oil of L. camara leaves (100 g) from France (Perpignan), Kenya (Nairobi), Sri Lanka (Kottawa) and Switzerland (Neuchâtel) were obtained by steam distillation for 6 hr using a Clevenger arm apparatus. Pinus sylvestris L. (Pinaceae) essential oil was from a commercial source. Odours of L. camara from Kenya and Switzerland were collected at room temperature by sucking charcoal-filtered air (400 ml min⁻¹) for 3 h through a 2 l desiccator containing leaves (20 g) or flowers (10 g) to a Pasteur pipette (5 mm inner diameter) containing 500 mg Porapak Q (60-80 mesh, Millipore Corporation, USA) from which volatiles were desorbed with 500 μl dichloromethane (DCM; Merck, analytical grade). Odour of Pinus mugo Turra subsp. uncinata (DC.) Domin (Pinaceae) foliage (100 g) was obtained similarly.

2.3. Gas chromatography linked electroantennographic detection

The tsetse fly antenna was mounted (Guerin and Visser, 1980) and used as an electrophantographic detector (Arn et al., 1975) in parallel with the flame ionisation detector of the gas chromatograph (GC-EAD) to locate biologically active constituents in odour extracts and in mixtures of volatiles. Analytes were injected on to a polar high-resolution capillary column (30 m FFAP, 0.25 mm i.d., 0.25 μm film thickness, BGB Analytik, Switzerland), carrier gas (H₂) at 50 cm s⁻¹, after 5 min at 40 °C the column was programmed at 5 °C min⁻¹ to 230 °C with column effluent split 50:50 to the FID and antenna (Steullet and Guerin, 1994). Chemical identity of biologically active compounds in L. camara extracts was determined by gas chromatography-coupled mass spectrometry (GC-MS as in Steullet and Guerin, 1994; column and conditions as in GC-EAD) using Kovat’s retention indices, mass spectrum library matches and electroantennograme (EAG) activity of synthetics. EAG activity of alpha-zingibrene, germacrene-D and beta-curcumene could not be confirmed as synthetic samples were not available. Except for 1-octen-3-ol (above), specific enantiomers of compounds identified in L. camara extracts with chiral center(s) were not determined, but known enantiomers of synthetic chiral compounds used are indicated in Fig. 3. The enantiomer of 1-octen-3-ol present in the odour of L. camara leaves was identified as R (−) using a 40 m chiral gas chromatographic column (50% gamma CD 6-O-TBDMS-2,3 di-O-n-But in PS086 with 4–6% diphenyl, Institute of Chemistry, University of Neuchâtel) 0.25 mm i.d., at 180 °C (isothermal), splitless injection at 180 °C, carrier gas He at 1.5 ml min⁻¹.

The responses of G. brevipaplis, G. pallidipes and G. f. fuscipes were compared by GC-EAD to 21 plant volatiles (listed in Fig. 3). These compounds, some of which were used for behavioural tests, were >95% pure as indicated by GC except beta-myrcene (90%) and (−) camphene (85%). All compounds were from Fluka, Buchs, Switzerland, except (−) bornyl acetate and (E)- beta ocimene from Firemenich, Geneva, Switzerland and (E)-2-hexenal from Aldrich, Switzerland. Due to the variation in the antennal responses between individual flies (at least 2 antennae were recorded per species), responses of a given antenna in a GC-EAD recording
were normalized by summing the responses in mV to the 21 products. The percent contribution of each compound to this sum was then square root transformed for analysis by 2-way ANOVA.

2.4. Olfactory thresholds

The olfactory thresholds of the three tsetse species for the chemostimuli identified in *L. camara* were established by EAG and GC-EAD (by injecting mixtures of products on to the column, as detailed above) to obtain antennal responses in log steps from 100 pg to 100 ng. Response curves were drawn from the electroantennogramme amplitudes recorded and regression analyses were applied to compare response thresholds.

2.5. Wind tunnel

The wind tunnel (170 cm long 60 × 60 cm; Rauscher et al., 1984), made of non-reflecting glass, had an airflow at 30 cm s⁻¹ and was operated in a climate chamber at 28 °C, 65 ± 5% RH. Overhead illumination with fluorescent tubes (36 W, >1 kHz, Philips) ran the length of the tunnel to produce 1000 lux on the floor. Tsetse flies took short hops of 0.25–0.5 m, reorienting at each landing to reach the odour source. To facilitate this, a 1.5 m long 20 cm diameter nylon net (1 mm) flight cylinder was stretched 40 cm above the tunnel floor to encompass the odour plume (see below). The tunnel was wrapped in white paper dotted with black patches as an optomotor cue with a slit on the experimenter’s side to facilitate observation.

Flies were placed, maximum 5 at a time, in transparent PVC release cylindrical cages (15 × 10 cm) the downwind end of which was covered with nylon netting (1 mm) and the upwind end with a metal mesh (1.3 mm) cover attached to the cage on a hinge to permit opening with a string on a pulley from outside the wind tunnel. The cage was placed horizontally on a stand at the downwind end of the flight cylinder with its lower side flush with the lower edge of the flight cylinder. At the start of a test the cover was lifted slowly to avoid mechanical disturbance of the flies and they were then exposed for 2 min to odour-free air followed by 2 min to the test odour. Responses of different numbers of flies of each tsetse species were recorded to treatments (min. 35 and max. 191). The responses to the odour extract of *P. mugo* and single components of *L. camara* leaf odour were tested with *G. pallidipes*, the species available to us in greatest numbers (*n* = number of flies tested, Table 1). The principal effect of treatments was measured as the percentage of activated flies attracted to the odour source, i.e. flies that flew past the funnels (see below) at the upwind end of the flight cylinder. Treatments were compared by the two-tailed Fisher’s exact test.

| Table 1 | Responses of tsetse spp. *G. brevipalpis*, *G. f. fusipes* and *G. pallidipes* to plant parts, plant odour extracts and to single plant volatiles and their mixtures in a wind tunnel |
|--------------|-----------------|----------------|-----------------|----------------|
| Species/Treatments | n (flies tested) | % Activation | % Attraction |
| *L. camara* leaves | | | |
| *G. brevipalpis* | 55 | 3.6 | 56.4 | 0.0 80.7 |
| *G. f. fusipes* | 85 | 7.1 | 47.1 | 1.2 70.0 |
| *G. pallidipes* | 120 | 1.7 | 26.7 | 0.0 37.5 |
| *L. camara* odour extract | | | |
| *G. brevipalpis* | 60 | 10.0 | 48.3 | 0.0 72.4 |
| *G. f. fusipes* | 60 | 13.3 | 56.7 | 5.9 61.8 |
| *G. pallidipes* | 110 | 7.3 | 21.8 | 0.0 58.3 |
| *P. mugo* needles | | | |
| *G. pallidipes* | 115 | 8.7 | 36.5 | 0.0 38.1 |
| *P. mugo* odour extract | | | |
| *G. pallidipes* | 40 | 7.5 | 27.5 | 0.0 36.4 |
| Single compounds | | | |
| *G. pallidipes* 1-octen-3-ol (1 ng) | 60 | 0.0 | 25.0 | 0.0 26.7 |
| *G. pallidipes* 1-octen-3-ol (100 ng) | 60 | 1.0 | 21.7 | 0.0 23.1 |
| *G. pallidipes* (-)-caryophyllene (10 µg) | 60 | 0.0 | 13.3 | 0.0 25.0 |
| *G. pallidipes* (-)-caryophyllene (100 µg) | 150 | 2.0 | 32.0 | 2.1 41.7 |
| *G. pallidipes* (-)-caryophyllene (10 µg) | 120 | 0.8 | 38.3 | 0.0 26.1 |
| *G. pallidipes* (-)-caryophyllene (100 µg) | 150 | 1.3 | 16.7 | 0.0 8.0 |
| Binary mixture | | | |
| *G. brevipalpis* | 85 | 3.5 | 23.5 | 0.0 80.0 |
| *G. f. fusipes* | 75 | 13.3 | 38.7 | 10.3 72.4 |
| *G. pallidipes* | 130 | 3.9 | 36.9 | 2.1 29.2 |
| Seven component mixture | | | |
| *G. brevipalpis* | 35 | 0.0 | 54.3 | 0.0 63.2 |
| *G. f. fusipes* | 91 | 7.7 | 41.8 | 13.2 81.6 |
| *G. pallidipes* | 191 | 3.7 | 46.1 | 1.1 59.1 |

a % Activation and upwind attraction during the initial 2 min exposure to a clean air source.

b % Activation and upwind attraction during 2 min exposure to volatiles from the treatments.

c 100 ng each 1-octen-3-ol + (-)-caryophyllene.
d 100 ng each of (+)-camphor, (+)-α-terpineol, (-)-β-caryophyllene, (-)-bornyl acetate, eugenol, citral (cis + trans) and 1-octen-3-ol.
e Of the substrates tested on all three tsetse species, attraction of *G. pallidipes* to the *L. camara* odour extract and to the seven component mixture was significantly higher than to *L. camara* leaves or to the binary mixture (p < 0.005, Fisher’s exact test).

2.6. Test stimuli and their delivery

The test stimuli were blown with charcoal-filtered air (350 ml min⁻¹) from a 1 l gas-wash bottle via a glass tube (4 mm id.) through the wind tunnel floor, 24 cm from the upwind end. This tube was bent downwind at...
35 cm from the floor and connected to a Teflon® funnel whose 4 cm diameter opening was covered with tin foil perforated with 16 randomly distributed 0.5 mm pinholes. A second such arrangement conducted the control air. In this manner the control and test funnel openings faced downwind beside one another into the upwind end of the flight cylinder. Computer-controlled solenoid valves switched the charcoal-filtered airflow between control and test flasks for the successive 2 min control and test periods of each test. The odour plume (visualised with ammonium acetate) filled the flight cylinder uniformly from 15 cm downwind of the Teflon® funnels to its end. (Further details of the wind tunnel are available on request from the corresponding author).

Augmentations of 100–300 ppm CO₂ were measured at the point of release within the wind tunnel of the odour from the gas-wash flasks containing the 10 g samples of L. camara and P. mugo foliage tested. The CO₂ levels were measured with an IR analyser (BINOS 1, Leybold-Heraeus, Germany; response time 1.8 sec). To control for any effects due to such CO₂ levels on tsetse behaviour, augmentations of 100, 250 and 350 ppm CO₂ were tested on G. pallidipes by releasing different flows from a pressurised gas cylinder containing 2% CO₂ in O₂. On delivery of these CO₂ levels and the volatiles from fresh L. camara leaves (10 g) or P. mugo needles (10 g), no shifts in humidity or CO₂ were detected at the insect release point inside the wind tunnel as measured with a HMI41 thermo-hygrometer (VAISALA, Finland; accuracy ±2%). The BINOS CO₂ analyser. Nevertheless, the control gas-wash flasks contained a filter paper disk impregnated with 200 µl distilled water. The porous polymer trapped odour extract of L. camara leaves or P. mugo needles was applied to a 7 cm diameter filter paper disk and placed in the gas-wash flask. Responses of G. pallidipes were recorded to synthetic 1-octen-3-ol (50:50 R:S; Merck, >97% pure) and β-caryophyllene presented singly at doses of 1 ng, 100 ng and 10 µg on the filter paper disk. Responses of all three species were recorded to binary mixture of the 1-octen-3-ol and β-caryophyllene and to a 7-component mixture of (+)-camphor, (+)-α-terpineol, β-caryophyllene, (−)-bornyl acetate, eugenol, citral (cis+trans) and racemic 1-octen-3-ol (all ≥97% pure) in DCM, each at 100 ng on filter paper. Control flasks for the plant odour extracts and the synthetic chemicals contained a filter paper disk impregnated with the same amount of DCM as employed to apply the test materials; in all cases, the solvent was allowed to evaporate before adding the filter paper to the gas-wash flask.

3. Results

In the wind tunnel experiments accounted for below the mean level of activation of G. brevipalpis, G. f. fuscipes and G. pallidipes during exposure to clean air in the initial 2 min control period was 5% and no greater than 13% of these activated flies ever flew to the clean air source. In this set-up, up to 50% of flies of the three tsetse species were frequently not activated by any test odours or, in the case of G. pallidipes, by CO₂ (up to an augmentation of 350 ppm at source). Despite this, flies of all the three species at first responded by activation in the release cage when exposed to volatiles from freshly removed leaves of L. camara; this activation reached 56% of the exposed flies in the case of G. brevipalpis, 47% for G. f. fuscipes and 27% for G. pallidipes as compared to activation levels of 4, 7 and 2%, respectively, to the clean air control (Table 1). Moreover, the proportions of these activated flies that flew to the point of release of the L. camara leaf odour in the wind tunnel reached 81, 70 and 37%, respectively, for G. brevipalpis, G. f. fuscipes and G. pallidipes, compared to 0, 1 and 0%, respectively, for the corresponding clean air controls (Table 1). By comparison, freshly removed needles of P. mugo activated 37% G. pallidipes (the only species tested) and 38% of these activated flies were attracted to the odour source (none for the clean air control; Table 1). The porous polymer adsorbed volatiles from leaves of L. camara proved attractive to all three tsetse species tested, attracting 72, 62 and 58% of the activated G. brevipalpis, G. f. fuscipes and G. pallidipes, respectively, compared to 0, 6 and 0%, respectively, for the corresponding clean air controls (Table 1). The volatile extract of P. mugo attracted 36% of the activated G. pallidipes and none to the clean air control (Table 1).

Volatile of L. camara that stimulate olfactory receptors of the three tsetse species were identified using their antennae as biological detectors in the fractionation of the leaf odour extract by gas chromatography. Two constituents of the porous polymer adsorbed L. camara odour, i.e. R-(−)-1-octen-3-ol and β-caryophyllene, induced the strongest electroantennogram responses from all three tsetse species (Fig. 1). Analysis of volatiles that accumulated over L. camara leaves (5 g) in a 50 ml bottle by direct injection of 2 ml of the headspace with a gas-tight syringe (McMahon et al., 2001) indicated 0.25 ng 1-octen-3-ol and 3.25 ng β-caryophyllene. L. camara morphotypes hybridise freely, but the tsetse antennal responses to steam distilled volatiles from L. camara growing wild in Kenya and in Sri Lanka, or cultivated in Switzerland and France showed (R)-(−)-1-octen-3-ol and β-caryophyllene to be the main tsetse chemostimuli in all distillates. Analysis of porous polymer adsorbed volatiles of L. camara flowers indicated 50 pg 1-octen-3-ol.
and 15 ng of \(\beta\)-caryophyllene, sufficient to stimulate tsetse antennal receptors (Fig. 2). 1-Octen-3-ol tested alone on \textit{G. pallidipes} in the wind tunnel induced 25, 22 and 13% activation at 1 ng, 100 ng and 10 \(\mu\)g, respectively (0, 2 and 0% for the controls), of which, respectively, 27, 23 and 25% reached the odour source (none for the controls; Table 1). Likewise, \(\beta\)-caryophyllene tested alone on \textit{G. pallidipes} induced 32, 38 and 17% activation at 1 ng, 100 ng and 10 \(\mu\)g, respectively (2, 1 and 1% for the controls), of which, respectively, 42, 26 and 8% reached the source (2, 0 and 0% for the controls; Table 1). A mixture of these two products proved more attractive for \textit{G. brevipalpis} and \textit{G. f. fuscipes}, matching the attractiveness of the leaves or leaf odour extract of \textit{L. camara}; this mixture was not as effective as the \textit{L. camara} leaf extract for \textit{G. pallidipes} (Table 1).

In addition to 1-octen-3-ol and \(\beta\)-caryophyllene, lipoxidation products (hexanal, \((E)\)-2-hexenal, hexanol, \((Z)\)-3-hexenol and \((E)\)-2-hexenol), mono- and sesquiterpenes (\(\alpha\)-pinene, \(\beta\)-pinene; \(\alpha\)-humulene, \(\alpha\)-zingiberene, germacrene-D, \(\beta\)-curcumene) and some aromatic constituents of \textit{L. camara} (3- and 4-ethyl phenol and 4-ethyl acetophenone) caused lesser but consistent responses from the antennae of the three species (details given for \textit{G. brevipalpis} in Fig. 1). These responses raised a question with regard to the importance of volatile secondary plant products as chemostimuli for tsetse in general. To answer this we recorded the antennal responses of the three species to equal quantities of 21 compounds representing end products of lipoxidation (short-chain aliphatic leaf volatiles other than 1-octen-3-ol), the isoprenoid pathway (terpenes), and to eugenol (derived from phenylalanine). With the exception of the responses of \textit{G. brevipalpis} to the early eluting monoterpenes, the relative electroantennogram responses of the three species to the test...
products were practically the same (ANOVA, $P > 0.05$; Fig. 3). Responses were also recorded for the three tsetse species to a similar range of volatiles in the essential oil $P. sylvestris$ and an odour extract of $P. mugo$, though these trees did not contain 1-octen-3-ol. The strongest chemostimuli were (+)-camphor, (+)-a-terpineol, b-caryophyllene, (/C0)-bornyl acetate, eugenol, and citral (cis+trans). The response recorded to different doses of these stimuli by the EAG and GC-EAD methods indicated that the olfactory threshold for 1-octen-3-ol was two orders of magnitude lower than for the other products. A mixture of (+)-camphor, (+)-a-terpineol, b-caryophyllene, (-)-bornyl acetate, eugenol, and citral (cis+trans) with 1-octen-3-ol added proved attractive to all the three tsetse species, and proved a better attractant than the binary mixture of 1-octen-3-ol and b-caryophyllene for $G. pallidipes$ (Table 1).

4. Discussion

Our findings show that tsetse flies possess receptor cells on their antennae for volatile end products of major biosynthetic and catabolic pathways of plants and that these sensory responses permit these insects to respond to $L. camara$ from a distance. 1-octen-3-ol, the most significant chemostimulant for tsetse in $L. camara$, is a previously described attractant for these flies in cattle odour (Hall et al., 1984) and is included in almost all odour baits for a range of tsetse species (Dransfield and Brightwell, 1992). We have quantified (by GC-EAD) the olfactory threshold of tsetse to be in the range of 0.1 ppt for this product, equivalent to detection threshold of male moths for their sex pheromones (Kaissling and Priesner, 1970). This sensitivity should permit tsetse to detect $L. camara$ from a distance. The sesquiterpene b-caryophyllene attracted tsetse flies on its own and when combined with 1-octen-3-ol in the wind tunnel and, as such, probably contributes to the attraction of tsetse to $L. camara$. Although our estimation of the antennal sensory threshold of these flies for b-caryophyllene is two orders of magnitude higher than to 1-octen-3-ol, the amount of this sesquiterpene was invariably at least an order of magnitude higher in all our $L. camara$ odour extracts. Interestingly, we have also found b-caryophyllene in the breath of cattle (unpublished data from this laboratory). Furthermore, phenols similar to the aromatic chemostimuli 3- and 4-ethyl phenol and 4-ethyl acetophenone of $L. camara$ have already been implicated in the attraction of tsetse flies to animal excreta (Bursell et al., 1988). It would appear that these flies, like other haematophasic arthropods, make parsimonious use of volatile chemostimuli by responding to a set of host volatiles when hungry and to a second—not quite unrelated—set from plants when replete. In fact the sensory responses of tsetse flies to plant volatiles parallel that of the carrot fly, a typical phytophagous dipteran, that likewise shows sensitivity to lipoxidation, isoprenoid and aromatic products of its host plants (Guerin et al., 1983).

At first glance it might seem enigmatic that tsetse should be attracted to plants, for unlike mosquitoes and phlebotomes that feed on nectar, tsetse flies feed exclusively on blood, and both sexes must do so regularly. However, tsetse flies do not survive temperatures above 40 °C (Hargrove, 1994), and their diurnal rhythm of host searching is already disrupted at temperatures above 35 °C (Torr and Hargrove, 1999). Under such extreme conditions tsetse flies seek refuge and plants mostly provide the appropriate microclimatic conditions for resting flies. Such cover is sought for longer in the day under adverse conditions of high temperature and low humidity (Torr and Hargrove, 1999). Satellite images show that vegetation cover and soil moisture are requisites for the maintenance of high fly numbers (Rogers, 2000). Our findings suggest that, in addition to the visual or other stimuli that plants may provide, these flies can actively seek vegetation based on odour. In addition to procuring shelter, being
associated with plants is usually a guarantee of proximity to preferred hosts for a blood meal.

Tsetse flies have recently colonised new sources of plant cover provided by coffee, tea, banana, sugar cane and exotic conifer plantations (Turner, 1981; Fournet et al., 1999). Movement of tsetse fly species away from river courses to the vicinity of villages has been facilitated by bush encroachment and these new shelters provided to tsetse are held responsible for sleeping sickness epidemics (Nash, 1969; Okoth, 1999). Once a plant has been colonised for cover, tsetse flies can live for months under appropriate microclimatic conditions, and infection rates with trypanosomes generally rises as the proportion of older flies increases in such populations (Leak and Rowlands, 1997). Emanations from the plant refuge that are of survival value for flies could serve to reinforce a learning process (Campbell-Lendrum et al., 1999) whereby experienced individuals would regularly return to the same refuge after a blood meal. The matter was aggravated when a weed like *L. camara* was introduced into Africa. This plant thrives in ecosystems disrupted by man and provides tsetse with key stimuli to successfully guide the return by fed individuals to its shade. Populations of tsetse can then increase and serve as foci for sleeping sickness epidemics.

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