I.8 Host Odour Attractants for Tsetse Flies

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Introduction

Tsetse flies, *Glossina* spp. (Diptera: Glossinidae), are haematophagous vectors of trypanosomes which cause sleeping sickness in man and a similar disease, nagana, in cattle. Tsetse are found only in Africa, but it is estimated that over 11 million km^2 are rendered virtually uninhabitable because of their presence, and that over 45 million people are at risk from human trypanosomiasis (Hagan and Wilmshurst 1975).

Although drugs are available for treatment of trypanosomiases, these tend to be expensive and require an extensive infrastructure for effective administration. Eradication of the vector can provide a long-term solution to the problem: in the past, this was achieved by bush clearance and extermination of alternative, wild host animals, but these methods have been replaced by ground spraying of persistent insecticides and sequential aerosol spraying of non-persistent insecticides from the air (Allsopp 1984).

In recent years, attention has focused on use of trapping devices for the survey and control of tsetse. These devices utilize mechanisms by which the flies find the host animals on which they depend for blood meals. Riverine species of tsetse of the *palpalis* group are typically restricted to a linear, relatively localized habitat along river gallery forests, and it seems that they rely primarily on visual cues to locate their hosts. In many areas, these species can be effectively surveyed and controlled with unbaited traps or insecticide-coated screens of appropriate shape and colour which simulate a host animal (e.g. Politzar and Merot 1984).

Savannah species of tsetse are more wide-ranging: although simple, visual traps can be used to survey the incidence of these species, such devices cannot give effective control at economically viable densities (e.g. Politzar and Merot 1984). Over the years, there have been numerous isolated reports of attraction of tsetse flies to the odours of host animals and their residues, but it was the work of Vale (1974) in Zimbabwe that established the importance of odour in attracting savannah species of tsetse to their hosts and to stationary baits. This provided a basis for work on chemical identification of the attractive components, and the successful application of odour-baited trapping devices in survey and control of savannah species of tsetse.

Identification of host odour attractants

Vale (1974) showed that the numbers of tsetse, *G. pallidipes* and *G. morsitans morsitans*, attracted to a simple model were increased by up to 50 times by odours from a single ox in an underground pit and that catches increased with the weight of the host animal used (Hargrove and Vale 1978). Starved animals were less attractive, but when such animals were returned to a fattening diet they rapidly became even more attractive than normal for a short period (Vale 1981).

Possible components of ox odour were screened as attractants for tsetse in the field (Vale 1980). Carbon dioxide increased catches of tsetse by between two and six times at release rates between 2.5 and 15 l min^{-1}. Acetone released at 0.3–30 g h^{-1} increased catches by up to six times, and the effects of these two chemicals were additive. Of the other chemicals tested, butanone could replace acetone as an attractant, but acetophenone and some low molecular weight carboxylic acids were found to be repellent to tsetse.

Subsequent work concentrated on trapping, isolating, and identifying the components attractive to tsetse in natural host odour. For trapping the odours, solid adsorbents were preferred since it was found that the attractiveness of ox odour could be reduced to the levels shown by carbon dioxide and acetone by filtering it through activated charcoal (Vale and Hall 1985*a*).

For detecting active components in the mixtures collected, electroantennography (EAG) was used, because in a field experiment, Vale (1982) showed that removal of the antennae from tsetse essentially eliminated their ability to respond to acetone and carbon dioxide, indicating that the receptors for these and, presumably, other attractants are located on the antennae. In the EAG technique, originally developed for detecting components of the sex pheromones of Lepidoptera, microelectrodes inserted into the antenna record a depolarization that occurs when chemoreceptors on the antenna are stimulated by an active chemical (e.g. Roelofs 1977).

Volatiles from cattle were collected by drawing air over the animals and through Porapak resin. The materials trapped were desorbed with dichloromethane and analysed by linked gas chromatography–electroantennography (GC–EAG) in which the effluent from the gas chromatography column is split between the gas chromatography flame ionization detector and a tsetse EAG preparation, thereby allowing simultaneous recording of the normal gas chromatography trace and the EAG activity of the components separated.

One minor component of ox odour gave a strong EAG response from *G. m. morsitans*; it was identified by mass spectrometry and by comparison with synthetic material as 1-octen-3-ol (Hall *et al.* 1984). This compound stimulated upwind flight of tsetse in a laboratory wind-tunnel (Bursell 1984), and doubled the number of tsetse attracted by ox odour in the field (Vale and Hall 1985a). The enantiomeric composition of the 1-octen-3-ol isolated from ox odour was found to vary between 80:20 and 92:8 *R*:*S* in different collections (Hall *et al.* 1984): no

difference was found between responses of tsetse to enantiomerically enriched samples in EAG, wind-tunnel or field tests.

Rates of production of acetone and 1-octen-3-ol by an ox as measured by air-sampling techniques were up to 24 mg h^{-1}, although typically 2–4 mg h^{-1}, for acetone, and up to 0.043 mg h^{-1} for 1-octen-3-ol. A mixture of carbon dioxide, acetone, and 1-octen-3-ol released at naturally-occurring rates of 2 l min^{-1}, 5 mg hr^{-1} and 0.05 mg hr^{-1} respectively, was found to be nearly as attractive as the natural odour from one ox for *G. m. morsitans* but only half as attractive for *G. pallidipes* (Vale and Hall 1985*a*).

In the above field experiments, electrified nets were used to kill tsetse which had been attracted to the odour source, and thus the results are a measure of attraction from a distance only. Catching flies in a trap involves attraction from a distance and stimulation of the attracted flies to enter the trap. Experiments in which traps were baited with carbon dioxide, acetone, or 1-octen-3-ol showed that all three odours attract flies from a distance, while carbon dioxide also increased the trap-entering response. Baiting a trap with combinations of all three attractants increased catches of tsetse by up to 64 times (Vale and Hall 1985*b*).

More recent work has shown that cattle urine is a powerful attractant for savannah species of tsetse, particularly *G. pallidipes* (Owaga 1984, 1985), and that it further increases the catches of tsetse in traps already baited with acetone and 1-octen-3-ol (Vale *et al.* 1986; Dransfield *et al.* 1986). Solvent extracts of the urine were also found to be attractive: after chromatographic (Hassanali *et al.* 1986) or chemical (Bursell *et al.* 1988) fractionation, the attractiveness was found to be associated with the phenolic fraction. This contained up to eight simple phenols: phenol, 3- and 4-methylphenol, 3- and 4-ethylphenol, 3- and 4-propylphenol and 2-methoxyphenol (Hassanali *et al.* 1986; Bursell *et al.* 1988). All except 2-methoxyphenol were active by EAG, and several were active in stimulating take-off and/or upwind flight in a wind-tunnel (Bursell *et al.* 1988). Field testing showed that none of the phenols was particularly attractive when used alone, but combinations of 4-methylphenol and 3-propylphenol were as attractive as the total mixture (Vale *et al.* 1988*a*; Owaga *et al.* 1988). 2-Methoxyphenol markedly reduced trap catches of tsetse. The phenols are slightly less effective in increasing the numbers of tsetse caught at electrified nets when compared with their effect with traps, indicating that the phenols also increase trap-entering responses (Vale *et al.* 1988*a*).

The phenols seem to account for most of the attractiveness of the urine, but some minor components of the neutral fraction were found to cause strong EAG responses from *G. m. morsitans* and *G. pallidipes*. These components were identified as indole, 3-methylindole, and two carotenoid metabolites, isomers of 3,3,5-trimethyl-4-hydroxy-4-(3-oxobutyl)cyclohexanone (Gough *et al.* 1987). Despite some promising indications in the wind-tunnel bioassay, no attractiveness has yet been demonstrated for these compounds in the field under a variety of conditions.

Acetone and 1-octen-3-ol have been shown to increase catches of *G. m. submorsitans* in Burkina Faso (Politzar and Merot 1984). None of the above compounds has shown any attractiveness for the riverine species *G. palpalis palpalis*. However, the riverine species, *G. tachinoides*, is attracted by the odour of host animals, and passage of the odour through a charcoal filter removes most of the attractive components (Merot *et al.* 1986). Carbon dioxide (Galey *et al.* 1986) 1-octen-3-ol, and the mixture of phenols found in cattle urine are attractive to *G. tachinoides*, but acetone is not attractive. The most important phenol appears to be 3-methylphenol (Merot, personal communication), which is the major phenolic component of bushbuck urine (Gough, unpublished results), the favoured host of *G. tachinoides*.

Use of tsetse attractants in survey and control

Of the known tsetse attractants, carbon dioxide, at doses of several litres per minute, is inconvenient to use on a large scale. However, baiting traps with acetone released at 100 mg h^{-1}, 1-octen-3-ol at 0.5 mg hr^{-1}, 4-methylphenol at 1.5 mg hr^{-1}.and 3-propylphenol at 0.2 mg hr^{-1} can increase catches of *G. pallidipes* by 20–30 times, making the traps much more sensitive and convenient monitoring devices for this species than traditional ox rounds.

The 1-octen-3-ol and the two phenols are dispensed from sealed polythene sachets which allow the compounds to diffuse out at a controlled rate. The acetone is currently dispensed from a bottle which has an appropriately-sized hole in the lid, but sealed dispensers are being developed. Studies of the trap-orientated behaviour of tsetse and their responses to different colours have led to the design of very effective traps which have a high specificity for tsetse (Vale *et al.* 1985).

For control, simpler and cheaper cloth screens impregnated with insecticide are used, which are also baited with acetone, 1-octen-3-ol, 4-methylphenol, and 3-propylphenol. In an initial trial on a 5km^2 island in Lake Kariba, an introduced population of tsetse was eradicated with insecticide-impregnated screens baited with acetone and 1-octen-3-ol only, at a density of 4 per km^2 (Vale *et al.* 1986*b*). In a subsequent operation on an area of 600 km^2 in Zimbabwe, tsetse populations were reduced by over 99.99% with similar odour-baited screens (Vale *et al.* 1988*b*). Since the introduction of the phenolic attractants, the method has become even more effective, and large-scale control operations using screens impregnated with deltamethrin and baited with acetone, 1-octen-3-ol, 4-methylphenol, and 3-propylphenol are in progress in Zimbabwe and Zambia. In Kenya, local tribesmen are using traps baited with acetone and jars of cattle urine for tsetse control (Brightwell *et al.* 1987).

Odour-baited traps and screens now provide a valuable complement to existing methods of control of savannah species of tsetse based on ground spraying of persistent insecticide or aerial spraying of non-persistent insecticide. Ground spraying makes heavy demands on labour and requires organization, while aerial

spraying requires sophisticated equipment and rigid scheduling. Traps and screens can be manufactured locally and deployed as resources permit and they operate continuously to monitor or reduce tsetse populations. The amounts of insecticide used on the screens are small, and environmental contamination is minimal. Furthermore, the synthetic odours and the designs of trap and screen are highly specific for tsetse.

The main problems and costs involved in using odour-baited traps and screens in tsetse control occur during the initial deployment and subsequent maintenance of these devices. These problems can be reduced if more attractive traps and screens are made available; such devices could then be placed at lower densities. Research into the identification of new attractants, and the improvement and simplification of the designs of traps and screens continues.

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