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Non – Insecticidal Control of Buffalo Fly using Behaviour–Modifying Systems

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1. Abstract

Current buffalo fly control methods rely heavily on the use of insecticides and problems with insecticide residues and resistance are occurring. This project investigated the development of non-insecticidal control of buffalo fly using behaviour-modifying systems, a potential alternative method. Natural odour attractants have been collected, identified, analysed, reconstituted using synthetic chemicals and tested for attractancy to buffalo flies. As a result of this, a mixture has been defined which is attractive to buffalo flies in laboratory experiments. Similarly, visual targets for day and night use have been developed and some of the parameters influencing the fly catch identified. These findings provide a solid foundation for the development of a system which effectively modifies the behaviour of buffalo flies. Further work with the objective of developing an effective buffalo fly attractant and target, thus minimising reliance on insecticides, is recommended.

2. Executive summary

Buffalo fly is cited as a major issue by beef cattle producers in northern Australia. Estimates on annual production losses were put at $80 million in Australia. Treatment costs for buffalo flies, excluding mustering, were estimated to be at least $20 million in Queensland. A reduction of buffalo fly populations below an economic threshold by using behaviour-modifying systems could potentially save producers these costs.

Current buffalo fly control methods rely heavily on the use of insecticides. Problems with insecticide residues and resistance to synthetic pyrethroids exist, with the latter resulting in declining efficacies for these insecticides. Chemical residues in excess of maximum residue limits of our major export markets were found with buffalo fly control chemicals used at recommended levels. The availability of non-chemical tools for the control of buffalo flies would lessen meat export problems and avoid the potential for total import bans on the basis of insecticide residues.

This project investigated the development of non-insecticidal control of buffalo fly using behaviour-modifying systems, which are one of the potential alternative methods for controlling buffalo flies. As an example, a system, which effectively attracts buffalo flies, has a great potential to reduce costs and production losses they cause, and to decrease or eliminate the risk of problems with residues in meat exports.

Project objectives

(i) To identify attractants, repellents and/or other sensory cues for use in non-insecticidal buffalo fly control systems.
(ii) to formulate recommendations for the commercial development of these systems.

Significant results

The literature on the behaviour, the role of attractants, arrestants and repellents and the population dynamics of the buffalo and horn flies was reviewed.

The behaviour of buffalo flies in locating and when residing on cattle has been investigated. Orientation of flies to animals occurred in freshly emerged flies, but the response increased to a maximum at 16-17 hours post emergence. Numbers of buffalo flies on animal in pens fluctuated throughout the day and night indicating that the flies regularly leave and return to their hosts.

Olfaction and vision in buffalo flies have been identified as important components in the location of host cattle and cattle dung. The work in this project aimed at modifying the behaviour of buffalo flies with chemical and/or visual means, so that the flies will not be able to locate cattle or egg laying sites. This would result in a disruption of the flies' life cycle, leading to lower fly populations, and thus assist in control. Substantial progress was achieved in the search for, and the assessment of, the effectiveness of chemical and visual cues, but further improvements in synthetic chemical attractants and in understanding the role of visual targets are still required.

A list of chemical compounds contained in odours emanating from cattle and their excretions was compiled from work done by overseas groups and by us using gas chromatography/mass spectrometry. These compounds were considered potential chemical cues in attracting buffalo fly to cattle.

The responses of buffalo flies to many single chemicals, mixtures of chemicals and bovine derived natural odours enhanced with synthetic components were assessed in an olfactometer,

a fly cage assay, in the behavioural observation facility for flies (BOFF) and in the field. Good responses were obtained for bovine odours, bovine odours enhanced with synthetic chemicals and some mixtures of synthetic chemicals. **A direct comparison of a synthetic mixture with odours from a steer showed that a similar response to the two stimuli could be obtained in the fly cage assay. This is a very encouraging result and an indication that the synthetic odour developed has the potential of efficiently attracting buffalo flies**.

We showed that buffalo flies, fly cage linings and extracts thereof (cuticular hydrocarbons) elicit a behavioural response in buffalo flies and determined their chemical nature.

Investigations of potential visual cues revealed buffalo flies oriented towards model cows and black rectangular targets in the BOFF and field situations. In the BOFF, there was an increase in the percentage responding when animal odour was superimposed on the visual targets. The addition of lures for other flies to visual targets in the field did not increase their attractivity to buffalo flies. Also, none of the other traps developed for stock associated flies caught buffalo flies.

The use of light traps to attract buffalo flies at night was evaluated. It was demonstrated that buffalo flies could orient towards a light source up to a distance of 60 metres. Light traps near penned cattle reduced fly numbers on cattle. However, in a small paddock the light trap had no apparent short term effect on fly numbers on cattle.

Conclusions

Good progress was made in this project towards the development of systems for non-insecticidal buffalo fly control. A synthetic mixture of components contained in cattle odour has been defined and shown to be attractive to buffalo flies in laboratory experiments. Similarly, visual targets for day and night use were developed and some of the parameters influencing the fly catch identified. These findings provide a solid foundation for the development of a system which effectively modifies the behaviour of buffalo flies. Commercialisation of an effective, non-insecticidal tool for fly control is feasible, as was demonstrated with Lucitrap. Thus, substantial benefits, could flow to the meat and livestock industry and the general community from the current findings, if they were further developed.

Recommendations:

1. That further research on the development of non-insecticidal, behaviour-modifying systems for the control of buffalo fly be conducted to:
   a) improve synthetic chemical buffalo fly attractants
   b) optimise current visual targets and investigate the mode of application
   c) evaluate integration of olfactory and visual cues into attractive, practical targets.

2. That an assessment of the potential of commercial development of the system(s) be made, and if found feasible, that commercialisation be initiated.

3. Background

Beef cattle producers in northern Australia frequently cite buffalo fly as a major issue (eg. UGA South Eastern Division 1996, Agrisearch/MRC survey 1993, Tick Fever Survey 1992, MRC North Australia Beef Survey 1990, South East Queensland Regional Beef Research Committee). The main concerns with buffalo flies are production losses, the risk of insecticide residues in produce, animal welfare considerations and the development of insecticide resistance in flies.

The development and implementation of non-chemical control methods for buffalo flies was considered an important and necessary step towards rectifying this problem by producers, the processing industry, consumer representatives and scientists at a forum on ticks, buffalo flies and residues in Rockhampton (1994). The project described in this report contains development work on behaviour-modifying systems for buffalo flies, one of the alternative, non-chemical control tools for these flies.

The background section deals with the pest insect, the production losses it causes, current and potential future control methods and provides a short introduction to behaviour-modifying chemicals and their use for insect pest control.

3.1 The pest

The buffalo fly, *Haematobia irritans exigua*, an introduced species, is difficult to separate morphologically from the American horn fly *H.irritans irritans.* They belong in the subfamily Stomoxinae of the family Muscidae. Buffalo fly and stable fly are the only members of this family in Australia whose mouthparts have been modified for piercing.

H.i. exigua occurs in the Oriental and Australian regions. The buffalo fly was introduced from Timor, entering mainland Australia near Darwin in 1838 from where it spread slowly to reach north western Queensland by 1928, Gympie in the early 1950s and Coffs Harbour in NSW in 1982. Flies were recorded as far south as Jerseyville near Kempsey in 1991 and also inland to Tenterfield (S.Spence pers comm).

The buffalo fly is an obligate ectoparasite of cattle. The adult flies live for 10 to 20 days and feed by sucking blood 10 to 40 times each day. The female fly requires a blood meal to mature her eggs, which are laid in batches of up to 26 under the faecal pad. The larvae feed in the dung and undergo three larval instars before pupating in or near the pad. Development from egg to adult fly takes a minimum of 8 days at 35^0C and this may extend to 32 days at 17.5^0C.

3.2 Economic losses

The direct effects of ectoparasites on their hosts has been extensively documented and include weight loss, reduced production of milk, eggs, meat, hide and wool (Lehmann 1993).

Early Australian studies on production losses resulting from buffalo fly infestation were inconclusive or showed no or little weight gain in protected steers. However, two recent studies using eartags containing diazinon which provide good control of buffalo flies over an extended period, showed substantial gains in treated animals. In northern Queensland, an increase in weight gain of 14% over 56 weeks was observed (GJ Sibson, pers comm). The second study in south east Queensland reported a 54% increase in live weight gain over 20 weeks (Spradberry and Tozer 1996).

Estimates of annual production losses were put at $80 million in Australia (Sutherst, pers. comm.). The treatment costs for buffalo flies, excluding mustering, were estimated to be at least $20 million in Queensland.

In the US, Kunz (1986) calculated production losses caused by horn flies may be worth $760 million. Control of the fly resulted in better weight gains, better food intake and conversion and better calf weaning weights. Increased milk production has also been reported when flies were controlled.

In addition to reduced weight gains, buffalo flies also transmit nematodes (eg *Stephanofilaria)* which cause sores on the animals. The sores, which vary in size from small areas to saucer sized lesions, result in permanent hide damage and thus in further economic losses.

3.3 Buffalo fly control

Current control methods rely heavily on the use of synthetic pyrethroid and organophosphate (OP) insecticides, although other groups such as the carbamate, bendiocarb and the macrocyclic lactones are available. Problems with insecticide residues and resistance to the synthetic pyrethroids exist, with the latter resulting in declining efficacies for these insecticides. However no resistance to the OPs has been detected. New application technology for the older groups (eg. diazinon ear tags) and new chemical groups (eg. methoprene; ivermectin) may only provide short term relief unless strategies can be developed to minimise selection for resistance.

Chemical residues in excess of maximum residue limits of major export countries were found with buffalo fly control chemicals used at recommended levels. Shorter application intervals with chemicals when resistance problems are encountered compound this problem. To safeguard meat exports, an Export Slaughter Interval was introduced to meet the requirements for export markets with a lower tolerance for chemicals used in Australia.

Non-chemical controls for buffalo fly currently include the use of dung beetles and a walk-through trap. The dung beetles reduce buffalo fly breeding through burial or spreading of dung pats. Different species are required to ensure optimal dung dispersion during the buffalo fly season. The use of the avermectins for internal parasite control may pose a problem for dung beetles as the chemical present in dung can kill developing beetles.

The walk-through trap developed by CSIRO removes buffalo flies when cattle walk through brushes inside a plastic domed structure. The flies then move upwards towards the light, are trapped by a false ceiling and die of dehydration. The animals need to be trained to use the trap. Although the trap is mainly aimed at the dairy industry, it can be used in extensive grazing where cattle use single watering points.

3.4 Potential alternative control methods

Potential non-insecticidal control methods include a buffalo fly vaccine, the use of buffalo fly parasitoids (biological control) and the use of behaviour-modifying systems. The latter approach could include fly attractants and traps, deterrents and/or repellents and antifeedants.

A buffalo vaccine would involve an antigen which is ingested by feeding flies and interferes with their life cycle. Research on a vaccine has been undertaken by CSIRO but the project is currently on hold.

Buffalo fly parasitoids are wasps which deposit their eggs on the fly pupae and the parasitoid larvae use the fly pupae as their food source destroying them in the process. To our knowledge, no work is currently in progress on the use of parasitoids in buffalo fly control.

Behaviour-modifying systems have been used as an alternative to insecticidal control for other livestock insect pests, eg tsetste fly, sheep blowfly, screwworm fly. These systems use chemicals and other devices (eg traps, targets) to mislead or confuse flies and prevent them from locating the host, mating or feeding on the hosts. Natural cues used by the flies for these processes are analysed, modified and then applied to the detriment of the flies. This project investigated the development of behaviour-modifying systems for the control of buffalo flies.

Such systems and their application to other insect pests are described in more detail in the next section.

3.5 Behaviour-modifying systems

A number of recent publications have reviewed the use of behaviour-modifying systems for insect management and control (Hummel and Miller 1987; Lewis 1984; Plimmer *et al.* 1982; Ridgway *et al.* 1990; Simeone and Siverstein 1990). An evaluation of insect trapping systems currently in use for agricultural and veterinary insect pests was carried out by Muirhead-Thomson (1991). For the biting flies of veterinary importance, it was shown that a wide range of trapping devices had evolved. The role of vision in these biting flies was reviewed by Allan *et al.* (1987). Principles of behavioural analysis for blood sucking flies and in particular tsetse flies have been outlined by Vale (1993).

For the use of behaviour-modifying chemicals in particular, Ridgway *et al*. (1990) concluded that there was reason for optimism that these chemicals could lead to reductions in the use of conventional pesticides and to significant expansion in the use of biologically based methods of pest control. For the tsetse flies *Glossina morsitans* and *G. pallidipes*, analysis of host-orientated behaviour led to a 10- to 1,000-fold improvement in the cost effectiveness of baits for surveys and control, and baits have now largely replaced air and ground broadcasting of insecticides (Vale 1993).

Successful examples of behaviour-modifying chemicals include mating disruption with pheromones in several moth species, and mass trapping of various fly species using kairomones (attractant originating from host or food source). Mass trapping of flies to reduce fly population density has been widely used, eg in Africa for tsetse flies, in North America for screwworm fly and in Australia for sheep blowfly. Tsetse fly populations were reduced by up to 95% by the use of a specifically designed trap made of blue and black cloth which was baited with a mixture of synthetic kairomones and buffalo urine. The numbers of screwworm fly were reduced with Swormlure pellets prior to the release of sterile screwworm in an eradication program. The pellets, containing a synthetic mixture of kairomones (Swormlure), a food source, a feeding stimulant and a toxin were spread from aeroplanes into the target area.

Our Brisbane based group had developed a synthetic attractant and a novel trap for the Australian sheep blowfly, *Lucilia cuprina*. The resulting trapping system which is now commercially available throughout Australia as Lucitrap, is selective for the sheep blowfly, user-friendly and needs to be serviced only once every three months. It has been demonstrated that Lucitrap can substantially reduce the population density of sheep blowflies on a property wide basis.

It was proposed that a similar approach could be taken with buffalo flies. Buffalo flies have to locate their host after emerging from dung pads, after ovipositing in fresh dung and any other time they leave the animal. They also have to locate fresh dung pads for successful oviposition. There is evidence from the horn fly that olfactory and visual cues both play a role in host location.
Experiments with an artificial cow (a black heated barrel) and the release of natural and synthetic cow-related odours elicited a considerable response from horn flies (Dalton et al 1978), suggesting such systems may be a viable control options.

The knowledge of buffalo fly behaviour in general, and of its response to olfactory and visual stimuli in particular, was very limited. Given the close relatedness with horn fly, it was considered necessary as a first step to review the behaviour of buffalo and horn flies and then to assess the relative merits of the different potential behaviour-modifying systems for their control.

4. Project objectives

By February 1997,

(i) to identify attractants, repellants and/or other sensory cues for use in non-insecticidal buffalo fly control systems.

(ii) to formulate recommendations for the commercial development of these systems.

5. Methodology

5.1 Fly colony and behaviour

5.1.1 Buffalo Fly Colony

A laboratory colony of buffalo fly was maintained throughout the project. This enabled experiments to be conducted with flies of standardised age and nutrition and relatively uniform size. The colony was established initially with flies collected from cattle near Ingham and Townsville. Throughout the project, the laboratory strain was supplemented with several further collections of flies from cattle in or near Townsville to ensure that the colony strain remained representative of the local field populations. The quality of the colony flies was monitored by measuring mean pupal weight and emergence rate. Batches with low pupal weight or emergence were not used for experiments. On one occasion in the second year of the project, flies from the colony strain were compared to wild flies collected from cattle near Townsville. Several behavioural tests designed to test fly vigour (Gover and Strong 1996) found no difference between the colony flies and the wild flies.

Flies were released onto two steers penned in metabolism crates in a windowless room (4m x 4m). The cattle had continuous access to water and received a daily ration of 10kg of lucerne pellets. A fluorescent light was suspended directly over and 0.5m above each steer's backline. The lights were on permanently. The room was maintained at 31°C and 65 % relative humidity (RH) by an evaporative cooler with heating capabilities. A total of 6 steers were used on a rotational basis with each steer spending 4 weeks in the room followed by 8 weeks on pasture. Dung dropped onto a tray behind the steer and was collected daily. Gravid females oviposited onto the fresh dung on the tray. Dung was collected and moved to an adjacent room maintained at 29°C. Dung was left undisturbed for 24 hours to allow all eggs to hatch. It was subsequently mixed and moistened if necessary to a consistency judged visually as 80 % moisture, a level considered optimal for larval development. It was then formed into pats on trays containing dry sand.
The pats were held at 29°C for a further 5-7 days, after which the pupae were retrieved from the sand by flotation. Pupae (500-1000 per day) were returned to the cattle room to maintain fly numbers on the cattle.

5.1.2 Age-Related Orientation of Buffalo Flies to Cattle

Newly-eclosed buffalo flies were collected in age cohorts of 100-300 flies varying less than 1h in time of emergence. They were held at 28°C in small cardboard pots with a supply of water until released, at varying times after emergence, into a small room containing a steer restrained in a crush in the centre of the facility. The numbers of buffalo flies on the animal and elsewhere in the room were counted at intervals of 5 minutes for 45 minutes. The flies were then cleared from the room and their total counted before a new cohort was released.

5.1.3 Fluctuations in Fly Numbers on Cattle

In each of 2 trials, 4 cattle were individually penned in cattle yards. The same cattle were used in both trials. Each side of each animal was photographed at intervals of 1 or 2 hours commencing in the afternoon and continuing until the following afternoon with the exception of a longer interval between 12 midnight and 6 am. Dusk and dawn fell close to 6pm and 6am respectively. The numbers of flies on the head, neck, back, belly, front leg and back leg were counted from each photograph.

5.2 Olfaction

5.2.1 Olfactometer

A choice type olfactometer developed and used by our group for sheep blowflies (Urech et al 1993) was initially used for the assessment of the responses of buffalo flies to olfactory stimuli (Figure 1). The design of this olfactometer was based on a horn fly olfactometer (Mackley et al 1983). It consisted of four separate holding chambers in which flies were placed (Figure 1). The flies had the choice to move upwind into either a control choice chamber (clean air) or a treatment choice chamber (air containing test odour) or remain in the holding chamber during the observation period (30 minutes). Entry into the control and treatment chambers was through a cone and thus irreversible. The numbers of buffalo flies in the control and treatment chambers were recorded every 5 minutes for 15 minutes.

The air was drawn through the olfactometer by an extraction fan. The air inlet for the control was drawn through activated charcoal filters into a temperature controlled box (which could be humidified) and then split to the four control choice chambers. Steer odours were obtained from an animal kept inside a metal framed cage (2.2x1.4x1.2 m) covered with polyethylene sheeting. The air was taken from the top of the cage and fresh air entered the cage through an opening (2.5 cm high) around the base. Odours from smaller sources were obtained by placing these sources into a temperature controlled box as used in the control stream. The air flows in the eight choice chambers were measured with a flow meter and adjusted to 0.4 m/s with in-line valves before each experiment.

A modification of the olfactometer was the replacement of the separate choice chambers by divided chambers of the same size as the holding chambers (see Figure 1). The divided chambers contained a separator wall extending the full length of the chamber.
This arrangement gave a larger area for odour and control streams to enter the holding chamber. In this setup, a stainless steel mesh with multiple cones was used as a one-way separator between holding and divided chambers.

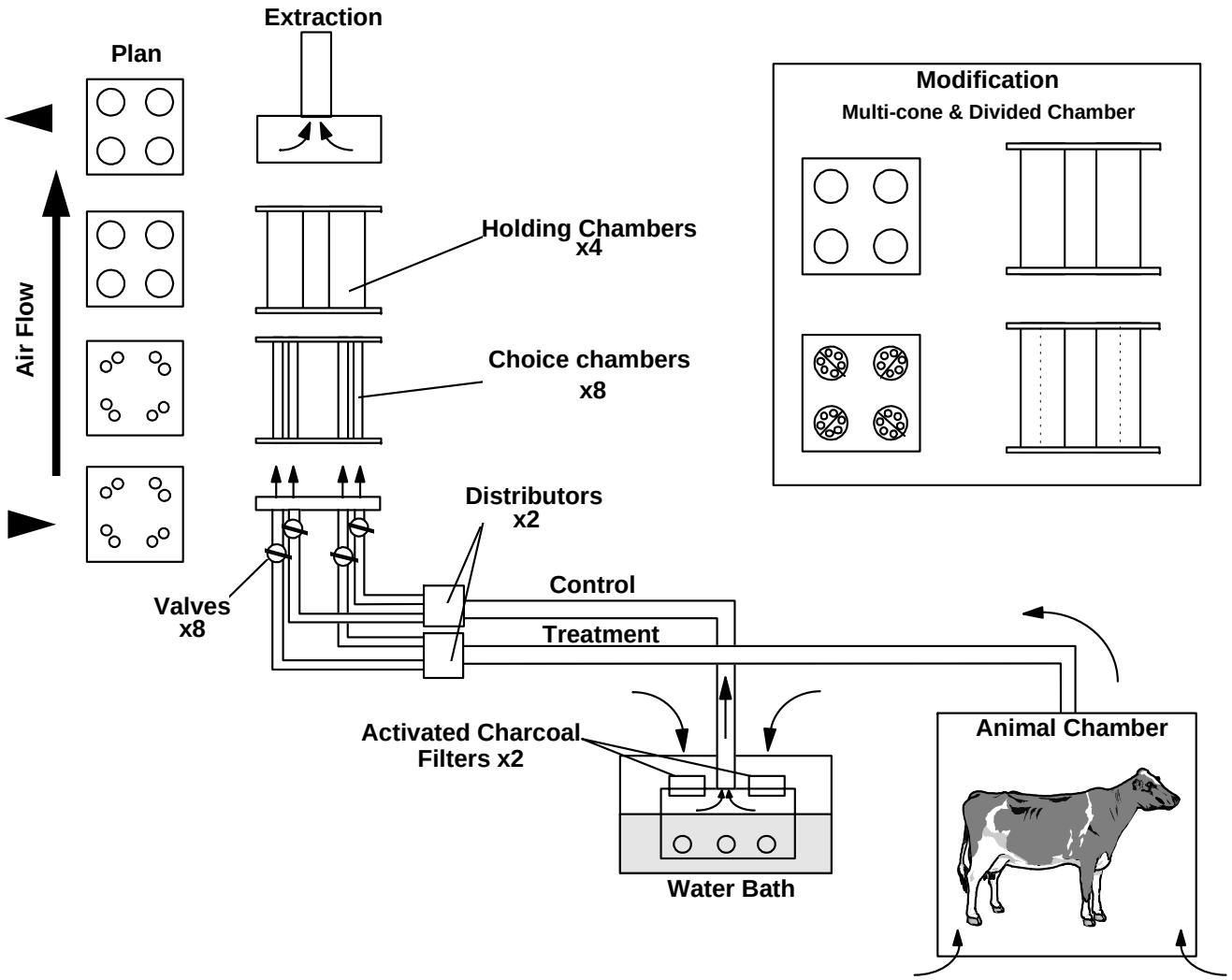


Figure 1: Schematic drawing of olfactometer used in olfactory assays for buffalo fly (only two of 4 chambers shown).

5.2.2 Fly cage assay

A metal framed fly cage (450x300x300 mm) with two opposing longitudinal perspex side walls, two walls and a ceiling made from fly screen and a removable steel floor was used for this assay (Figure 2). Two air streams (5 L/min) are introduced into the fly cage through glass tubes and directed at the centre of the circular target areas (∅ 100 mm) on each of the opposing perspex walls. Buffalo flies were introduced into the cage and left to acclimatise for 5 minutes. A UV light (365 nm), located centrally above the cage, was switched on for 3 to 5 seconds to attract the flies to a central position equidistant from both perspex sides prior to the start of the experiment. The response of the flies was measured by counting the number of flies congregating within the target areas at one minute intervals to ten minutes, and then at 15 minutes.

The results were presented graphically (cf Figure 6) showing the percentage of the buffalo flies in the cage responding to the odour (bars) and control (dots) at different times (minutes) after the start of the experiment. Each group of bars represents one experiment or replicate if the odour was the same. The graph also shows the relative humidity of the control (thin line) and odour (thick line) air streams.

The cage was placed in a fully lit, air conditioned room (30^{o}C) with a constant, directional air flow to remove the odours dispensed into the cage (cf Figure 3).

Charcoal filtered air was delivered via a diaphragm pump with an exchangeable filter at the inlet. The air passed through a temperature controlled humidifying chamber and bottle containing odour sources. A T-junction with a side arm containing a needle valve was used for adjusting the air bleed to the outside and thus regulating the air flow to the cage. An identical air delivery system was used for the control stream. Temperature and humidity of the air streams to both sides were measured. Standard tests on odours were carried with air streams matched in temperature and humidity.

Odour sources were introduced into the air stream inside a bottle (500 ml) through which the air passed. Solid odour sources (eg faeces) were placed in jars which were then put inside the bottle. Pure chemicals, diluted in an inert carrier such as paraffin oil or glycerol/water (1:1), were applied to filter paper strips which were suspended in the bottle. The odour source was renewed for each run (15 minutes).

Unlike in the olfactometer assay, odours from a small steer were obtained by placing the steer in an enclosed small temperature controlled chamber (animal holding annexe) adjacent to the experimental room (Figure 3). After 1 hour equilibration period, air was drawn from the room and pumped to the cage as above.

Initial experiments to explore the flies' responses in the olfactometer and cage assay were carried out. Parameters investigated were temperature, light conditions, orientation of olfactometer, odour sources and age and physiological status of flies.

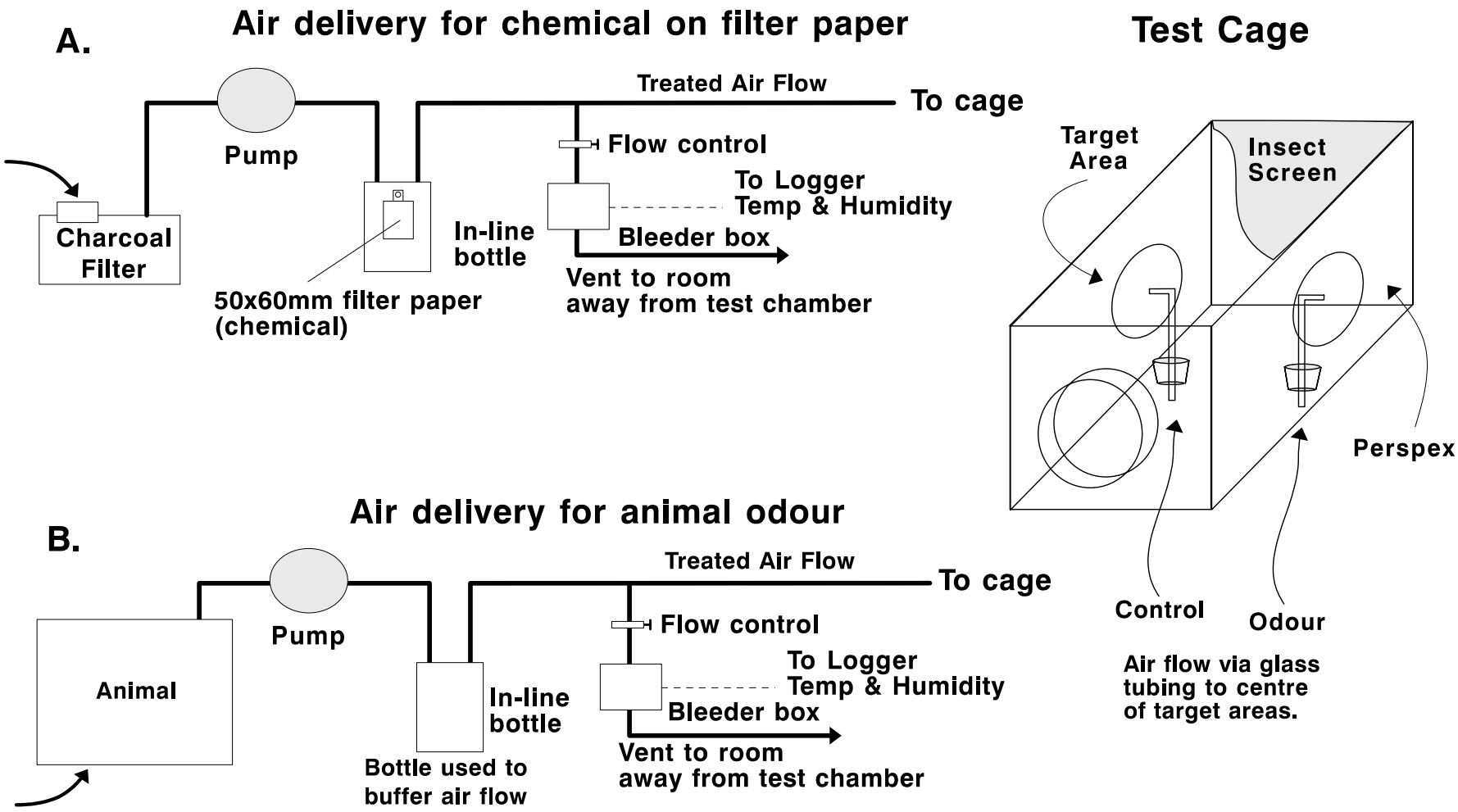


Figure 2: Fly cage and air delivery system used in olfactory assays for buffalo fly.

5.3 Vision

5.3.1 Visual targets

5.3.1.1 Efficacy of target in absence of cattle

In order to present a visual stimulus with no olfactory components, a black target (90cmx90cm with bottom 30cm above ground) coated with clear adhesive ("Stickem Special Pest Glue") was positioned in a flat paddock with no cattle. Flies were released 10m upwind of the target. Newly-emerged (<24hours old, unfed) and mixed age (collected off colony animals and starved for 3-4 hours) groups of flies were tested in an attempt to simulate newly-emerged and on-cattle fly populations. 100-200 flies were released on each occasion except on the single occasion which simulated natural emergence when 700 pupae (from which 680 flies emerged) were positioned around the trap. Emergence of these flies occurred from 4 pm to 12 midnight with peak emergence from 6-9pm. The number of flies captured on the target was assessed at least 2hrs after release. Each release was replicated on different days with the exception of the natural emergence simulation.

5.3.2 Light traps

5.3.2.1 Light Trap - First Field Trial

Cattle at the Oonoonba Veterinary Laboratories, Townsville, were allocated into 2 groups of 14 so that the groups were similar in terms of age and previous fly burdens (assessed by the mean of 3 counts on different days). The groups were held in separate paddocks and were penned each night in separate pens 26 x 4m. The pens were separated by 250m which included a number of buildings and trees. Preliminary mark-recapture trials revealed minimal dispersal of flies between the groups. The light trap was installed on the outer edge of one pen halfway along the long side. The trap consisted of 2 x 40W blacklight (UV and visible wavelengths) fluorescent tubes illuminating a white sticky target (1m high x 1.2m wide). The lights were suspended 2.8m above the ground and the target fixed immediately beneath it. The light was switched on before dusk and off after dawn. A similar target without a light was installed in the other pen.

Flies were counted on both sides of each animal each day before the start of the trial and throughout the trial. The number of flies on the sticky boards was counted each day. After 6 nights the light was removed. It was installed in the other pen and 9 days later the light was run for a further 4 nights over the other experimental group.

5.3.2.2 Light Trap - Second Field Trial

The second trial was similar to the first but with the following modifications. The board and light were moved lower (lights 1.4 m above the ground). A wire grid (5 x 5cm apertures) was placed in front of the board to protect the light. This also gave the cattle something to brush against to assist with disturbing the flies (although no cattle were observed to do this). The light was set so that it alternated between on and off at 15 minute intervals.
This was done to minimise the opportunity for the flies to habituate to the light and also because changes in light appear to be best for stimulating the flies to rise from the animals. Only one 40 W blacklight fluorescent tube was used.

5.3.2.3 Field Trials of Light trap with Unrestricted Cattle

Two trials similar to the second field trial were conducted. However the cattle were not penned at night, the control target was omitted and the treatment was not reversed within each trial. The light trap was positioned centrally on the short side of a paddock (100 x 250m) and 15 m from a water trough which preliminary observations had shown that the cattle visited during the night and day. The paddock was flat with only a few trees. The light could be seen from anywhere in the paddock. The light was operated on a continuous 15min:15min light:dark cycle between 7pm and 6am. Each trial consisted of 4 nights trapping (although the trapping was interrupted in the second trial by heavy rain which threatened to disrupt the power supply to the light).

5.3.3 Distance and phototaxis

Mixed age flies of both sexes and were netted off the colony animals and approximately 20 flies were placed into each of 3 small (12cm x 7.5cm diameter) PVC cages with mesh ends and a central divider which created 2 chambers in each cage. The cages were positioned 1m above the ground such that the mesh openings faced directly towards or away from the light (a naked, ie no reflective target attached, 40W blacklight fluorescent tube). The flies were first attracted to the end of each cage furthest from the light. The dividers were lifted for 10 seconds and then replaced and the number of flies which had moved to the other end of the cage were counted. This procedure was done initially with the light off to provide a baseline response ("dark response") and then with the light on to measure the degree of phototaxis ("light response"). The procedure was conducted at 10m intervals from 10-60m and then repeated with a new batch of flies starting from 60m and reducing to 10m. The light was moved to the opposite end of the transect for the second batch. The data for each distance were then averaged. The trials were conducted at night in a flat area of bare earth with minimal background light.

5.3.4 Effect of intermittent light cycles on light trap collections

A single steer was placed in a 6 x 12m pen. The pen was surrounded with hessian to a height of 2.2m to reduce external influences and maintain a consistent background for the duration of the trial. A light trap with a low target as used in previous field trials was placed midway along one of the short sides. A 40W blacklight fluorescent tube was used and later a 20W white (ie visible wavelengths only) tube. Each afternoon, 100 flies from the colony (ie mixed ages and sexes) were marked with fluorescent dust and released onto the steer. The flies which settled on the steer after 30 minutes were counted. This number rather than the number released was used as the starting population and it varied from 61 to 94 with a mean of 80. Light cycles tested were a) continually on b) 1 minute light/1 minute dark c) 15 minutes light/15 minutes dark, and d) no light. A different cycle was tested each night until each had been tested twice in the case of the 40W blacklight and 3 times in the case of the 20W white. The light was turned on at 7pm and off at 8am. The marked flies captured were counted the next day. With the 40W blacklight all cycles (except the no light which caught negligible numbers throughout), caught >90% of the flies observed on the steer prior to the start.

Consequently the blacklight was discontinued in favour of the white as a smaller catch for the control treatment (continually on) was needed for any differences between cycles to be detected.

5.3.5 Electroretinograms

Laboratory reared female *H. i. exigua*, about 4 days old, were used; they were fed on blood about an hour before the start of each experiment. The fly was held immobile, head upright, in "Blu-Tack", exposing the compound eyes. One platinum electrode was inserted in the head whilst another was positioned to touch the surface of one of the compound eyes. The potential recorded between the electrodes was monitored, via a "Grass Instrument Company" P16B pre-amplifier, on a "Tektronix" storage oscilloscope and "Delta" computer data logger.

Photic stimulation of the recorded eye was provided for, through a flexible fibre optic guide, illuminated with designated wavelengths selected in an "LKB" UV/visible spectrophotometer. Stimulus access to the light guide was controlled by a custom-made, electromagnetically-operated vane. This system allowed production of precise, square wave stimulation and was triggered by a pulse from the data logger.

Three types of potentials were evoked from the insects: (a) a phasic interneurone "on" spike; (b) a tonic retinula cell receptor potential; and (c) a phasic interneurone "off" spike. Only the retinula cell potential (b), can be strictly considered to be a retinogram. Three measurements were made at each wavelength, using 6 sec pulses interspersed with 60 sec recovery periods; starting at 300 nm and moving up in 5 or 10 nm steps, to 650 nm. At the start and after every three wavelength increment a standard wavelength was used, so that variations in the sensitivity of the preparation during the several hours duration of the experiment could later be corrected for. The energy of the various wavelengths exiting the fibre optic was measured with a calibrated phototransistor.

5.4 Vision and olfaction combinations

5.4.1 Trials of traps developed for other stock-associated Diptera

Three traps developed for other livestock-associated Diptera were tested for their suitability for buffalo fly, namely a sticky trap baited with a commercial tsetse fly lure (Agrisense tricomponent sachet) and acetone, a modified Williams trap for stable fly (Broce 1988) and a modified Manitoba trap (without carbon dioxide) for tabanids (Adkins et al 1972). Each trap was tested separately. All tests were conducted with unfed, newly-emerged flies <23hrs old (held prior to use at 20 ^{o}C and 85% RH). Flies were released 10-20m from the traps (downwind of the blowfly trap, upwind of the other traps. 100 or 200 flies were released at a time. Most releases were conducted in the afternoon to simulate natural emergence which peaks in the late afternoon and evening. Collections were assessed 15-24 hours after release. Each test was replicated 2-4 times on successive days.

5.4.2 Behavioural observation facility for flies (BOFF) trials

A series of experiments were conducted in the BOFF (behavioural observation facility for flies) to help establish the relative importance of vision and odour in attracting buffalo flies. The BOFF (Figure 3) is a temperature controlled (30^{0}C) room approximately 8x5x3 m, with an annexe capable of presenting a steer visually and /or olfactorily (APA). The APA and experimental room are separated by a glass partition, but are connected through a closeable air duct.

Approximately 50 mixed sex, blood deprived, 4 to 6 day old flies were released into the BOFF at the opposite end to the animal presentation annexe (i.e. 7 m from the APA). The

APA allowed visual and olfactory components to be presented separately or in combination to the test insects.

The buffalo flies could thus be exposed to the following stimuli: a. no stimulus (i.e. empty APA); b. vision only; c. odour only; d. vision plus odour; e. target (SCOVOS) only and f. target plus odour. The target was a life size two dimensional plywood model cow, 1.5m long and 1.4m high and painted with black and white markings (SCOVOS = synthetic cow with visual and/or olfactory stimuli).

The number of flies caught on a grid of clear sticky strips attached to the glass partition of the APA was used to assess the attractiveness of the stimulus. The assessment time allowed varied from 15 to 30 minutes.

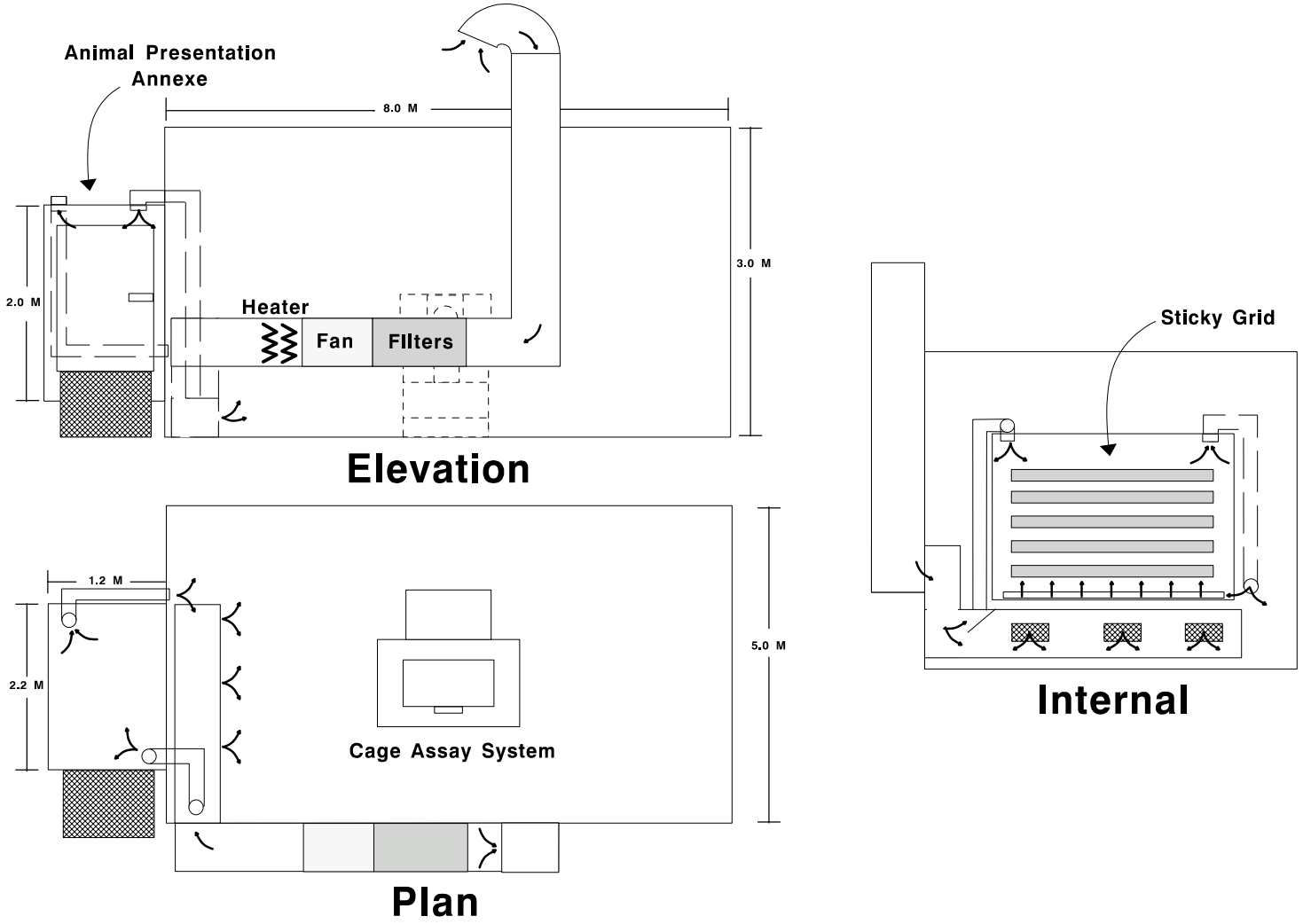


Figure 3: Behavioural observation facility for flies (BOFF) and animal presentation annexe (APA) used in olfactory and visual assays for buffalo flies.

5.4.3 SCOVOS field experiments

Both sides of SCOVOS were covered with black plastic sheets to which a thin layer of Tanglefoot (sticky polybutene) was applied. If lures were used, they were attached to the top of the support post at the front legs.

Field experiments were carried out in clear, flat paddock at ARI, Yeerongpilly. A known number of buffalo flies (50-80) were released remotely from two jars placed on the ground 12 m up- and downwind from the model by an operator at least 20 m away from jars and model. The flies in one jar were marked with a fluorescent dye so that the origin of the caught flies could be determined (no wild flies were present in paddock). After a fixed time (1-1.5 h), the flies caught on the sticky panels were counted and removed from the model. The experiment was normally repeated four times a day (2 replicates of 2 treatments).

5.4.4 Tsetse fly lures

Two preliminary field experiments evaluating tsetse fly lures against buffalo flies were conducted at Brian Pastures Research Station, Gayndah. Pairwise comparisons between black, vertical sticky targets (120x60 cm rectangle, 80 cm above ground) with and without tsetse lure were carried out in paddocks carrying cattle with buffalo flies. The tsetse fly lure was swapped between the targets, which were 1 km apart, every 24 hours and the flies caught during that period were collected. The fly numbers were transformed (square root), the means calculated and subjected to analysis. The results given are the backtransformed mean buffalo catches per 24 hours.

5.4.5 Lucitrap and tsetse fly lures

A replicated 4x4 Latin square experiment including a black rectangle (see above), a rectangle baited with tsetse lure, a Lucitrap and Lucilure (commercially available trapping system for sheep blowflies) and a Lucitrap with tsetse lure was run at Brian Pastures Research Station. The four treatments were swapped and the flies collected every 24 hours. The catches were square root transformed and analysed.

5.5 Chemical analysis of odours

Qualitative and quantitative analyses of volatile components were carried out either by gas chromatography (GC)/mass spectrometry (MS) or GC fitted with a flame ionisation detector (FID).

GC/MS was performed on a VG Trio GC/MS with electron impact ionisation. The GC was fitted with a 30m DB5 capillary column (J&W, Alltech) and a temperature program starting at 40 ^{o}C and finishing at 26O ^{o}C was used. The mixtures were applied to the GC column either by thermal desorption or split injection of a solution.

GC/FID was performed on a HP5335 GC fitted with an autosampler and an integrator. The mixtures were applied to a 15m DB5 column by split injection of hexane solutions. Quantitation was by the area under curve.

Volatile chemicals contained in air were collected by drawing the air through a metal tube containing Tenax TA with a constant flow pump (80 ml/min).

Organic compounds are adsorbed on Tenax TA and can then be desorbed either with an inert gas or a solvent for analysis. Thermal desorption was done with a thermal desorption device (ATD50, Perkin Elmer) using nitrogen at 150 ^{o}C for 10 min. The nitrogen passes through a cold trap (-20 ^{o}C) where the organic compounds are condensed. Transfer of compounds to the GC column is then achieved by rapid heating of cold trap.

5.5.1 Cattle odours

A metal framed polyethylene cage (cf Olfactometer section) was placed over a small tethered steer and supported 25mm above ground to allow for air intake. A hose connected to an extraction fan was connected to the centre of the cage ceiling to provide adequate air circulation inside the cage. The air contained in the cage was drawn through a Tenax tube (1 or 2 hours). Desorption was achieved by thermal desorption.

5.5.2 Buffalo fly cuticle hydrocarbons

20 buffalo flies were placed in hexane (1 ml) containing tetracosane (C_{24} hydrocarbon) for16 h. The hexane was run through a short column of silica gel to remove any polar components. The column was washed with fresh hexane (1ml). The combined hexane fractions were evaporated to dryness. Prior to injection into GC (1 ul), hexane (50ul) was added to the residue and the tube vortexed for 30 sec.

The position of double bonds was determined as described by Carlson et al (1989). Briefly, the substrate, dimethyl disulfide (DMDS) and a iodine solution were heated to 38 ^{o}C for 24 hours. The DMDS adduct was analysed by GC/MS which provided characteristic fragmentation patterns which allowed the determination of the original location of the double bond along the carbon chain.

6. Results and Discussion

6.1 Fly colony and behaviour

The literature on the behaviour, the role of attractants, arrestants and repellents and the population dynamics of the buffalo and horn flies was reviewed as part of this project. The full review with over 140 references is contained in Appendix A.

The review concluded that olfactory and visual cues played an important role in host and oviposition site location in buffalo flies. The work carried out in this project focussed on finding, analysing and applying olfactory and visual cues which could be used as part of a behaviour-modifying system for the control of buffalo fly.

6.1.1 Buffalo fly colony

The buffalo fly colony at OVL provided pupae of consistent quality. The mean pupal weight was between 3 and 4 mg. The flies emerged and were maintained on bovine blood at ARI until they were used in experiments.

6.1.2 Age-related orientation of buffalo flies to cattle

Upon their release into the room, most flies flew initially to a light fitting on the ceiling containing the two fluorescent tubes illuminating the room, and subsequently moved to the animal or to the walls of the room, usually accumulating on the animal with time. Some

orientation to the steer was seen in flies as little as 1-2 h old, the youngest cohort tested. At this age, a maximum of approximately 10% of the flies released into the room had settled on the animal 45 minutes after their release. The orientation response increased with increasing age of flies up to a maximum of 16-17 h after eclosion, with some 40-45% of released flies of this age found on the animal during the second half of the 45 minute counting period. The observed decline in responsiveness to the steer of flies older than this is probably attributable to a deterioration in their condition as a result of prolonged deprivation of blood.

These results indicate that a proportion of even recently eclosed buffalo flies are responsive to sensory stimuli from potential host animals, and should therefore be susceptible to trapping.

6.1.3 Fluctuations in fly numbers on cattle

It is generally thought that flies remain on the host cattle except for brief flights to oviposit on cattle dung or to cross to another host. It is likely that flies already on cattle would be more difficult to attract to a trap than flies which were away from their hosts, especially for traps which mimic visual and olfactory cues from cattle. Any indication that the flies leave their hosts regularly rather than occasionally would improve the potential for traps which mimic the hosts.

During light trap experiments on yarded cattle, it was noticed that the numbers of flies on cattle appeared to decrease at night but increase again by the following morning. On one occasion, significant numbers of flies were collected at night with a sweep net from the fence and grass around a single penned steer when the numbers on the animal were low. These flies had all fed recently on blood and included both sexes and various ages of females. It appeared that some flies were leaving the steer for short periods. This preliminary observation prompted these 2 trials to examine the diurnal fluctuation in fly numbers on cattle.

In each trial, particularly the first, (Figure 4), fly numbers on each of the cattle varied considerably indicating that there was constant movement of flies to and from the cattle.

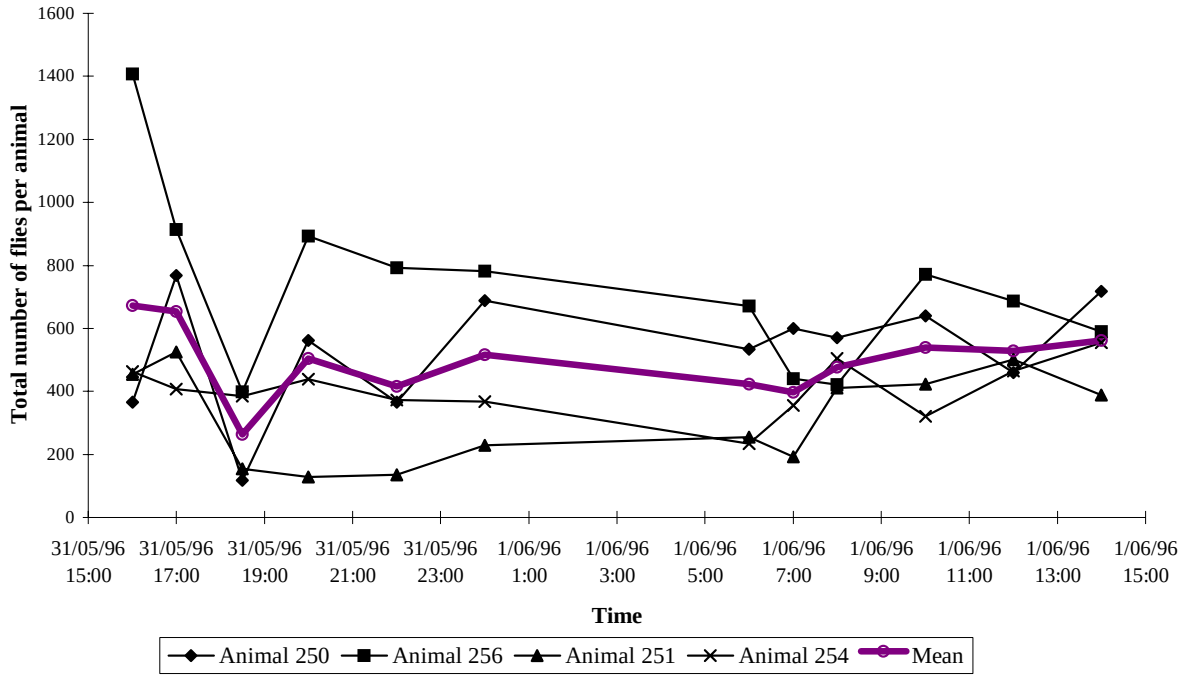


Figure 4 Fluctuations in fly numbers on 4 cattle over a period of 22 hours (First trial)

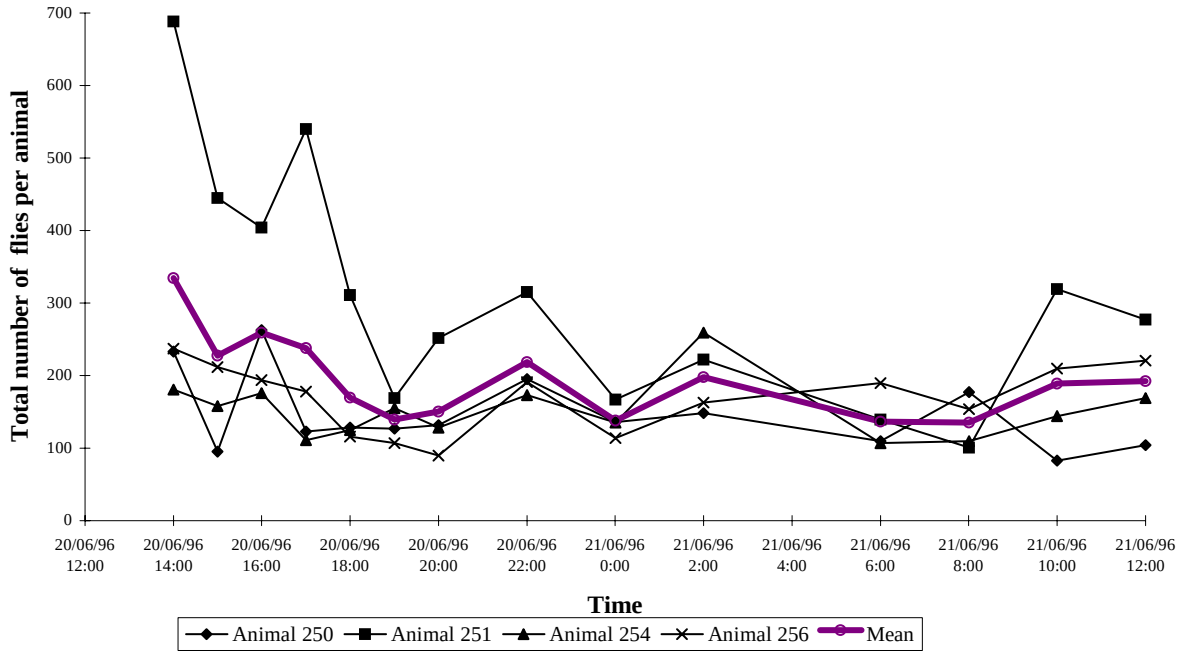


Figure 5 Fluctuations in fly numbers on 4 cattle over 22 hours (Second trial)

The means of all cattle varied to a lesser degree. The trial cattle were the only hosts within about 200m so it is likely that any movement to and from non-trial cattle was minimal. Consequently the mean is essentially one quarter of the total local population on the four trial cattle and consequently when the mean drops, (such as it did after dusk in the first trial), it suggests that flies are leaving the cattle not simply to cross to other cattle, but for a more extended period. Conversely, when the mean increases it suggests that flies are returning to the cattle from somewhere other than the other cattle.

However, the stability of the mean, relative to the numbers on the individual cattle suggests that most flies which left one animal went to one of the others relatively quickly.

The most striking feature in the trials was the pronounced drop in the number of flies on 3 of the 4 cattle in the hours after dusk in the first trial. This was consistent with the observations which prompted the trials. However the response was not as pronounced in the second trial (Figure 5). Consequently it cannot be said at this stage that there is any consistent diurnal pattern of movement of flies to and from the animals. Any fluctuations may depend on other factors such as temperature.

In both trials but particularly in the first there was a change in the areas of the host preferred by the flies. During the night there was a general movement by the flies from the upper areas of the host such as the head and shoulder to the lower areas, namely the belly and legs. Perhaps movements of this type could explain why in light trap trials discussed elsewhere in this report, the low light trap worked better than the high trap.

6.2 Olfaction

6.2.1 Olfactometer

6.2.1.1 Physiology and age of flies

The colony flies have continuous access to bovine blood while they are held for experimentation. There is evidence that they will not respond well to olfactory stimuli if they have recently fed. The responses of buffalo flies which had access to water only or glucose solution only for 16 hours, to water only for 2 hours prior to experiment, and to blood till the start of experiment, were compared in the olfactometer using bovine faeces as the attractant. The most consistent response was obtained from the flies which had been on water only for 16 hours. The flies which had access to blood up to the start of the experiment responded very poorly. Replacement of blood by water approximately 16 hours before an experiment became standard protocol.

The responses of flies of different ages were compared using dung and whole animal odours. No consistent difference was detected between newly emerged, unfed flies (<48 hours) and flies 4 to 8 days old and between 4, 6 and 9 day old flies. In some experiments the responses to the odour and the control were higher for the newly emerged flies, but the difference in responses between odour and control was similar. The survival of older flies was better during the experiment and it was more difficult to provide a constant supply of newly emerged flies. The standard age of flies to be tested was set at 4 to 6 days.

6.2.1.2 Characteristics of olfactometer

The choice type olfactometer had been used vertically for horn flies (Mackley et al 1983) and horizontally for Australian sheep blowflies (Urech et al 1993). In experiments with horizontal olfactometer orientation the flies showed a preference for the “upper” choice chamber over the “lower” one. The vertical orientation used by Mackley, where the flies move upwards from the holding chamber into the choice chamber, was then investigated. Results with a vertical pathway were better than with a horizontal. The most consistent responses, and the best defined separation between odour and control were obtained with a vertical arrangement, where the flies were placed in the holding chamber located above the choice chambers. Thus, the flies moved downwards against an upward moving stream of air carrying the odour.

Buffalo flies are strongly positively phototaxic, that is they readily and quickly move towards any source of light. The illumination of the olfactometer was a crucial factor in any evaluation of the olfactometer. The initial experiments were conducted in a completely darkened room with a red fluorescent tube inserted in the centre of the olfactometer as had been reported for horn flies and sheep blowflies. However, it was noticed that the flies in the holding chambers oriented towards the central light, particularly towards the end of the fluorescent tube where the red coating was thinner. Some reduction in the flies' orientation towards the light was achieved by removing the warmth generated by the fluorescent tube by circulating air through the cylindrical lamp fitting. Further experiments were conducted in complete darkness (with only an end point response measurement), but no response to olfactory stimuli was observed under these conditions. Central lightning of the choice chambers only (dark holding chambers), brought more flies into the choice chambers, but the selectivity for the treatment over control chambers was considerably lower. The best results in terms of selectivity for the odour and overall response were obtained in a brightly and evenly lit room. Thus, the olfactometer was placed in the middle of a room, equidistant from fluorescent ceiling lights.

Preliminary experiments had also indicated that temperature and in particular humidity of the air stream had an influence on the responses of buffalo flies. Buffalo flies showed a preference for air streams with a higher humidity. Thus, it was important to match the relative humidity between odour and control streams. The humidity difference between the two unmatched streams was large when an animal was used as the odour source. Water could of course be one of the attractive ingredients of "cattle odour". However, we decided to carry out our search, mainly aimed at organic attractants, with matched humidity air streams.

Inconsistencies in the responses of buffalo flies between the chambers and experiments, led us investigate the use of a less restrictive separator between holding and choice chambers. The multi-cone and divided chamber system appeared to improve the consistency and the extent of the responses to a certain degree and it became the standard arrangement for olfactometer testing in the latter stages.

6.2.1.3 Steer and steer related odours

The initial experiments with steer odours were carried out against a non-humidified control air stream in the olfactometer with separate choice chambers. The responses were generally good with approximately 40 to 70% of the flies entering the odour eminating choice chambers with very few flies (less than 10%) in the control choice chambers.

When the humidity of the control air stream was increased to match the level of steer odour stream, the overall response and the difference between odour and control chambers became much smaller and less consistent. In some cases, there was even a preference for the control chamber over the odour chamber. The consistency and overall response was somewhat improved when the separate choice chambers were replaced by the divided chambers and multicones (cf Figure 1). With this arrangement, responses to steer odour was typically between 40 and 80% while the control response was 5 to 20%.

However, there were experiments where the responses to steer odours were low (10 to 40%) with corresponding control responses at 0 to 30%. Experiments with such a low response and little discrimination between odours appeared to occur more often during winter. A logical explanation for this observation was never found even with careful elimination of variable parameters. The same phenomena has been observed with other insects by us and other researchers. The use of a negative ion generator in the air stream, as suggested by Prof Butler at the University of Florida, did not consistently improve the observed responses.

The response of buffalo flies to steer related odours was also assessed. Faeces from animals on pasture or grain fed elicited a higher response to the odour (20 to 40%) than control (10% to 20%). Again variability between experiments was quite high, possibly due to differences in faeces. Bubbling the air stream through an aqueous slurry of faeces did not improve the flies' responses when compared to solid faeces. A small, but positive response (20 to 25% odour; approx 10% control) was obtained with the odours given off by fresh rumen fluid.

The inconsistencies and low responses obtained with the olfactometer in some experiments, led us to develop an alternative behavioural bioassay which was hoped would increase the responses of buffalo flies and reduce sensitivity to unknown influences. The following section contains the results and discussion of the experiments carried out with the fly cage assay.

6.2.2 Fly cage assay

6.2.2.1 Characteristics of fly cage assay

The fly cage assay was developed with the hypothesis that the responses of buffalo flies to olfactory stimuli would be more readily detected if the flies had to make less effort to respond. In the olfactometer the flies have to "squeeze" through cones, making the registered response irreversible. Preliminary observations had indicated that buffalo flies held in a cup will readily move to an area on the wall to which an air stream carrying an attractive odour (breath, steer odour) was directed. This was further developed by placing a vertical wall in a fly cage and blowing an odour carrying air stream onto the wall. Buffalo flies kept in the cage congregated around the area where the air stream hit the wall. Interestingly, when the wall was removed and the same air stream dispensed through a funnel with fly mesh over its wide end, no fly responses to the odour were observed.

One fundamental difference between the olfactometer and the cage assay was the (ir)reversibility of the flies' responses. In the olfactometer the flies entering the choice chamber could not return to the holding chamber, thus the numbers of flies in the choice chambers were increasing or stayed constant during the course of the experiment. In the fly cage assay, the flies could freely move into and out of the designated target area, thus increasing, constant or decreasing numbers of responding flies could be obtained. The assay does not establish whether the flies present in target area at one count are the same or different flies from the previous count.

In the fully developed fly cage assay, two odour streams are introduced into a fly cage through glass tubes and continuously dispensed onto the two opposing perspex sides of the cage (cf Figure 2). The odours are ventilated from the cage, placed in a room with a constant, directional airflow, through the other screen walls of the cage. Through the use of test and control air streams of matched temperature and humidity, a behavioural response of the flies is observed. The flies move to, and remain in the area where an attractive test odour (eg whole cattle odour) meets the cage wall. When the odour flow is stopped, the flies redistribute in the cage.
The quantitative output from the assay is the percentage of buffalo flies present in the cage which are within the target area on the opposing perspex sides of the cage at various times after the introduction of the odour stream (cf Figure 6).

The initial testing and comparisons of the cage assay were carried out with steer odour. The flow rates of the air streams were set to attract an optimal number of flies to a circular area of 100 mm diameter. When the flow was too low the flies would clump around a small area which

made fly counting difficult. With too high a flow rate the flies did not approach the centre of the circle.

Before the start of each experiment a UV light (365 nm) located centrally above the cage was switched on for a short period. This resulted in a centring of the flies prior to the start of experiment. By delivering steer odours to both sides, it was established that neither of the sides was inherently preferred by the flies (no bias). When clean air (as used in a control stream), either at ambient humidity (50%) or humidified (70%), was pumped to both sides nil or very small responses (5%) to either side were observed.

A comparison between the olfactometer and the cage assay showed that the latter gave a higher response to the odour stream than the olfactometer. The responses to the control were similar in both assays, resulting in a better discrimination between odour and control in the cage assay. The variability between experiments also appeared to be lower in the cage assay than the olfactometer.

A comparison was carried out between competitive and non-competitive experiments in the cage assay. In the former, the odorous and the control air stream were delivered at the same time to opposing sides of the cage. In the non-competitive assay, the two streams are delivered one after the other (alternate) to the same side. As expected the observed responses of the flies were larger in the non-competitive assay, since there is at any one time only one target area "active". This increase in response was more pronounced for the control stream than the odour stream. Therefore, the discrimination between odour and control was better in the competitive assay, which was selected as the assay for our screening work.

6.2.2.2 Steer and steer related odours

Good responses of buffalo flies towards the side where steer odours were presented were obtained in the cage assay (Figure 6). In most experiments, 60-80% of all flies present in the cage were located in the specified area on the wall at 15 minutes. In the corresponding area on the opposing wall in the control stream (charcoal-filtered air of matched temperature and humidity), typically 0-10% of the flies were observed. Thus, there was a high response to steer odours and a good discrimination between odour and control. The retention of the flies in the target area when exposed to steer odours for a prolonged interval (140 minutes) was also determined. With five minute reading intervals, a 60% response to the odour was reached after 10 minutes (5% on control). This level was maintained fairly constantly for 45 minutes (control 10%) and then dropped to 40-50% (control 10-15%). After 100 minutes the odour response was 40% (control 5-10%) where it stayed till the end of the experiment.

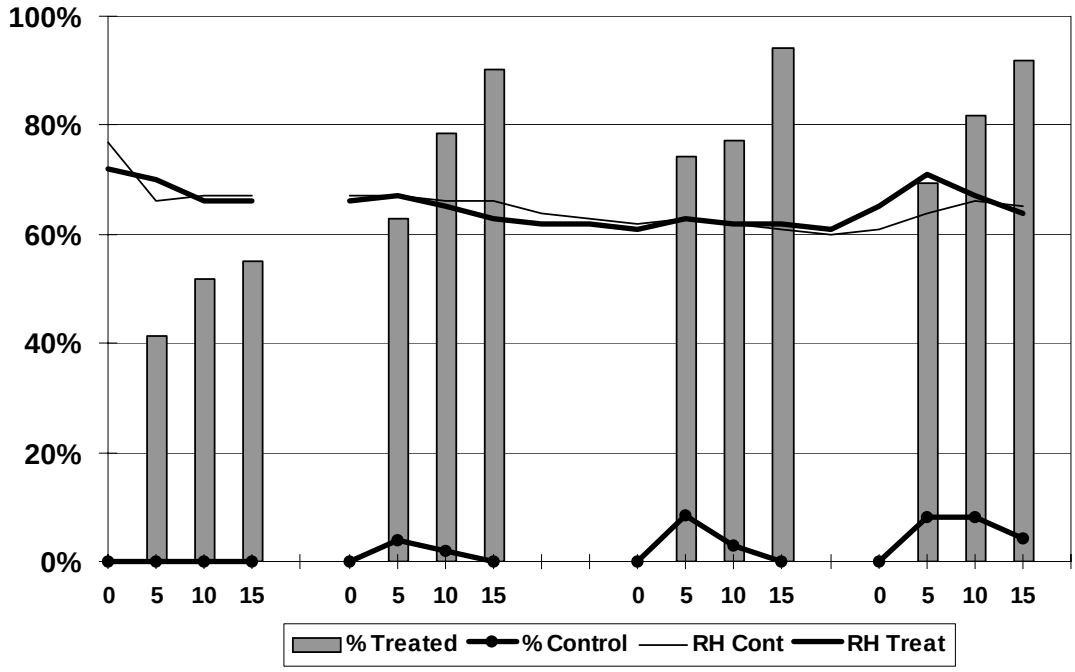


Figure 6: Percentage of buffalo flies responding to steer odour (grey bars) and control (black dots) air streams and relative humidity (RH) for odour (thick line) and control (thin line) streams against time (minutes). Each block is a new replicate.

Odours originating from different parts and excretions from cattle were tested in the cage assay with the aim of locating the principal source of the components contained in cattle odour. Bovine faeces gave responses between 40 and 80% to the odour side (approximately 10% on control), confirming the olfactometer result. The odours given off by fresh urine from a heifer elicited responses of 20 to 40% on the odour side with controls of less than 10%. Bovine blood, fresh and aged for 2 days at room temperature, applied to filter papers in the air stream resulted in a small response of 20 to 40 % to the odour side and 0 to 15% on the control side.

Odours collected from within a head mask applied to a steer, and presumed to consist largely of the animal's breath, also showed a strong response in the assay with 50 to 75% and 15 to 40% of the flies on the odour and control sides respectively. The reasons for the relatively high responses to the control side in the assays with cattle breath are not certain. The midside of a steer was covered with a large funnel from which air was drawn from the funnel stem and introduced to the cage. This "steer body odour" attracted approximately 60% of the buffalo flies with 5 to 20% on the control side.

It was evident from these experiments that a wide variety of cattle related odour sources are capable of attracting buffalo flies. All the animal related sources elicited a positive orientation response in the cage assay. None of the sources was outstanding on its own and the odours collected from the complete animal were the best attractant. However, none of the tested sources can be ruled out as at least a contributor to the overall attractivity to buffalo flies.

6.2.2.3 Fly associated odours

The release of chemical components by insects, inducing attractancy or aggregation behaviour of individuals of the same species, has been demonstrated for many insect species. Examples of such compounds are sex and aggregation pheromones. Tests were carried out to explore the potential presence of attractive components in buffalo flies.

Air drawn from a buffalo fly rearing cylinder, lined with absorbent paper and holding 2000 to 3000 live buffalo flies which had been maintained in the cylinder for 5 days, elicited a high response (60 to 70%) with very few flies on control (<6%). Live buffalo flies (1000) placed inside a clean container in the air stream resulted in responses of 20 to 60% (control <5%). The soiled lining from the rearing cylinder, cut into pieces and placed in a bottle in the air stream gave responses of 40 to 60% and less than 3% for control.

This clear evidence that odours produced by buffalo flies and their excreta were attractive to buffalo flies, led us to investigate the chemical components contained in fly excreta and on the surface of buffalo flies. Long chain hydrocarbons (C_{20} to C_{35}) are commonly found in the waxy layer protecting the fly's cuticle (cuticular hydrocarbons). These hydrocarbons often play a role in the communication within species, eg mating, and are thus pheromones. It is possible that the cuticular hydrocarbons could be used to interrupt vital stages in the flies' life cycle.

The cylinder lining containing the fly excreta were extracted sequentially with hexane and methanol. After evaporation of the bulk of the solvent, the extracts were applied to filter papers and tested in the cage assay. There was no response to the hexane extracts, a small (10%) response to the methanol extracts and a 20% (versus <2% control) response to a combination of both extracts. Chemical analysis of the extracts showed that the hexane fraction contained mainly monounsaturated and, to a lesser extent, saturated hydrocarbons (C_{23} to C_{27}), with the same compounds in methanol plus non-identified lower boiling point components. It was not known whether the low response obtained in the cage assay for these extracts was due to the absence of certain chemicals in the extracts which were initially present, or due to low release rates of the cuticular hydrocarbons from the filter paper.

The chemical analysis of the buffalo fly cuticular hydrocarbons is reported in a later section.

6.2.2.4 Screening of single chemicals

The chemical components of cattle odour identified by other groups and by us were tested in the cage assay. A serial dilution of a single, pure chemical was made in an appropriate, high boiling carrier and applied to filter paper strips. The paper strips were suspended in a bottle through which the air stream flowed, picking up the test chemical. A high boiling carrier was chosen to provide a liquid matrix which did not contribute to the odour. the carrier spread over the same area of filter paper independent of test substance concentration and stayed on filter paper for the duration of experiment. The serial dilutions were made in paraffin oil for fat soluble substances (a stock solution was made in olive oil if the substance did not dissolve in paraffin oil at high concentration) or in glycerol/water 1:1 for substances soluble in aqueous systems.

Table 1 contains the classes and the compounds tested, and if a response was observed, the percentage responses to odour and control side in the fly cage.

Table 1: Class, compounds and percentage response to odour and control for test substances in buffalo fly cage assay.

Class	Compound	Response (%)	
		odour	control
Alcohols	*n*-butanol	13	4
	2-butanol	-	-
	iso-butanol	12	4
	ethanol	27	9
	1-octenol	-	-
Amines	ammonia	22	10
	dimethylamine	-	-
	ethanolamine	-	-
	methylamine	17	4
	trimethylamine	-	-
Carbonyls	butanal	17	3
	butanone	-	-
	carbon dioxide	-[A]	-[A]
	3-nonanone	14	8
Carboxylic acids	butanoic acid	-	-
	2-methylpropanoic acid	20	9
	3-methylbutanoic acid	14	17
	lactic acid	-	-
Hydrocarbons	*(Z)*-9-tricosene	-	-
Indoles	indole	40[B]	5
	2-methylindole	-	-
	oxindole	-	-
	skatole (3-methylindole)	60[B]	4
Phenols	2,4-dimethylphenol	-	-
	3,4-dimethylphenol	-	-
	4-methylphenol	-	-
	phenol	-	-
	3-*n*-propylphenol	-	-
Sulfur compounds	dimethyl disulfide	-	-
	dimethyl sulfoxide	-	-
	3-methylthiophene	-	-

[A] Responses to carbon dioxide when combined with increased humidity were observed

[B] Inconsistent results; see text

The responses to single chemicals were either small or not detectable with the exception of indole and skatole. Responses below 10% were arbitrarily listed as a nil response, as this was considered the cut off level for random movement to target area. The majority of the responses to single chemicals were observed at only one or two of the serial dilutions. This seemed to indicate that there was a narrow concentration window in which the buffalo flies responded positively to the stimulus of these chemicals. At concentrations below the optimum, there was normally no response to either odour or control. At higher substance concentrations more flies were often located on the control than the odour side. A possible explanation for this observation, is that the flies get activated by chemical in the cage, but due to the high concentration in the odour stream the flies orient towards the clean control stream.

The responses of buffalo flies to single chemical stimuli were not always reproducible. This is in contrast to the regular and repeatable responses of the flies to odours sourced from a steer. One possible explanation is that the entire animal odour provides a multiple and thus robust stimulus to the fly, whereas single chemicals only stimulate a narrower range of receptors, and whether or not a behavioural response is observed depends on other nervous system "switches" in the fly.

The reasons for the observed intra- and inter-run inconsistencies with single chemicals are not known at this stage. Moreover, during the winter months there was a general drop in the responsiveness of the flies even to animal odour. The buffalo flies are bred, maintained and tested under controlled conditions (temperature, humidity, light, diet) and a reasonable uniformity in their behaviour could be expected under these circumstances. An attempt was made to overcome the flies' "winter blues" by placing a negative ion generator in the air stream (as suggested by Prof Jerry Butler at the University of Florida) but no substantial improvement in the response was obtained.

It was also noted that with single chemicals the arrestment of responding flies in the target area continuously supplied with odour, was not as persistent as with whole animal odour. In contrast to the steer odour experiment (see above),with single chemicals the maximum response was often obtained before 10 minutes with a noticeable decline of flies within the target area at 15 minutes.

Indole and skatole, a methyl substituted indole, elicited high responses of buffalo flies in a narrow concentration window (indole: 10^{-1} dilution and neat 500 mg/ml stock solution in olive oil; skatole: 10^{-2} dilution of 200 mg/ml stock solution in olive oil). The high responses to these two substances were reproduced many times over a period of several months. The flies responses to skatole and indole became much smaller and then virtually disappeared. At the time this was explained by a general lack of the buffalo flies to response during the winter months. The response to steer odour also decreased but never completely disappeared. When the response to steer odour improved again during spring time, the responses to skatole and indole never recovered much above the 10% baseline. Although a satisfactory explanation for this observation can not be provided, there is a suspicion that a small change to the system may have caused the drop in response. In the early stages of testing, silicone rubber tubing was used to carry the air streams to the olfactometer. This was in time replaced by more inert teflon tubing to minimise any possibility of chemical contamination through adsorption and subsequent desorption of chemicals (eg from steer odour) on the porous silicone material. However, it is still hard to contemplate that any such contamination would have carried through a range of experiments where no steer odour was used.

It was established in later work (see below) that skatole or indole are a crucial ingredient in mixtures which are attractive to the buffalo fly. Thus the observations made in the cage assay for skatole and indole would have some validity.

The hydrocarbon tested, *(Z)*-9-tricosene, is a sex pheromone of the house fly and other flies, and it is the only commercially available unsaturated C_{20+} hydrocarbon. A monounsaturated C_{23} compound, initially believed to be *(Z)*-9-tricosene, was the major component in extracts of gravid buffalo fly. However, it was later shown that this was a different isomer (see section on chemical analysis of buffalo fly cuticular hydrocarbons).

This initial screening of single chemicals in the cage assay had provided us with a list of potential candidates to be included in attractive mixtures. The testing of mixtures in the buffalo fly cage assay is described in the next section.

6.2.2.5 Testing of chemical mixtures

Mixtures prepared from single chemicals which had provided some responses were tested in the cage assay. From previous work with other insects, it is expected that multicomponent attractants will elicit behaviour which is at least additive in relation to the single component responses. Often these effects are synergistic, that is, the overall effect is much bigger than the sum of the individual component responses. With a choice of about a dozen chemicals and their relative and absolute concentrations, there is a large number of combinations of potential attractants. The initial concentration of the components used in the mixtures was selected at the maximum response in single chemical screening. However, this may not necessarily be the optimal concentration in the mixture. Thus there is a need to test mixtures with variable concentrations of single components.

The first synthetic mixtures tested were the attractants reported in the literature for other flies, eg tsetse fly and screwworm fly. Responses of about 20% (control 5 to 10%) were observed on exposure to high dilutions ($10^{-5\ to-\ 6}$) of tsetse attractant, but they were not consistently reproducible. Swormlure, the screwworm fly attractant, did not elicit any responses in buffalo flies.

The combination of bovine derived odours and pure chemicals gave promising results. The response to the bovine derived odours was greatly enhanced by the addition of one or several of the chemicals which had been found to elicit a response in buffalo flies. Combinations of skatole with rumen fluid and dung respectively, resulted in a marked increase in the response of the flies over the response to the individual components of the combinations. The results from such an experiment with cattle dung and chemicals are shown in Figure 7. The responses to the odour stream were low for skatole (A), skatole plus ammonia (B), skatole plus octenol (C), skatole plus ammonia plus octenol (D) and for cattle dung alone (E). Combinations of dung with chemical mixtures A, B, B (higher concentration of ammonia) and D in experiments F to I respectively, showed a substantial increase in response. This indicates that the augmentation (if component already present in material) or addition of selected synthetic chemicals increases the response of the flies to these materials. This observation suggests that there is a potential for synthetic attractants to compete with or overcome naturally occurring olfactory attractants from cattle.

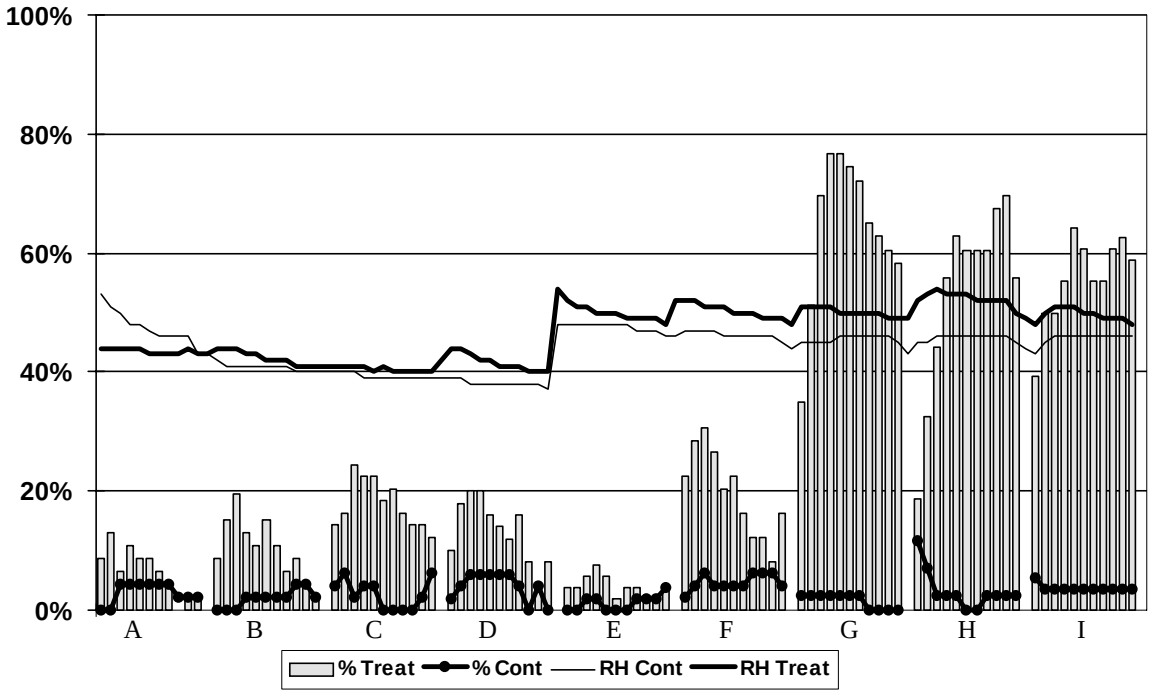


Figure 7: Percentage of buffalo flies responding to odour (grey bars) and control (black dots) air streams and relative humidity (RH) for odour (thick line) and control (thin line) streams against time (minutes) in fly cage assay. Each block is a separate experiment with data at 1, 2, 3, ..10, 15 minutes. Odours were from left to right: A: skatole; B: skatole + ammonia; C: skatole + octenol; D: skatole + ammonia + octenol; E: cattle dung; F: skatole + dung; G: skatole + ammonia + dung; H: skatole + ammonia (high concentration) + dung; I: skatole + ammonia + dung + octenol.

Synthetic mixtures were tested extensively in the cage assay. A combination of skatole and ammonia gave an increased response when compared to the single chemical responses, although this was not the case in every experiment. Other candidate chemicals were then added at different concentration to the skatole/ammonia mixture and the flies' response to the new mixture observed. Compounds which resulted in increased responses included butanol and 2-butanol, whereas no increase was observed with indole, nonanone and 3,4-dimethylphenol. No clear difference in responses was seen between the addition of butanol or 2-butanol.

Further single chemicals were then added to the skatole/ammonia/butanol mixture. These included three amines: methylamine, dimethylamine and trimethylamine. All amines gave an improvement in the fly cage assay responses, with the best improvement obtained with methylamine.

The skatole/ammonia/butanol/methylamine (SABM) mixture was at this stage the best and most consistent combination used in the fly cage assay. Investigations were conducted on the necessity for the individual ingredients and on possible substitution of components with more effective analogues, in order to improve the responses of the buffalo flies. Replacement of skatole in SABM by indole or 2-methylindole resulted in a substantial drop in the responses. The removal of ammonia from the mixture also gave a reduction in the observed responses, although to a much lesser extent than the substitution of skatole.

The addition of butanoic acid (Ba) to the SABM mixture provided a further improvement in response.

Other chemicals that were added to SABM but did not result in a better response included hexanal and 4-methylphenol. The concentration of the individual components of SABMBa were optimised for fly cage response by altering the concentration of one component at the time. Through this procedure the mixture containing skatole at 10^{-2} dilution in paraffin oil of a 200 mg/ml stock in olive oil, ammonia at 10^{-1} dilution of 28% aqueous solution in glycerol/water 1:1, butanol 10^{-2} in paraffin, methylamine 10^{-4} of 25-30% aqueous solution in glycerol/water and butanoic acid 10^{-5} in paraffin oil was selected as the best combination to elicit a buffalo fly response in the cage assay.

A direct comparison of SABMBa with the odours from a steer showed that a similar response to the two stimuli could be obtained. This is a very encouraging result and an indication that the synthetic odour developed so far has the potential of attracting buffalo flies quite efficiently. However, it does not mean that the synthetic mixture is equivalent to a steer in attracting buffalo flies in the field or even an insectary. The cage assay provides most likely a good indication of short range attractancy which may only be the last step in getting flies to a trap or target in a more natural environment. Further improvements to SABMBa mixture are being investigated by using the fly cage assay and other tests with the mixture will be carried out in the fly behaviour observation facility and the field.

6.3 Vision

6.3.1 Visual targets

6.3.1.1 Efficacy of target in absence of cattle

Simple visual targets such as black rectangles have been successfully used in conjunction with attractive odours in tsetse fly traps (Wall and Langley 1991). This experiment was conducted to investigate whether such targets could be useful for buffalo fly and whether visual cues alone have some potential for trapping buffalo fly.

Table 2 Released flies recaptured by black target

Flies	Release	Mean % Captured	Range	Replicates
Newly-emerged	4pm	8	5-13	4
Mixed Age	4pm	23	11-32	3
Mixed Age	7pm (dark)	2.5	2-3	2
Natural emergence	4pm-12 midnight	5	5	1

Buffalo fly did respond to the simple target which presented only visual cues (Table 2). The target was less attractive to newly emerged flies than to mixed age groups of flies which had already been exposed to cattle. The target was less successful at night for mixed age groups of flies than in the afternoon.

6.3.2 Light traps

Caged buffalo fly are strongly positively phototaxic. This response is also elicited briefly in buffalo fly on cattle when the flies are disturbed and could also be expected in flies dispersing between hosts. Consequently the potential of light as an attractant was investigated.

Preliminary tests using a single penned animal showed that a trap consisting of a 40W blacklight fluorescent light run throughout the night and shining onto a white sticky target could catch enough flies to reduce the number of flies on the penned animal substantially. Field trials were conducted to investigate whether this attractancy was sufficient to reduce the fly numbers on entire herds of cattle.

6.3.2.1 Light Trap - First Field Trial

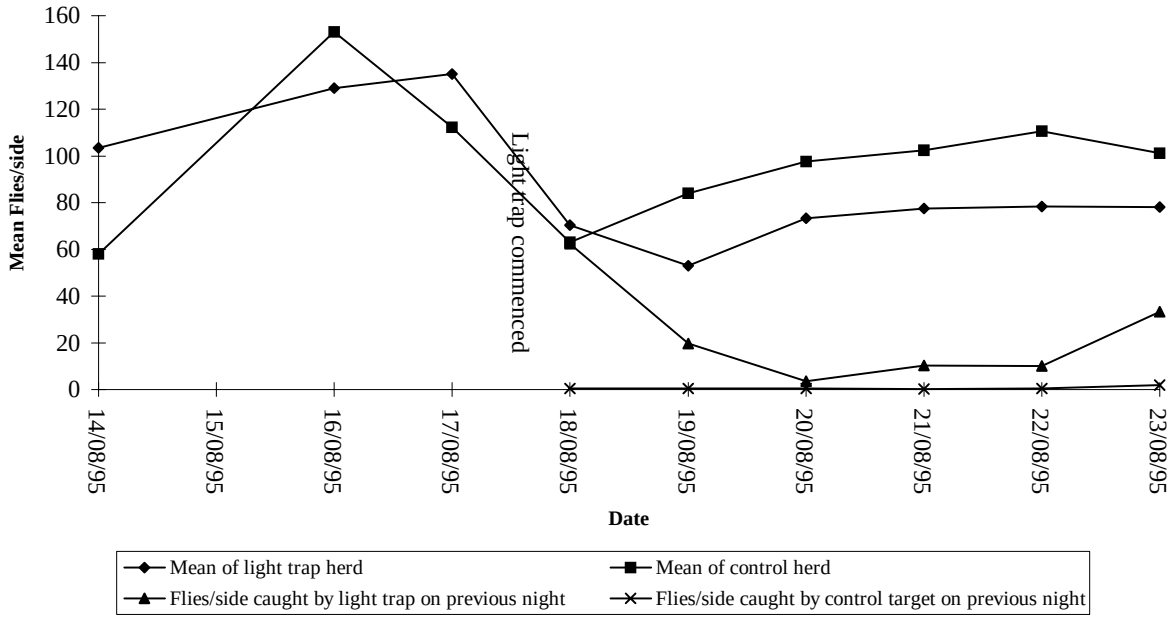


Figure 8 First series of first field trial of light trap with penned cattle

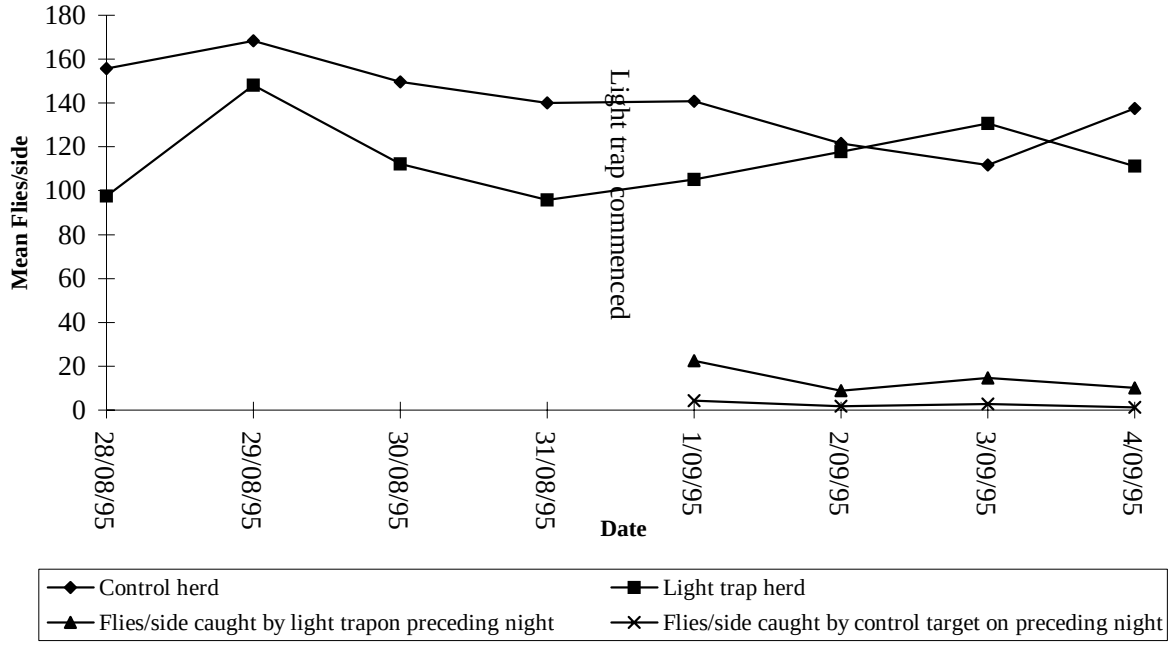


Figure 9 Second series (light trap swapped to other herd) of first field trial of light trap with penned cattle

Trap catches in Figure 8 and 9 were expressed as catches per animal side (ie total catch / 28) to make them comparable to the counts of the fly populations on the cattle. The light trap caught significant numbers of flies and appeared to exert a modest reduction in the population in the first series but not on the second. There was no apparent selectivity in the sex or age (assessed by gonotrophic development of females) of flies caught.

6.3.2.2 Light Trap - Second Field Trial

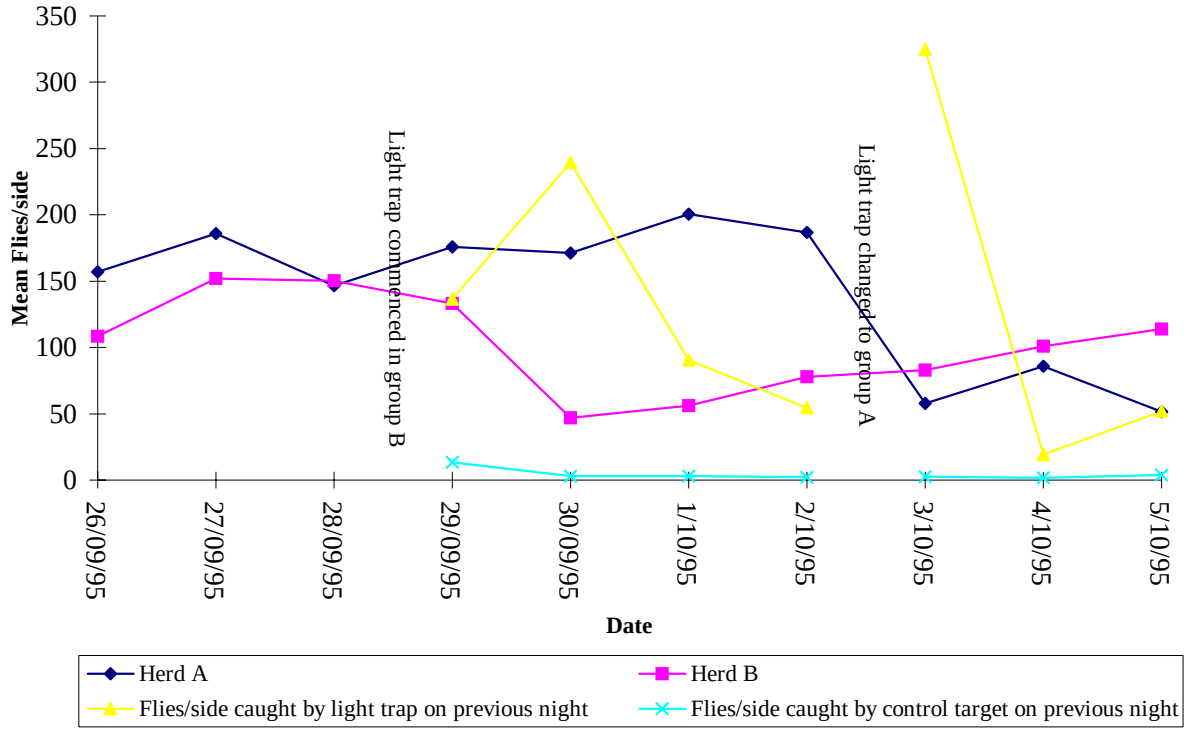


Figure 10 Second field trial of light trap with penned cattle

This trial caught far more flies than the first trial and succeeded in reducing the populations on both groups of animals (Figure 10). At the time it was thought that the intermittent light was primarily responsible for the improvement however other work showed that this was unlikely and consequently the improvement may have been due to lowering the target. This is contrary to experience with other insects where light trap efficacy generally improves as height increases.

A perplexing and currently inexplicable aspect to both these trials was the poor correlation between the numbers of flies trapped and the changes in the populations on the cattle. Larger reductions in the populations would have been expected when large numbers were trapped. Since all cattle on the property were included in the trial, any immigration would have been detected as a loss from the other group. Newly emerged flies could have accounted for some of the discrepancy. However a strong possibility is that conventional counts per animal side underestimate the fly population.

6.3.2.3 Field Trials of Light trap with Unrestricted Cattle

These 2 experiments tested how the light trap, which had been successful with penned cattle, worked when the cattle were not confined close to the trap.

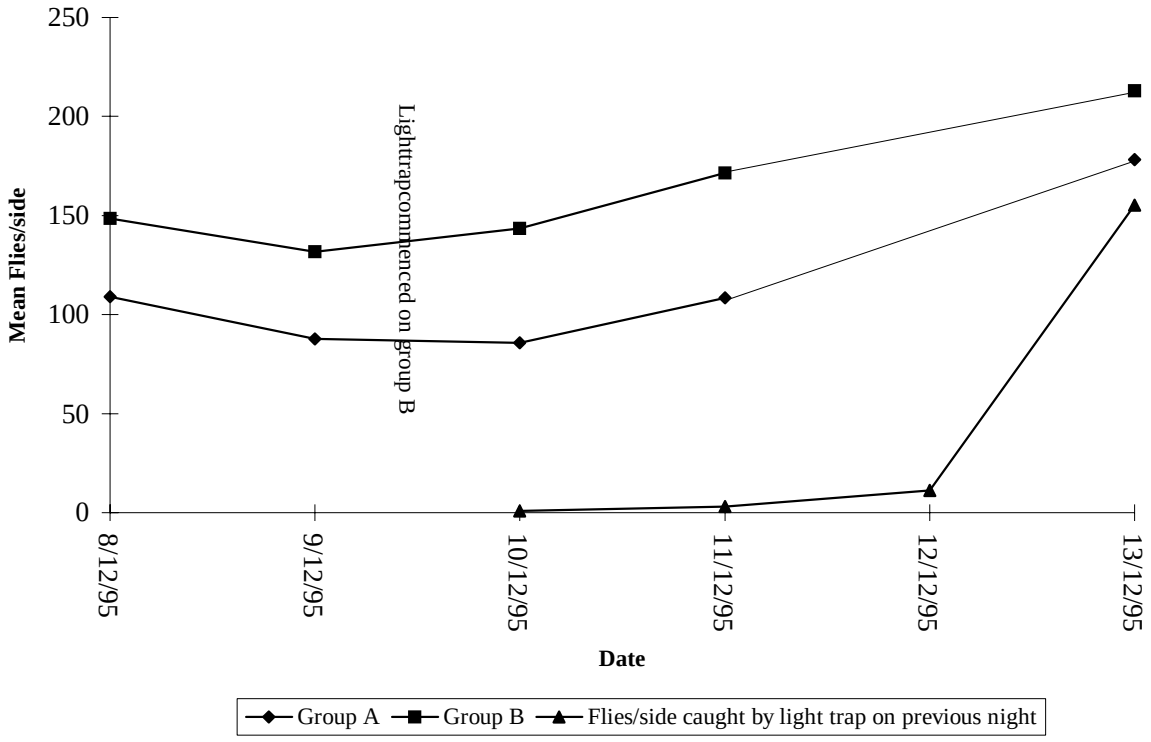


Figure 11 First field trial of light trap with unrestricted cattle (trap on Group B)

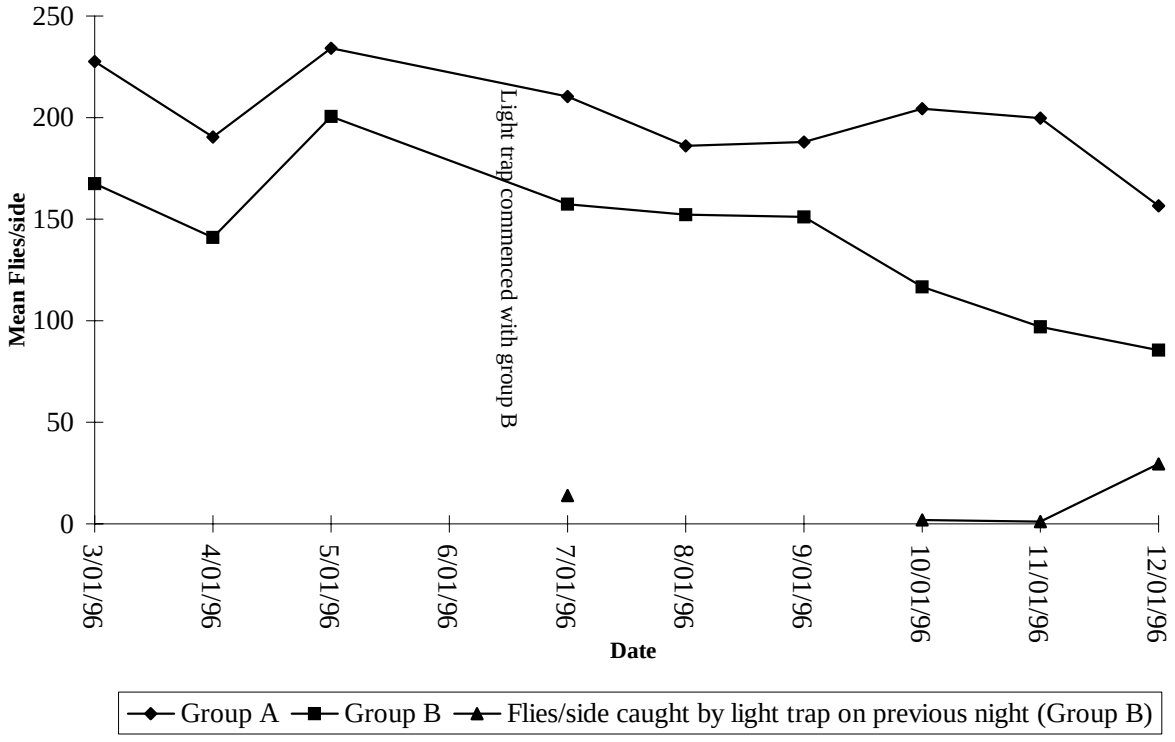


Figure 12 Second field trial of light trap with unrestricted cattle (light trap was not run on nights of 7-8/1 and 8-9/1 due to heavy rain)

Trap collections were lower than those obtained with penned cattle and the fly populations on the cattle were not reduced (Figures 11 and 12).

Cattle appeared to neither favour nor avoid the trap. Clearly the light trap was effective over only a limited distance and the cattle did not stay close enough to the trap for long enough.

6.3.3 Distance and phototaxis

This experiment attempted to quantify the distance over which the flies would respond to the light used in the light trap in order to estimate how close the cattle would have to be to a light trap for it to be effective.

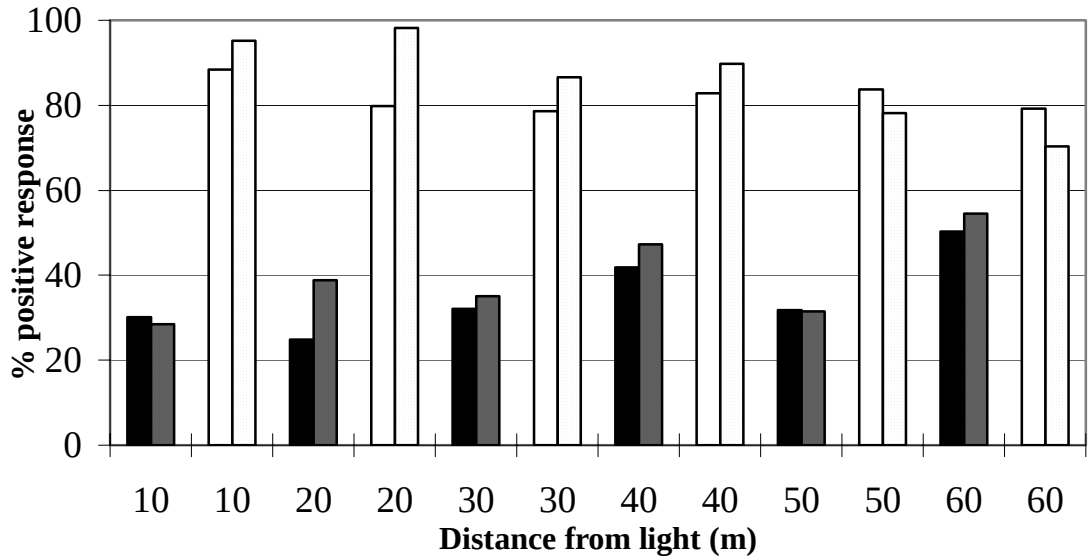


Figure 13 Mean response of caged flies to absence and presence of light at various distances from a light on 2 separate occasions. (Responses to dark are heavily shaded, responses to light are minimally shaded).

The caged flies were consistently attracted to the light at all distances tested up to 60m from the light (Figure 13). In a similar, later experiment the responses were largely similar and again extended to the maximum distance tested, namely 80m. These results would suggest that flies should respond to a light trap from a considerable distance. However the failure of the field trials with unrestricted cattle suggest that this is not the case for flies on cattle. The strong phototaxis displayed by caged flies is apparently not matched by the flies in the field.

6.3.4 Effect of intermittent light cycles on light trap collections

Earlier field trials of light traps strongly suggested that intermittent light was more effective than constant light (Figures 8-10). This experiment attempted to confirm this and tested whether the duration of the light and dark periods in the intermittent light cycle also affected light trap efficacy.

Table 3 Effect of light cycles on percentage of flies caught by light trap (20W white fluorescent light source) run close to a single penned steer

Cycle	Mean % Caught	Range	Replicates
Constantly on	36	23-45	3
Constantly off	7	3-10	3
1:1 min light:dark cycles	33	26-43	3
15:15 min light:dark cycles	33	25-47	3

With the 20W white light, none of the intermittent light cycles improved the overall catch. This conflicts with the earlier field trials but this experiment with the single steer offers the more reliable test as it was a direct comparison under more controlled conditions with no other variables such as trap height.

Light traps appear to have the potential to reduce buffalo fly infestations if the cattle remain close enough to the trap for long enough. Exactly how close and how long are required have not yet been quantified but for the light sources tested so far it would appear to be desirable to have the cattle within about 15m of the trap for about 1hour (or more).

6.3.5 Electroretinograms

Compound eyes are the major sense organs of adult buffalo flies, providing sensory information essential for:

(a) location of resources such as cattle for feeding, the opposite sex for reproduction, and fresh dung pads for egg-laying;

(b) location of the direction of the “sky” for dispersal, orientation and escape from predators and other causes of disturbance.

Measurement of the spectral sensitivity of the retinula cells in the compound eyes shows to which wavelengths of light or ultraviolet they are most sensitive; in other words, what the fly is best adapted to see. This is essential background for the design of optic sources, targets and traps to be used in non-toxic control measures for buffalo flies, through management of their behavioural characteristics.

A degree of variability in spectral sensitivity was found, depending on where the photic beam was directed on the compound eye and on the positioning of the electrodes. There was also a degree of variability between insects.

As can be seen in Figure 14, there are two outstanding classes of retinula cells in the compound eyes of female *H. i. exigua*: (i) ultraviolet sensitive cells, responding maximally at 325 nm; and (ii) blue-green sensitive cells, responding maximally at 535 nm.

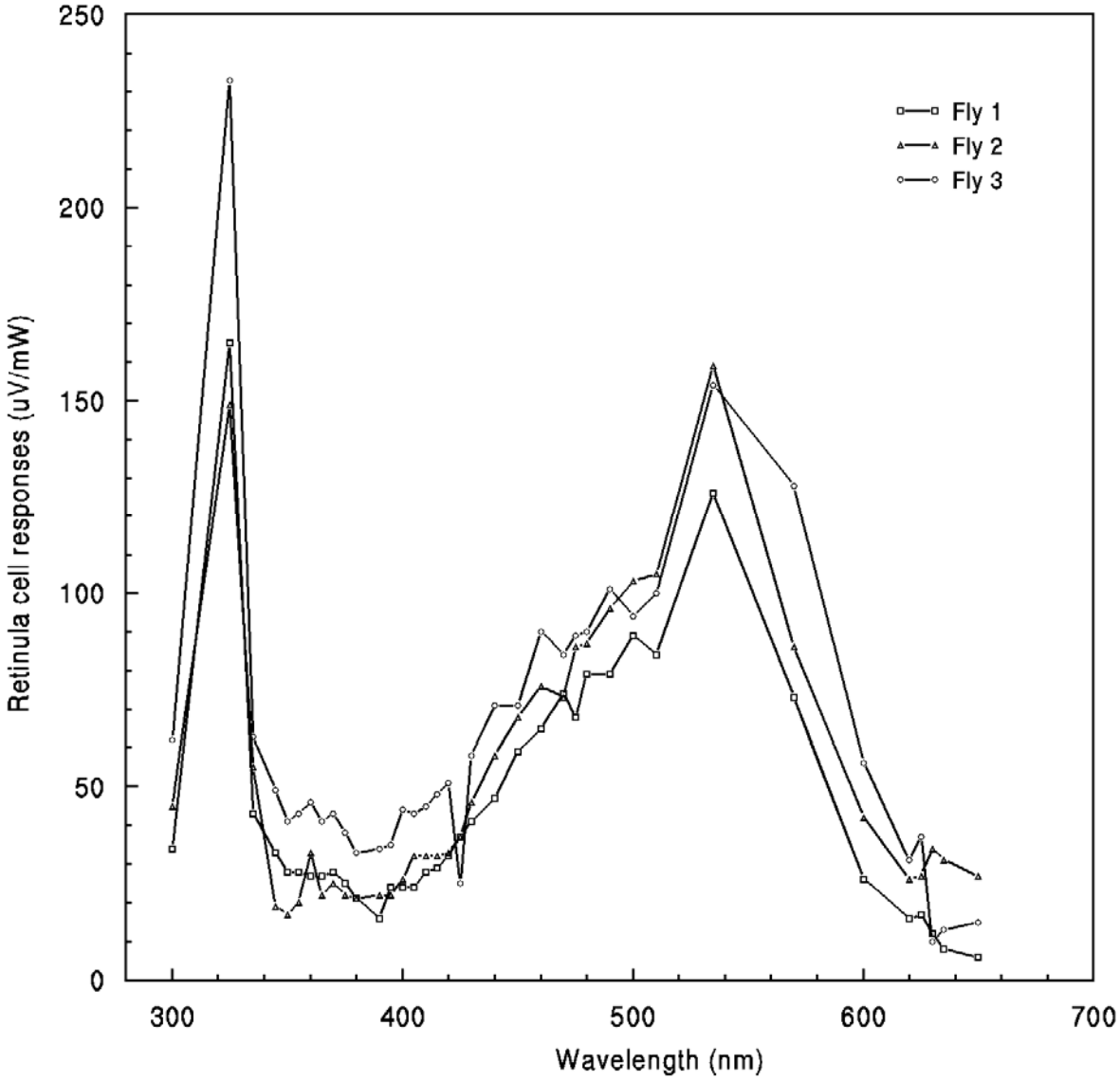


Figure 14: Spectral sensitivity of retinula cells of buffalo fly compound eye. Data from three female flies.

The broadness of the base of the blue-green cell peak, extending from violet to red, suggests there may be other types of cells involved. There is some indication of minor peaks at 460 nm, 500 nm and 625 nm. Similarly, at the base of the ultraviolet peak, there are minor peaks at 360 nm and 375 nm. Minor peaks may represent a relatively small number of retinula cells of different spectral sensitivity or they may be the result of interference by non-receptor pigments in the eyes or may have been produced by some feature of the stimulus or recording equipment.

In conclusion, two predominant classes of photic response characterise the compound eyes of *H. i. exigua* females:

(i) Sharply defined sensitivity to ultraviolet “light”, peaking at 325 nm. Input from these cells is likely to be involved in “sky orientated” behaviours such as dispersal and alarm. 325 nm would be the primary wavelength to test in experiments aimed at attracting disturbed buffalo flies off cattle at night (when there is no competition from the sun).

(ii) Broad sensitivity from violet to orange-red, markedly peaking in the blue-green at 535 nm. Input from these cells is likely to have a major role in normal navigation of buffalo flies, being critical for orientation towards mates, hosts and oviposition sites.

The behavioural roles of visual stimulation by the two major wavelengths is being further investigated. This information is expected to play a significant part in the rational design of combined olfactory/visual manipulation for non-toxic management of the flies.

6.4 Vision and olfaction combined

6.4.1 Trials of traps developed for other stock-associated Diptera

Capture rates were poor for all traps with means of 0%, 2% and 0% for the sticky trap + tsetse lure + acetone, the modified Williams trap and the modified Manitoba trap respectively.

The sticky trap offered minimal visual cues. Later work described elsewhere in this report combined tsetse lure + acetone with a visual target and it remained ineffective. The modified Williams trap relied on reflection of incident UV light as its attractant but it seemingly did not produce sufficient contrast between itself and the background levels to elicit useful capture levels. The modified Manitoba trap used a black target as the attractant. Later work showed that a black target was a useful attractant. However the buffalo fly did not display the negative geotaxis necessary to ascend the funnel above the target and be captured in the trap. This characteristic would work against some other established trap designs, eg some of the designs for tsetse fly, which also rely on the flies travelling up funnels to be collected.

6.4.2 BOFF Trials

In locating their host, biting flies respond to a complex and changing array of chemical and physical cues. For the buffalo fly, vision and olfaction are seen as an important components for host location. However the relative importance of these components is unclear.

A total of 9 experiments were run in the behavioural observation facility for flies (BOFF). The maximum responses at the stated time intervals is shown in Table 4.

The combination of vision and odour attracted the highest numbers of flies with 32-75% (mean 47.2%) being caught 15-30 minutes after release. Vision and odour separately attracted similar numbers of flies (35.3 and 34.4 % respectively).

The target (SCOVOS) failed to attract buffalo flies, with the combination of SCOVOS and odour similar to odour alone. The addition of movement to SCOVOS failed to improve its performance (11.4 and 10.3% with and without movement respectively).

Table 4. Percent *H. i. exigua* caught on sticky grid on glass partition between BOFF and APA.

Expt. No./ Ass'mt Time	Control	Vision only	Vision plus odour	odour only	SCOVOS	SCOVOS plus odour
190/15	5.8	28.8	43.9	33.3 34	_	_
192/20	12.9	36.9	38.9 36.4	36	_	_
195/30	20.7		64		5.8	47.8
196/30	41	54.4	63.6			
198/25	20.4	40	74.5		10.3	
199/25	11.8	41	44.4		12	42
202/25	7.4	5.8	36.4		3.6	13.7
203/25		40.4	42.6 58			
206/25	9.4		22.2 41.3			
Mean Attracted	16.2	35.3	47.2	34.4	7.9	34.5

In the olfactometer experiments (see above) odour from buffalo flies themselves were shown to be attractive. In trial 203 where odour from flies was added to the combination of animal vision and odour, a slight increase in attraction was achieved (50.3 and 58.4% for animal and animal plus flies respectively).

These trials indicate that both olfaction and vision are important in host location for buffalo flies, and that our current target lacks the relevant visual stimuli.

6.4.3 "Model cow" field experiments

A summary of the buffalo flies caught in the Yeerongpilly trials is given in Table 5.

Table 5: Mean percentages of buffalo flies caught on sticky sides of model cow in paddock.. The flies were released 12 m upwind and downwind from model cow, respectively.

Treatment	Percentage of flies caught released from			Number of
	upwind	downwind	total	experiments
All targets	33	24	29	14
Visual target only	27	21	25	7
Visual target + lure	39	27	33	7

On average 29% of the flies released 12 m from the target were caught on the target after 1 to 1.5 hours. This is far more than would be expected from a random dispersion of the released flies which would give a catch of 2% with no allowance for vertical dispersion (eg assuming that all flies will stay below 1.4 m).

This result clearly indicates a positive orientation of the buffalo flies towards the model cow. A higher percentage of the upwind released flies were caught compared to the downwind released flies, suggesting that there is some drifting of flies with the wind. The visual target plus lure had higher catches than the target alone. This difference was however not significant (P>0.05). If the lure is responsible for the increased catch rate this should be demonstrated by an increase in the catch of the downwind released flies as only they are exposed to the odour plume. However, the increase was observed for the up- and downwind released flies.

Results from individual experiments are given in Table 6.

Table 6: Percentages of buffalo flies, released 12 m upwind and downwind from model cow, caught on sticky sides of model cow in paddock in various experiments.

Treatment	Percentage of flies caught, released upwind	downwind	total	No of replicates Age of flies days
Model cow + lure	39	18	29	5
Model cow + lure + acetone	41	20	31	2-3 d
Model cow	25	18	22	2
Rectangle, 1.2x0.6m, black	38	21	30	7 d
Model cow	19	13[A]	16	2
Model cow + dung	31	9	20	9 d
Model cow	19	28	24	2
Model cow + lure + acetone[B]	44	27	36	7 d
Model cow[C]	20	19	20	2
Model cow + lure + acetone[B,C]	20	27	24	9 d

[A] 1 replicate only; [B] high release rate;
[C] flies released along animal axis, 12 m from head and tail respectively.

The addition of tsetse lure and acetone or dung did not seem to consistently increase the catch of buffalo flies on target. In the case of tsetse lure and acetone these observations agree with the findings in fly cage assay that the tsetse lure is not attractive to buffalo flies.

6.4.4 Tsetse fly lures

The mean buffalo catches on the sticky targets in the pairwise comparison at Brian Pastures Research Station are presented in Table 7.

Table 7: Mean catches of buffalo flies on black rectangle with and without lure and number of flies on cattle at Brian Pastures Research Station

Treatment	Trial 1	Trial 2
Black rectangle	10.3	2.0
Black rectangle + tsetse lure	6.6	3.0
Buffalo flies on cattle (per side)	20-500	20-200
Number of observation periods	9	11

In accordance with previous results, it was found that the tsetse lure did not increase the catch of buffalo flies. The differences between the treatments within trial were not significant ($P > 0.05$). The number of buffalo flies on cattle during the experiment were between 20 and 500 and 20 to 200 flies per side in trial 1 and 2 respectively. These figures clearly illustrate the point that a strong attractant combined with appropriate application technology is required if an impact on fly numbers in the field is to be achieved.

6.4.5 Lucitrap and tsetse fly lures

At Brian Pastures Research Station the rectangular target with and without tsetse attractants and the Lucitrap with Lucilure and tsetse attractant were tested for their attractivity for wild buffalo flies. The targets/traps were placed in similar locations in cattle paddocks and rotated at 24 hour intervals according to a random 4x4 Latin square design. The rectangular targets caught a mean of 7.4 and 11.4 buffalo flies per 24 h with and without tsetse attractants respectively, which was significantly more than the Lucitrap which with either lure did not catch any buffalo fly during the trial. This is not unexpected, as the behaviour of the buffalo fly may prevent it entering a trap like Lucitrap even if it is attracted by the odour. This experiment has confirmed that Lucitrap is not suitable for use with buffalo flies. It should be noted that the targets are not very effective either, as 50 to 350 buffalo flies per side were counted on cattle at the station during the experiment.

6.5 Chemical analysis of odours

6.5.1 Cattle odours

Chemical analysis of odours emitted by cattle have been carried out in research projects on tsetse fly attractants (Torr et al 1995, D R Hall pers comm). The compounds were detected with a variety of adsorbing and desorbing techniques and included alcohols, amines, carbonyls (aldehydes, ketones, carbon dioxide), indoles, phenols, sulfur compounds and others such as water, epoxides and terpenoid compounds. Some of the detected components were integrated into a powerful and successful tsetse fly attractant.

Our technology for adsorbing and desorbing chemicals present in odours is somewhat different from the ones used by Torr and Hall. The adsorption is onto an inert stationary phase which has an affinity for chemicals of widely varying polarities and boiling points. Desorption from the support was achieved by thermal desorption under an inert gas and direct loading of desorbed material onto a gas chromatography column. The advantage of this procedure is the absence of solvents which can interfere with the analysis of the most volatile components.

We detected in the odour taken from a steer largely the same compounds as described by Torr and Hall. In addition, we found more components than they had reported belonging to the carbonyl and sulfur groups, and some compounds from chemical classes which they had not reported, namely carboxylic acids and esters.

Representative components from these classes were then tested in the fly cage assay.

6.5.2 Buffalo fly cuticle hydrocarbons

The olfactory attractancy for buffalo flies to fly cage paper lining, live flies and extracts of the soiled paper lining led us to investigate the chemical nature of this stimulus.

Chemical analysis of the paper lining extracts revealed the presence of mainly monounsaturated and saturated hydrocarbons with a straight carbon chain length of greater than twenty. These type of compounds have been identified and reported to act as pheromones in other fly species. Other groups had reported the importance of insect produced hydrocarbons in their communication and mating. Therefore, we decided to investigate the chemical nature and potential use of the hydrocarbons contained in the cuticle of the buffalo fly.

Buffalo flies of different colonies (CSIRO, DPI), feeding (*in vitro, in vivo*), sex and age were extracted with hexane containing an internal standard (saturated C_{24} hydrocarbon) to obtain the cuticular hydrocarbons. After purification, the hydrocarbons were analysed by gas chromatography/ mass spectrometry (GC/MS) and GC/flame ionisation detection. The inclusion of an internal standard allowed a quantitation of the detected components. The location of the double bond was determined by reacting the unsaturated hydrocarbon with dimethyl disulfide, followed by GC/MS analysis of the resulting adduct. The results are summarised in Table 8.

The results showed that there are differences between flies of different age and sex. The newly emerged flies had a small amount of cuticular hydrocarbons, and they were mainly C_{27} compounds. When the flies were 2 to 3 days old, the amount of cuticular hydrocarbons had increased and shorter chain length hydrocarbons (C_{23} and C_{25}) had been formed. The amount of hydrocarbons and the synthesis of shorter chain compounds continued with increasing age, as shown by the 13 and 20 day old female flies which had been fed on blood but kept off the animal. The percentage of gravid flies was only 20 and 50% respectively.

Buffalo flies were collected off an animal when they were three days old. At this stage 80% of the female flies were gravid. In both sexes, 90% of the total measured hydrocarbons were C_{23}, and the major components were monounsaturated compounds. Females and males had the same three monounsaturated C_{23} compounds (tricosenes), but the quantities present were substantially different in the sexes. The major component in the female buffalo fly was the *(Z)*-11-tricosene (meaning that the double bond was located at carbon 11 and that the stereochemistry was Z), followed by Z7 and Z5. In the male, *(Z)*-7-tricosene was the most abundant compound ahead of Z11 and Z5.

Table 8: Buffalo fly cuticular hydrocarbons. Amount of cuticular hydrocarbons (ug/fly)[A,B] in hexane extracts of buffalo flies of various ages and physiological states (laboratory strains):

Age, sex, nos	21:1	21:0	Z11-23:1	Z7-23:1	Z5-23:1	23:0	Z9-25:1	Z7-25:1	25:0	Z9-27:1	Z7-27:1	27:0	27br	29	Comments
newly emerged female, 20		▯							0.083	0.21	▯	0.188	0.213?	▯	ex DPI in vitro fed
newly emerged male, 20		▯							0.083	0.135	▯	0.143	0.03?	▯	ex DPI in vitro fed
2-3 days female, 20				0.115	▯	▯	0.08	0.25	0.22	2.73	0.638	0.618		0.183	ex DPI in vitro fed
2-3 days male, 20		▯		0.34	0.053	0.085	0.065	0.13	0.218	1.90	0.418	0.478		0.113	ex DPI in vitro fed
13 days female, 20	▯?	0.15	4.73	1.53	0.208	2.73	0.263?	0.085	0.568	0.165	▯	0.758		▯	ex DPI in vitro fed 20% gravid
3 days female, 20		0.090	2.32	0.738	0.123	0.705	0.062	▯	0.115	▯	▯	0.173			ex CSIRO in vivo fed 80% gravid
3 days male, 20		0.048	0.77	2.03	0.328	1.03	▯	▯	0.200	▯?	▯	0.14		▯	ex CSIRO in vivo fed
mixed age female, 20		0.24	4.08	0.535	0.075	1.20	0.090?	▯	0.25	0.163	▯	0.36		▯	ex CSIRO in vitro fed 50% gravid

[A] Amounts calculated from ratios of components and internal standard (C24:0) (GC/FID areas under peaks)

[B] Hydrocarbon nomenclature: carbon chain length:number of double bonds; prefix provides position and stereochemistry (assumed to be Z) of double bond;

▯ present but no quantitation available

? tentative assignment

In 2-3 days old DPI flies a compound with m/z 376 (equivalent to $C_{27}H_{56}$) was also present, but GC retention time is not in accordance with diene.

The above measurements were made on extracts from 20 female and male flies. To determine how significant this observed difference was, 5 flies of each sex were individually extracted and analysed. The mean concentration of *(Z)*-11-tricosene in the 5 female buffalo flies was 2.3 ug/fly with a standard error of 0.16. The corresponding mean and standard error for *(Z)*-7-tricosene in males were 2.02 and 0.10 ug/fly. A similarly small variability within sex was found for the other detected hydrocarbons. This confirms that the large difference in hydrocarbon components was due to the gender of the fly.

Another interesting, and potentially important point, is that the major cuticular hydrocarbon components in buffalo and horn flies are different. Mackley et al (1981) reported that *(Z)*-9- and *(Z)*-5-tricosene were the two major components in male and female horn fly cuticular hydrocarbons respectively. The discrepancy between Mackley's and our results raises the point about how closely related the buffalo and horn flies really are. The phenotopic differences are very small and the two flies have been classified as subspecies of *H. irritans.*

7. Success in achieving objectives

A review, prepared for this project, on the potential role of behaviour-modifying systems in buffalo fly control concluded that olfactory and visual attractants presented the best chance of achieving control. Consequently, the work carried out during the term of this project focussed on detecting, analysing and applying olfactory and visual cues used by buffalo flies.

The responses of buffalo flies to many single chemicals, mixtures of chemicals and bovine derived natural odours enhanced with synthetic components have been assessed in an olfactometer, a fly cage assay, in the BOFF and in the field. Good responses were obtained for bovine odours, bovine odours enhanced with synthetic chemicals and some mixtures of synthetic chemicals. It was shown that buffalo flies, fly cage linings and extracts thereof (cuticular hydrocarbons) also elicited a behavioural response in buffalo flies and the chemical nature of these compounds has been determined.

It has been demonstrated that buffalo flies orient positively toward certain targets (cattle, a model cow and black rectangles of approximate steer size) during day time, and light sources at night. The wavelengths of light to which the buffalo fly's compound eyes are most sensitive have been defined. These characteristics of the buffalo fly's vision and its behaviour when exposed to visual stimuli will assist in the construction of an effective visual target.

Positive orientation of buffalo flies could be demonstrated for olfactory and visual stimuli. However, the combination of olfactory and visual stimuli proved the most attractive to buffalo flies. This suggests, that both stimuli will have to be incorporated into the development of an effective buffalo fly trap or target.

The literature review carried out at the start of this project narrowed the focus of the project work to olfactory and visual attractants. In this context, the first objective, to identify attractants, repellents and/or other sensory cues for use in non-insecticidal buffalo fly control systems was achieved. Natural odour attractants have been collected, identified, analysed, reconstituted using synthetic chemicals and tested for attractancy to buffalo flies. As a result of this, a mixture has been defined which was attractive to buffalo flies in laboratory experiments. Similarly, visual targets for day and night use have been developed and some of the parameters influencing the fly catch identified. These findings provide a solid foundation for the development of a system which effectively modifies the behaviour of buffalo flies.

The formulation of recommendations for the commercial development of the behaviour-modifying systems, as outlined in objective 2, could not be achieved as the research on olfactory and visual cues had not progressed far enough to make this step a feasible option. However, a future commercialisation of the current findings is still likely. For such a strategy to succeed, the potency of the olfactory and visual attractants must be improved above their current level. The funding of work with these objectives is contained in the recommendations of this project.

8. Impact on Meat and Livestock industry

Buffalo fly is cited as a major issue by beef cattle producers in northern Australia (cf Background). Estimates on annual production losses were put at $80 million in Australia. Treatment costs for buffalo flies, excluding mustering, were estimated to be at least $20 million in Queensland. A reduction of buffalo fly populations below an economic threshold by using behaviour-modifying systems, could potentially save the producers these costs. A trapping system developed by our group for the Australian sheep blowfly, which is commercially available (Lucitrap), has been shown to reduce sheep blowfly populations.

Current buffalo fly control methods rely heavily on the use of synthetic pyrethroid and organophosphate (OP) insecticides, although other groups such as the carbamate, bendiocarb and the macrocyclic lactones are available. Problems with insecticide residues and resistance to the synthetic pyrethroids exist, with the latter resulting in declining efficacies for these insecticides. Chemical residues in excess of maximum residue limits of major export countries were found with buffalo fly control chemicals used at recommended levels. Shorter application intervals with chemicals when resistance problems are encountered compound this problem. The availability of non-chemical tools for the control of buffalo flies would lessen the problems encountered with meat export and avoid the potential for total import bans on the basis of insecticide residues by foreign countries.

A system, which effectively attracts buffalo flies, has a great potential to reduce costs and production losses, and to decrease or eliminate the risk of problems with residues in meat exports.. The commercialisation of an effective, non-insecticidal tool for fly control is feasible, as was demonstrated with Lucitrap. It is anticipated that with further development of the outputs from this project (chemical and visual attractants) a commercial system could also be attained for buffalo flies. Thus, substantial benefits, as outlined above, could flow to industry from the current findings.

9. Conclusions

This project investigated the development of non-insecticidal control of buffalo fly using behaviour-modifying systems. These systems are one of the potential alternative methods to the currently used insecticides for controlling buffalo flies. Problems with insecticide resistance and residues in produce made the development of such systems high priority.

The literature on the behaviour, the role of attractants, arrestants and repellents and the population dynamics of the buffalo and horn flies was reviewed as part of this project (Appendix A).

The behaviour of buffalo flies in locating and when residing on cattle has been investigated. Orientation of flies to animals occurred in freshly emerged flies, but the percentage response increased to maximum at 16-17 hours post emergence.

Numbers of buffalo flies on animal in pens fluctuated throughout the day and night indicating that the flies regularly leave and relocate their hosts.

Olfaction and vision in buffalo flies have been identified as important components in the location of host cattle and cattle dung. The work in this project aimed at modifying the behaviour of buffalo flies with chemical (olfactory) and/or visual means, so that the flies would not be able to locate cattle or egg laying sites. This would result in a disruption of the flies' life cycle, leading to lower fly populations, and thus assist in control. The search for and the assessment of the effectiveness of chemical and visual components for this purpose were the main purpose of this project. Substantial progress was achieved in both areas, but further improvements in synthetic chemical attractants and in the understanding of the role of visual targets are still required.

A list of chemical components contained in odours emanating from cattle and cattle excretions was compiled from work done by overseas groups and by us using gas chromatography/mass spectrometry. The listed compounds were considered potential chemical cues in attracting buffalo fly to cattle.

The responses of buffalo flies to many single chemicals, mixtures of chemicals and bovine derived natural odours enhanced with synthetic components have been assessed in an olfactometer, a fly cage assay, in the BOFF and in the field. Good responses were obtained for bovine odours, bovine odours enhanced with synthetic chemicals and some mixtures of synthetic chemicals. Single chemicals and previously described attractant mixtures for other fly species (tsetse fly, screwworm fly, Australian sheep blowfly) gave no or very low responses. A direct comparison of a synthetic mixture with the odours from a steer showed that a similar response to the two stimuli could be obtained in the fly cage assay. This is a very encouraging result and an indication that the synthetic odour developed so far has the potential of attracting buffalo flies quite efficiently.

It was shown that buffalo flies, fly cage linings and extracts thereof (cuticular hydrocarbons) also elicit a behavioural response in buffalo flies and the chemical nature of these compounds has been determined.

The investigations of potential visual cues revealed that buffalo flies oriented towards model cows and black, rectangular targets in the BOFF and field situations. In the BOFF, there was an increase in the percentage responding when animal odour was superimposed on the visual targets. The addition of lures developed for other flies to visual targets in the field did not increase their attractivity to buffalo flies. Also, none of the other traps developed for stock associated flies caught buffalo flies.

The use of light traps to attract buffalo flies at night was also considered. It was demonstrated that buffalo flies could orient towards a light source up to a distance of 60 metres. Light traps near penned cattle reduced the fly numbers on the cattle. However, in a small paddock the light trap did not have any apparent short term effect on the fly numbers.

Good progress has been made in this project towards the development of systems for non-insecticidal buffalo fly control. A synthetic mixture of components contained in cattle odour has been defined and shown to be attractive to buffalo flies in laboratory experiments. Similarly, visual targets for day and night use have been developed and some of the parameters influencing the fly catch identified. These findings provide a solid foundation for the development of a system which effectively modifies the behaviour of buffalo flies.

10. Recommendations

The following recommendations take into consideration the current state in the development of non-insecticidal behaviour-modifying systems for the control of buffalo flies, the likelihood of developing and commercialising an effective system, and the anticipated value of such a system to the meat and livestock industry:

1. That further research on the development of non-insecticidal, behaviour-modifying systems for the control of buffalo fly be conducted with the following objectives:

   a) To improve the synthetic chemical attractants for buffalo flies

   b) To optimise current visual targets and to investigate their mode of application

   c) To evaluate integration of olfactory and visual cues into an attractive and practical target for buffalo flies.

2. That an assessment of the potential for commercial development of the system(s) resulting from the research is made, and if found feasible, that commercialisation be initiated.

11. Bibliography

Adkins TR, Ezell, WB, Sheppard DC, Askey MM (1972) A modified canopy trap for collecting Tabanidae (Diptera). *J. Med. Entomol*. **9**:183-185.

Allan SA, Day JF and Edman JD (1987) Visual ecology of biting flies. *Annu. Rev. Entomol.* **32**: 297-316.

Broce AB (1988) An improve Alsynite trap for stable flies, *Stomoxys calcitrans* (Diptera: Muscidae). *J. Med. Entomol*. **25**:406-409.

Carlson DA, Roan CS, Yost RA and Hector J (1989 Dimethyl disulfide derivatives of long chain alkenes, alkadienes, and alketrienes for gas chromatography/mass spectrometry. *Anal. Chem.* **61**:1564-1571.

Dalton LW, Kinzer HG, Reeves JM and Atmar JW (1978) Host location in the horn fly: roles of heat, carbon dioxide, water vapor, and cow-produced odors in attraction. *Southwest. Entomol.* **3**: 147-153.

Gover J, Strong L (1996) Effects of ingestion of dung containing ivermectin on aspects of behaviour in the fly *Neomyia cornicina. Physiol. Entomol*. **21**:51-58

Hummel HE and Miller TA, eds. (1987) Techniques in Pheromone Research. Springer-Verlag, New York.

Kunz SE (1986) Integrated pest management and Dipteran pests in the new world. *In:* Parasitology - Quo Vadit, pp. 659-664, Howell MJ (ed.), Australian Academy of Science, Canberra.

Lehmann T (1993) Ectoparasites: Direct impact on host fitness. *Parasitol. Today* **9**: 8-12.

Lewis T, ed. (1984) Insect Communication. Academic Press, London.

Muirhead-Thomson RC (1991) Trap responses of flying insects. The influence of trap design on capture efficiency. Academic Press Limited, London.

Plimmer JR, Inscoe MN and McGovern TP (1982) Insect Attractants. *Ann. Rev. Pharmacol. Toxicol.* **22**: 297-320.

Ridgway RL, Silverstein RM and Inscoe MN, eds. (1990) Behaviour-modifying chemicals for insect management. Marcel Dekker, New York.

Simeone RM and Siverstein JB, eds. (1990) Semiochemicals and pest control - prospects for new applications, Journal of Chemical Ecology edn., vol. 16. Plenum Press, New York.

Spradbery JP and Tozer RS 1996) The efficacy of diazinon impregnated ear tags against buffalo fly and resulting weight gains and diazinon residues in meat and milk. *Aust. Vet .J.* **73**:6-10.

Urech, R, Green, PE, Franke, F, Mulder, JC, and Roberts, C (1994) Behavioural responses of *Lucilia cuprina* (Wiedemann)(Diptera: Calliphoridae) to olfactory stimuli: Evaluation of an olfactometer. *J. Aust. ent. Soc.* **33**:137-141.

Mackley JW, Carlson DA and Butler JF (1981) Identification of the cuticular hydrocarbons of the horn fly and assays for attraction. *J. Chem. Ecol.* **7**: 669-683.

Torr SJ, Hall DR and Smith JL (1995) Responses of tsetse flies (Diptera: Glossinidae) to natural and synthetic ox odours. *Bull. Entomol. Res.* **85*:***157-166***.**

Vale GA (1993) Development of Baits for Tsetse Flies (Diptera, Glossinidae) in Zimbabwe. *J. Med. Entomol.* **30**: 831-842.

Wall R., Langley, P. (1991) From behaviour to control: the development of trap and target techniques for tsetse fly population management. Agric. Zool. Reviews 4:137-

12. Appendices

Review of the role of behaviour-modifying systems in buffalo fly control

Milestone reports (excluding budget reports)

THE ROLE OF BEHAVIOUR-MODIFYING SYSTEMS IN BUFFALO FLY CONTROL

INTRODUCTION

A number of recent publications have reviewed the use of behaviour-modifying systems for insect management and control (Hummel and Miller 1987; Lewis 1984; Plimmer *et al.* 1982; Ridgway *et al.* 1990; Simeone and Siverstein 1990). An evaluation of insect trapping systems currently in use for agricultural and veterinary insect pests was carried out by Muirhead-Thomson (1991). For the biting flies of veterinary importance, it was shown that a wide range of trapping devices had evolved. The role of vision in these biting flies was reviewed by Allan *et al.* (1987). Principles of behavioural analysis for blood sucking flies and in particular tsetse flies have been outlined by Vale (1993).

For the use of behaviour-modifying chemicals in particular, Ridgway *et al*. (1990) concluded that there was reason for optimism that these chemicals could lead to reductions in the use of conventional pesticides and to significant expansion in the use of biologically based methods of pest control. For the tsetse flies *Glossina morsitans* and *G.pallidipes*, analysis of host-orientated behaviour led to a 10- to 1,000-fold improvement in the cost effectiveness of baits for surveys and control, and baits have now largely replaced air and ground broadcasting of insecticides (Vale 1993).

As pointed out by Donald (1994) organic farming with its prohibition against synthetic inputs is no solution to global food security. Sustainable chemicals of the future must have a narrower more specific spectrum of activity, be non-persistent and rapidly degraded to harmless metabolites. The use of behaviour-modifying chemicals for insect control would appear to embrace these specifications.

Haematobia irritans - Horn and buffalo flies

Production losses

The direct effects of ectoparasites on their hosts has been extensively documented and include weight loss, reduced production of milk, eggs, meat, hide and wool (Lehmann 1993).

In the US, Drummond *et al*. (1981) estimated that annual losses in production to arthropod pests exceeded $3000M. Byford *et al*. (1992) put this figure at $2,260M for losses due to ectoparasites. Kunz (1986) produced estimates exceeding $760M for horn fly (*H.i.irritans*) alone. Control of the fly resulted in better weight gains, better food intake and conversion, and better calf weaning weights. Increased milk production has also been reported when flies were controlled. Drummond *et al*. (1986) reviewed the effects and control of arthropods pests of livestock. Although the horn fly was seen as the major arthropod pest of pastured cattle in the US and Canada, due to the variable effects on production reported in the literature, no overall estimate was made, and the need for additional data was suggested.

The economic impact of the buffalo fly (*H.i. exigua)* on producers is difficult to assess. Early Australian studies have been inconclusive (Arundel and Sutherland 1988) or remain unpublished. In Australia costs exceeding $80 M annually in direct production losses alone, have been claimed (Sutherst pers comm). Depressed prices at saleyards due to fly lesions result in additional economic losses.

The buffalo fly also has the potential for increasing the risk of establishment of the screwworm fly should it enter Australia. In a recent QDPI producer survey, buffalo fly was ranked as the most important animal health problem facing the dairy and beef producers (1992 Tick Fever Survey). This is supported by the MRC-QDPI Producer Survey.

Taxonomic Studies

Stable and buffalo flies belong in the subfamily Stomoxinae of the family Muscidae. They are the only members present in Australia whose mouthparts have been modified for piercing. Zumpt (1973) reviewed the world literature on the subfamily and provided information on the biology, habits, economic significance and control. A revision of the Australian Muscidae was undertaken by Pont (1973). *Haematobia* is regarded as the most specialized genus in terms of morphology as well as biology (Zumpt 1973). *H.irritans exigua*, an introduced species, is the only subspecies in Australia and it is difficult to separate morphologically from the American horn fly *H.irritans irritans.* The only feature reported by Zumpt (1973) for separating the two subspecies was the bristling on the male hind tarsi. Skidmore (1985) recognised the two as separate species, *H.exigua* and *H.irritans*, although acknowledging their close affinities. The horn fly was introduced to North America from Europe between 1884 and 1886 (McLintock and Depner 1954). While the horn fly is reported to undergo a diapause in the pupal stage, no such mechanism is known to occur in the buffalo fly.

Ferrar (1979) described the immature stages of dung-breeding muscid flies in Australia and provided keys to the larvae and puparia. Keys to the adult flies were given by Pont (1973).

Distribution of H.i.exigua

H.i. exigua occurs in the Oriental and Australian regions. The buffalo fly was introduced from Timor, entering mainland Australia near Darwin in 1838, from where it spread slowly to reach north western Queensland by 1928 and reached Gympie in the early 1950's. Letts (1962) and Seddon (1967) provide detailed discussions on the introduction and spread of the fly. Williams *et al*. (1985) documented the spread of the buffalo fly from Bororen in Queensland in 1974 to Coffs Harbour in NSW in 1982. There are recent reports of flies as far south as Jerseyville near Kempsey in 1991 and also inland to Tenterfield (S.Spence pers comm).

Biology/ Life Cycle

Numerous authors have described the general biology of the buffalo fly (see (Cook 1980; Ferrar 1979; Kettle 1984; Skidmore 1985; Zumpt 1973)). *H.i.exigua* is an obligate ectoparasite of cattle that leaves the host only to oviposit in fresh faeces. The female fly requires a blood meal to mature her eggs, which are laid in batches of up to 26 under the faecal pad. The emerging larvae feed in the dung and undergo three larval instars before pupating in or near the pad. Development from egg to adult fly takes a minimum of 8 days at 35^0C and this may extend to 32 days at 17.5^0C.

Control/ Resistance

Current control methods rely heavily on the use of synthetic pyrethroid and organophosphate (OP) insecticides, although other groups such as the carbamate, bendiocarb and the macrocyclic lactones are available. Problems with insecticide residues and resistance to the synthetic pyrethroids exist, with the latter resulting in declining efficacies for these insecticides. However no resistance to the OP's has been detected. New application technology for the older groups (e.g. diazinon ear tags - Ciba) and new chemical groups (e.g. methoprene - Zoecon; ivermectin - MSD) may only provide short term relief unless strategies can be developed to

minimize the selection for resistance. Available non-chemical methods of control include the use of walk-through buffalo fly traps and dung beetles.

To enable an understanding of how behaviour-modifying systems might be used in control of the buffalo fly, the following sections review the general behaviour, the role of attractants, arrestants and repellents and the population dynamics of the buffalo and horn flies.

BEHAVIOUR OF THE BUFFALO FLY

In this section, the following aspects of buffalo and horn fly behaviour are considered: dispersal and host finding, host preferences, feeding behaviour, mating and other intraspecific interactions and oviposition. The behavioural responses of buffalo and horn flies to sensory stimuli were considered particularly relevant to this project, and therefore are discussed in separate sections dealing with attractants, arrestants and repellents.

Dispersal and Host Finding

Much evidence has been accumulated that buffalo flies and horn flies can disperse over long distances. Ferrar (1969) studied the development of two separate reinfestations of cattle on Magnetic Island (North Queensland) following the eradication of buffalo fly there. These reinfestations implied a movement of buffalo flies more than 7 km from the nearest cattle on the mainland, perhaps with the assistance of prevailing winds. Horn flies have also been shown to disperse and locate hosts over distances ranging from 50 m to several km (Byford *et al.* 1987; Chamberlain 1982; Eddy *et al.* 1962; Guillot *et al.* 1988; Hoelscher *et al.* 1968; Kinzer and Reeves 1974; Kunz *et al.* 1983; Marley *et al.* 1991; Sheppard 1994).

Sheppard (1994) demonstrated dispersal of up to 5 km in a wooded area and, considering the difficulties of detecting dispersed horn flies, suggested that the capacity of horn flies to disperse, particularly when assisted by strong winds, may have been underestimated in previous studies. However, horn flies are not dependent upon winds to transport them over long distances, and can cover considerable distances to locate hosts upwind or crosswind in moderately windy weather (Chamberlain 1981; Eddy *et al.* 1962; Kinzer and Reeves 1974; Marley *et al.* 1991; Sheppard 1994). Nevertheless, a downwind bias in dispersal has been reported on occasion, in both the absence (Chamberlain 1985) and presence (Chamberlain 1984; Eddy *et al.* 1962) of hosts. Strong winds (e.g. > 16 km/h) are reported to have a deleterious effect on host location (Kinzer and Reeves 1974).

Macqueen and Doube (1988) examined recruitment of buffalo flies to an initially uninfested cow held in isolation at least 50 m from the nearest paddock containing cattle. During four out of five sampling periods, 86% of female buffalo flies arriving on the cow were newly emerged. This is in contrast to the proportion of newly emerged buffalo flies in the local population (18.4%). Some 90% of buffalo flies emerged between 12.00h and 20.00h; most newly emerged females arrived on the cow between 16.00h and 08.00h.

With horn flies, Guillot (1988) and Chamberlain (1982) also found evidence of a higher propensity of recently eclosed horn flies than of older flies to migrate to hosts. However, Marley *et al*. (1991) found that the age composition of horn flies migrating to a herd 400 m away from the nearest hosts did not differ from that of the source population, the mean density of which ranged from 87-270 flies/side/animal during the study period. Those authors attempted to explain the discrepancy between these and previous results of other workers by suggesting that the earlier studies examined movement over too short a distance (generally < 200 m) to identify

true migratory behaviour, and their results were therefore biased mainly on the relatively short-range "appetitive" searching of newly-emerged flies within an area.

Kinzer and Reeves (1974) found that laboratory-reared 1 - 12h-old and 20 - 28h-old horn flies were equally successful in finding a host approximately 90 m away. However, it is still not clear to what extent flies that have located a suitable host subsequently leave it and find another host. Obviously, females must locate the same or an alternative host after ovipositing on dung, perhaps daily after the preoviposition period, but doubt remains as to how frequently flies must re-locate hosts under other circumstances. This question is likely to have important implications for the probability of trapping *Haematobia* as a control measure.

In the study by Macqueen and Doube (1988) discussed above, the majority of females captured on the isolated sentinel animal during one sampling period were parous (and therefore had fed on another host). During this period, a group of cattle had "camped" for the night 100-200 m away from the sentinel, and the authors suggested that flies were attracted from these to torches used by experimenters in sampling flies on the sentinel during the night. The relevance of this isolated result to the normal behaviour of parous buffalo flies is therefore in doubt.

Kinzer and Reeves (1974) found that 4 - 6d-old laboratory- reared horn flies released on a host were equally likely to move away from it as newly-emerged flies; approximately 70% of each class could no longer be found in the release area after 11h. However, in experiments of this kind, the possibility of disturbance to the flies' normal behaviour pattern as a result of the release procedure must be considered in interpreting the results. Chamberlain (1982) found that (wild-caught) horn flies of a range of ages were less likely to disperse after being placed on an animal than (laboratory-bred) newly eclosed flies. Movements of wild flies any sizeable distance from the host appear to be quite limited (approximately 1% transferred to another host 50 m away), suggesting that older flies, at least, do not frequently leave a host and move to another some distance away. Interchange of horn flies among adjacent hosts in a herd, however, appeared to be common; more than half of the flies initially on an animal may transfer to other hosts less than 3 m away within 24h (Chamberlain 1982).

On balance, the above studies indicate that the propensity of buffalo flies and horn flies that have located a host to disperse further is probably limited. However, sufficient inconsistencies exist to preclude a definite conclusion on the subject in the present state of knowledge.

Chamberlain (1981, 1985) has demonstrated that the probability of a horn fly's locating a host is many times greater than would be the case if searching flies relied upon fortuitous encounter; i.e. active orientation from some (undetermined, and doubtless variable) distance occurs to potential hosts. Experimental investigations of the sensory stimuli involved in host finding by horn flies have been conducted. This work is reviewed in the following section.

Host Preferences

In northern Australia, buffalo flies have been recorded as attacking cattle, buffalo, horses, mules, donkeys and occasionally humans (Tillyard 1931). However, these host species are by no means equally susceptible to infestation, and among major domestic animals in Australia, only cattle and to a much lesser degree, horses are considered to be affected significantly (Seddon 1967).
In a summary of work previously published elsewhere, Krijgsman and Windred (1933) stated that buffalo flies oriented to skin odour of various animals in the following order of preference: Friesian cattle > Zebu cattle > buffalo > horse > dog. However, in the original paper (Krijgsman and Windred 1930) the order of preference was given as cattle of both breeds = buffalo > horse and dog.

Doube (1984) studied the relative numbers of buffalo fly infesting mature steers in herds both mixed and homogeneous for breed. There were approximately five times as many buffalo flies on the most susceptible animals of the mixed herd as on the least. However, water buffalo carried as many flies as cattle in that herd, and there was no clear evidence of differences amongst cattle breeds in this regard. Amongst homogeneous herds, kept in the same locality, Brahmans had fewest buffalo flies, Brahman ´ British crossbreds more and $^{7}/_{8}$ British: $^{1}/_{8}$ Brahman the most. The lack of consistency in the findings for mixed and homogeneous herds was not explained.

Tugwell *et al*. (1969) demonstrated an inverse relationship between the proportion of Brahman blood in cattle in small mixed herds and their burden of horn flies. However, Ernst and Krafsur (1984) found no significant differences in horn fly infestation among four British and European breeds of cattle in a mixed herd; only 3.3% of the total variance in fly numbers on animals was attributable to host differences. By contrast, in another study of the susceptibility of British and European breeds to infestation by horn fly, Steelman *et al*. (1991) found that Chianina cattle had £ 50% of the horn fly burden of the other breeds. Steelman *et al*. (1993) also found differences in the susceptibility of individuals within breeds; numbers of horn fly on Chianina cows varied by at least 4.5 fold. In general, then, available information favours the existence of significant differences in the host preference of *Haematobia* amongst at least some breeds of cattle, although these differences may be confounded by other factors on occasion.

One possible such factor is host colour. For many years, it has been stated that horn flies prefer dark to light-coloured animals as hosts. Franks *et al*. (1964) summarised the results of earlier observations and conducted experiments to test the role of host colour in this regard. They found that crossbred heifers were preferred in the order red > black > white and that on white animals dyed black on one side, horn flies settled preferentially on the dyed side. Tugwell *et al*. (1969) demonstrated a significant effect of animal colour on horn fly burden, after allowing for breed differences; amongst animals having low percentages of Brahman blood, there were significantly more horn flies on black cross-bred cattle than on red. However, breed composition differed between these two classes, raising the possibility that factors other than host colour were involved in the observed preferences. This possibility is inherent in most comparisons of this kind between naturally coloured animals which may differ in relevant characteristics besides colour. Nevertheless, the work of Franks *et al*. (1964) with dyed animals suggests that host colour may be a significant factor in host selection by horn (and perhaps buffalo) flies.

Tillyard (1931) reported early observations that buffalo flies preferred bulls to bullocks or cows in Java and northern Australia. Similarly, bulls were reported to carry greater numbers of horn flies than cows or calves in Canada and Denmark, although a report to the contrary exists from Venezuela (references in (McLintock and Depner 1954)). Such reports led Dobson *et al*. (1970) to inject steers experimentally with testosterone propionate; those receiving 125 mg weekly or 250 mg two-weekly carried approximately 50% more horn flies than castrated steers. Higher doses, however, showed some tendency to reduce horn fly burdens. Subsequently, Christensen and Dobson (1979) showed that bulls and (for a limited time) testosterone-treated steers carried more horn flies than untreated steers. Bulls had significantly larger sebaceous glands than steers and both bulls and treated steers had larger sebaceous gland cells than untreated steers.
The latter parameter in particular was considered to be an indicator of lipid synthesis and hence sebum production. Without further investigation, however, it is not clear how increased production of sebum results in higher horn fly numbers.

Indeed, almost nothing is known about the mechanism(s) that bring about the observed "preferences" of flies for particular colours or breeds of host animal. Obvious possibilities are increased orientation of host seeking flies to animals emitting optimal quantities and

combinations of attractant olfactory or visual stimuli, but other factors such as varying residence times of flies or even differential survival on different animals may be involved.
Responses of hosts to infesting flies may be one factor influencing residence time. Much further work is required for an understanding of the behavioural processes concerned.

Feeding Behaviour

H. irritans are invariably considered to be obligate blood-feeders, which spend most of their time, both day and night, on the host, feeding intermittently (MacQueen and Doube 1988; McLintock and Depner 1954; Tillyard 1931). Harris *et al.* (1974) used an electronic feeding recorder to investigate the frequency and duration of feeding by horn flies held in small cages on a steer. At an ambient temperature of 26^0C and under a 12h light - 12h dark cycle, females fed a mean of 38.4 times in 24 hours, males 24 times in 24 hours. Total feeding times for this period were 163 min and 96 min for females and males, respectively. With both sexes, feeding was spread evenly throughout each 24 hour period. Uncaged flies on an animal were found to feed more than 24 times in 24 hours.

Early laboratory experiments with buffalo flies in Java, summarised by Krijgsman and Windred (1933), showed that skin odour of potential host animals stimulates movement towards the odour source, followed by proboscis extension and piercing movements. Fresh blood and serum elicited similar behaviour, together with sucking when the liquid was contacted. If direct contact was prevented, piercing movements were observed.

Krijgsman and Windred (1933) also observed that female buffalo flies tested in the laboratory approximately one hour after their removal from hosts in the field exhibited piercing and sucking behaviour on fresh buffalo, cow and horse dung. It was not established how much of this material was ingested, or whether this behaviour occurred in the field. These authors also reported that approximately 30% of buffalo flies trapped on fresh dung in the field in Java were males; the activities of these flies on the dung were apparently not investigated. However, Macqueen and Beirne (1975) trapped very few male horn flies on fresh dung in Canada.

Tillyard (1931) stated that buffalo flies in the laboratory oriented to green leaves, which they then pierced and sucked. He speculated that this behaviour may prolong the survival of flies in the field in the absence of suitable hosts. However, there appear to be no reports in the literature of feeding by horn or buffalo flies on other than mammalian hosts in the field.

Mating and Other Intraspecific Interactions

Little information is available on mating behaviour in buffalo flies, other than the unpublished observations of J. Anderson (personal communication). With both sub-species of *H.irritans*, mating is considered normally to occur on the host (McLintock and Depner 1954; Tillyard 1931), although copulation on vegetation has also been observed with the horn fly (Bruce,WG 1964) cited in (Zorka and Bay 1980)). Courtship and mating of horn flies in the laboratory has been described by Zorka and Bay (1980).
The male initiates the process by approaching the female, usually from behind, tapping her abdomen with his prothoracic tarsi, and then mounting her dorsally. From here he contacts the prothoracic legs of the female and raises and lowers them alternately before moving posteriorly and attempting copulation.

Pheromonal stimulation is apparently involved in male courtship, as solvent extracts of the cuticle of female horn flies have been shown to elicit this behaviour towards dead or tethered males in laboratory bioassays (Bolton *et al.* 1980). The monoolefins *Z*-5-tricosene, *Z*-9-pentacosene and *Z*-9-heptacosene were amongst compounds identified in solvent extracts of

female cuticle, and synthetic forms of these were found by these workers to stimulate male courtship, a combination of these compounds being the most effective.
It was not clear whether components of the female mating pheromone helped to elicit the orientation of males to females from a distance.

The possibility of pheromone-mediated interactions amongst females is shown by the finding that monoolefin hydrocarbons of female (but not male) cuticle stimulated slight but significant positive orientation by females in a laboratory olfactometer (Mackley *et al.* 1981). Involvement of this pheromone in aggregation of females on hosts or dung appears possible.

Horn flies held at 32^0C in laboratory cages mostly mated within four days. Those held on a cow did so rather sooner, within two days (Harris *et al.* 1968). In this study, females normally mated only once. Individual males caged with ten females inseminated a mean of 4.5 females within seven days, beginning one to two days after emergence (Harris *et al.* 1968).

Oviposition Behaviour

Gravid females of both sub-species fly down from the host to newly-dropped dung, on which they lay their eggs within five minutes (MacQueen and Doube 1988; McLintock and Depner 1954). Laboratory experiments have shown that buffalo flies orient to the dung of host animals in response to its odour; the stimulatory effectiveness of the dung in this regard decreased as its age increased (Krijgsman and Windred 1933). The order of preference obtained in these experiments was buffalo > cow > horse. The odour of dog dung was not attractive. Macqueen *et al*. (1980) found that under laboratory conditions, buffalo flies could be induced to oviposit most reliably on dung if it was preheated to 39^0C, which approximates bovine rectal temperature. This temperature preference may be one factor ensuring that oviposition occurs only on fresh dung. Another may be the rapid formation of a dried crust on deposited dung, reducing odour emission (Hammer 1942) cited in (McLintock and Depner 1954)).

McLintock and Depner (1954) reported from their own and other studies, that after alighting on a pat, horn flies move under its sides, depositing their eggs in groups of four to six under the sides of the pat or on the grass or soil on which it rests. This choice of oviposition site presumably minimises desiccation of the eggs before hatching.

Kunz *et al*. (1970) found that horn flies in Texas would oviposit in dung throughout the day and night. Similarly, Macqueen and Beirne (1975) found no clear diel pattern in the numbers of female horn flies trapped on fresh cattle dung in the field in Western Canada; in all experiments, oviposition continued throughout each 24 hour period.

ATTRACTANTS AND ARRESTANTS

The use of chemical messengers, one of the modes of biological communication, appear to be significant in most groups of animals. In insects this is reflected in their highly developed olfactory and gustatory systems, with the chemical environment being a dominant modality mediating food choices, avoidance of danger, location of a sexual partner and the choice of a habitat for their progeny (Stadler 1984). Odours from host animals for example mediate the availability of food, oviposition sites and other functions. These behaviourally mediated interactions are dependent on precise chemical messages (semiochemicals) in a species (pheromones) or between species (allelochemicals) and on their olfactory systems (Mustaparta 1984).

The term semiochemical was proposed by Law and Regnier for these types of chemical messengers and included all natural chemicals involved in inter- or intraspecific communication (Dickens and Payne 1981). The classification of semiochemicals (behaviour-modifying chemicals or infochemicals) can be based on their communicative role or the response/s that they elicit (Table 1). The same chemical may also elicit more than one of these responses. The problems of compounding such quite different kinds of behaviour under one chemical label such as "arrestant" and "attractant" have been discussed by Kennedy (1978).

Table 1 Classification of Semiochemicals

Communicative role (Dicke *et al.* 1990)	**Responses elicited** (Dethier *et al.* 1960)
Allelochemic (Interspecific)	Arrestants
Allomone	Locomotor stimulant
Kairomone	Attractant
Synomone	Repellent
Apneumone (non-living)	Feeding, mating, ovipositional stimulant
Homeochemic (Intraspecific)	Feeding, mating, ovipositional deterrent
Pheromone	

Both contact chemoreception (gustation) and olfaction are involved in the perception of chemical messengers in insects but there is not always a clear distinction between the two modes in the literature. The role of contact chemoreceptors in the selection of mammalian hosts has been little investigated (Stadler 1984). Although contact stimulation probably plays a role in host-selection behaviour, Galun (1976) believed that olfaction was much more important.

An insect attractant has been defined as a chemical which causes insects to make orientated movements towards its source (Dethier *et al.* 1960). Barton-Browne (1977) redefined an attractant as a chemical or mixture of chemicals which, acting in the vapour phase, cause an insect to behave in ways which result in its moving toward the source of the material or towards a zone of preferred concentration. Kennedy (1978) suggested that in practice it appeared that orientation towards an odour source from a distance was usually by non-chemical cues, the chemical only conditioning the orientation response to them.

An arrestant was defined by Dethier *et al*. (1960) as a chemical that causes kinesis reactions that, in the absence of orientation cues, often causes the insect to aggregate near the chemical source by decreasing the speed of locomotion (orthokinesis) or appropriately affecting the rate of turning (klinokinesis). What arresting effect a chemical has depends on how these two mechanisms are combined. An arrestant may be a sex pheromone or a kairomone emanating from food or oviposition sites.

Aggregation may be viewed as the end result of movement reactions that reduce the distance between individuals in their environment.
Such clustering may be brought about by a combination of attraction and arrestment and result from responses to other individuals (conspecifics) or to chemical and non-chemical cues arising from other sources.

For sex and aggregation pheromone communication, attraction has been defined as the net displacement of one individual towards the chemical source. Conversely arrestment is the lack of net displacement toward or away from the chemical source. Both may be viewed as part of a continuum caused by pheromone mediation of quite disparate movement reactions such as klinotaxis and anemotaxis.
Attraction and arrestment are only outcomes not mechanisms and they are a summary of the change in spacing between an individual and the chemical source (Carde and Baker 1984).

Semiochemicals that interfere with aggregation and/or induce dispersal have been variously termed inhibitors, disruptants, repellents or antiaggregants (Vite and Baader 1990). Overall the behavioural effect of a chemical depends on the context, including other chemical and non-chemical cues, and is decided by central integration as well as receptor specificity (Kennedy 1978).

Chemical Attractants

Distance orientation of biting flies to their hosts have been reviewed by Sutcliffe (1987). Distance responses have been defined by Kennedy (1977) as those occurring where the odour gradients are too disrupted and shallow to permit a chemotactic approach and any taxes depend on directional cues provided by other features of the environment such as wind or a visual target. In biting flies distance orientation was defined in terms of the development of receptivity to the host (appetitive search), activation of the blood-feeding behavioural package and host-location (Sutcliffe 1987). The number of sensory modalities invoked in the insect tended to increase as the host was approached. Once hosts are located, several characteristics, including size, group size, body covering, defensive behaviour and activity rhythms, may influence the subsequent choice of an individual host or host species. Chemicals attractive for biting flies have been listed by Sutcliffe (1987) and Moore (1993).

Buffalo flies have been shown to respond to odours from host skin and faeces by positive orientation towards their source (Krijgsman and Windred 1933)(see Host Preferences, above). Horn flies have also been found to orient upwind in a laboratory olfactometer to odours collected from the skin of a cow and of a human arm (Kinzer *et al.* 1970). The response of 4h-old and 8 - 12h-old flies to human skin was compared: only the older flies showed significant upwind movement, suggesting that newly-emerged horn flies may not become responsive to host odour for several hours. Perhaps this may result from a period of post-eclosion quiescence. Hargett and Goulding (1962) found that horn flies, held in containers, oriented in still air to an area of screen through which odours of bovine hair and of solvent extracts of this could diffuse.
Gamal El-Din (1972) demonstrated a positive response of horn flies to washings of the body of host and non-host animals. Buffalo and cow washings were preferred to horse, donkey, sheep and camel; the last two were the least preferred.

Experiments with an artificial cow (black 114 l barrel, horizontally placed on legs, controllably heated, with sticky sides) in a large insectary (27 x 27 m) showed that a release of steer odour from the artificial cow greatly increased its attractivity to horn flies,when compared to the emission of various combinations of heat and CO_2 (Dalton *et al.* 1978). It was also demonstrated that an increase in the release rate of steer odour led to higher fly attractivity. The authors concluded that steer odour was the most important factor in the flies' orientation towards the artificial device.

Few studies of the effects of components of host odour on the behaviour of *Haematobia* have been undertaken. Doube and Macqueen (1978) trapped small numbers of buffalo flies in the field using electrified grids surrounding a source of carbon dioxide. Tugwell *et al*. (1966) obtained significant landing responses of wild horn flies to targets baited with carbon dioxide, under both daylight and night conditions. Kinzer *et al*. (1978) investigated the factors affecting

the number of flies caught on an artificial cow. The release of CO_2 (20 l/min) or CO_2+CH_4 (methane, 5 l/min) from the "cow" greatly increased the horn fly catch compared to "shape only" or "shape and heat cow". Release of water vapour in addition to the other chemicals from the "cow" did not improve its attractiveness. The artificial cow (heated, CO_2 release) was also tested against a heifer.
The catches were comparable at 0600 h, but at 1900 h the heifer was much more attractive to horn flies (for possible interpretation see next section). One limitation of these field experiments were the close proximity of the competing targets to each other (15 m), relative to the distance between fly release and targets (270 m). The observed preferences could have been obtained as a result of short range effects. It was also clear from other experiments with the same devices (Dalton *et al.* 1978) (see above) that cattle odour contained behaviourally significant components besides carbon dioxide.

Mackley *et al*. (1981) analysed the crude lipid extracts from horn flies which had elicited a positive response from female horn flies in an olfactometer. They determined the active components to be monoolefins, namely Z-9-tricosene, Z-5-tricosene, Z-9-pentacosene and Z-9-heptacosene. Female horn flies showed a significant, positive response in an olfactometer to the first and second of these components.

A range of chemicals originating from cattle had been found to effectively attract tsetse flies, and some of these have been tested against stable flies. Holloway and Phelps (1991) supplemented insecticide-impregnated blue cloth with some of these components and registered the number of stable flies attracted to this target. 1-Octen-3-ol (0.6 mg/h) increased the catch by about a factor of 2, but 4-methylphenol, 3-*n*-propylphenol and binary and tertiary mixtures of these components did not lead to any increase when compared to the unbaited trap.

Hargett and Goulding (1962) found that horn flies did not discriminate behaviourally between two airstreams of 88% and 19% relative humidity presented in a laboratory olfactometer in the absence of other significant stimuli. This conclusion received some support from results of field experiments (Dalton *et al.* 1978) which suggested that the release of water vapour from artificial devices, in the absence of host odour, had no clear effect on captures of horn flies released in the field. However, Gamal El-Din (1972) found in laboratory studies that horn flies strongly preferred moist air containing host or dung odours to similarly odourized dry air for positive orientation. Host or dung odour in dry air was nevertheless preferred to moist air without host or dung odour.

Methodology

The methodology utilised to investigate the response of horn flies to odours in the laboratory involved various designs of olfactometers. Kinzer *et al*. (Kinzer *et al.* 1970) determined the ratio of flies moving upwind in parallel control and treatment tubes. A T-shaped glass tube, with balanced air streams entering both ends, test and control respectively, was used by Gamal El-Din (1972). The flies were introduced from the bottom end of the T and then had to make their choice when they reached the crossing of the 3 branches. Mackley's olfactometer (Mackley *et al.* 1981) consisted of multiple holding chambers from which the horn flies moved vertically, upwind into one of two choice chambers which were separated from the holding chambers by cones restricting back-movement of the flies. A cage olfactometer, where flies were photographed as being in either one of two air streams was used by Chamberlain (1987) to investigate the effect of heat, light and airflow on horn flies.

To assess buffalo and horn fly populations in the field, counting the number of flies on (part of) cattle, often with the help of binoculars, is commonly used (Byford *et al.* 1987; Dobson *et al.* 1970). Doube and McQueen used an electric grid to kill buffalo flies attracted by CO_2 (1978) and, to investigate arrival on a isolated host, aspirated flies off a cow into jars using a vacuum

cleaner (1988). Sticky surfaces (Stickem on burlap or Kraft paper) on black, horizontally placed drums was used to catch landing horn flies (Dalton *et al.* 1978; Kinzer *et al.* 1978; Tugwell *et al.* 1966).

Gamal El-Din (1972) established through sectional amputation that the olfactory receptors were located in the flagellum of the antennae.

Electromagnetic energy attractants

Two different wavelength windows seemed to play a role in host finding process of the horn fly: the ultraviolet (UV)/ visible range (200 - 700 nm) and emissions in the near infrared (heat) part of the spectrum (780 - 3000 nm). The visual ecology of biting flies was reviewed by Allan *et al*. (1987). With regard to the horn fly, they stated that "the role of vision in host orientation may be of only minor importance in this diurnal, host-associated species".

A strongly positive phototactic response by buffalo flies was noted by Krijgsman and Windred (1933) during their laboratory behavioural experiments. Hargett and Goulding (1962) studied the orientation of horn flies to light of a range of wavelengths. The response was strongest in the ultraviolet region (350 - 390 nm) and decreased with increasing wavelength, finally disappearing in the red region (greater than 670 nm). Morgan (1966) found that horn flies in the laboratory were most attracted to "blacklight blue" lamps (emitting in the range 290 - 500 nm), less to "blacklight" lamps (emitting 290 - 600 nm) and less still to "daylight" lamps (290 - 700 nm). A blacklight blue lamp in a large cage with a heifer was preferred to the heifer by a majority of flies.

Agee and Patterson (1983) studied the spectral sensitivity of horn fly compound eyes using electro-retinography. The electro-retinogram obtained was generally similar in form to that obtained for the muscids *Stomoxys calcitrans* (stable fly) and *Musca autumnalis* (face fly), with major peaks at 360 nm (ultraviolet) and 490 nm (blue/green). In the presence of host cattle in the field, horn flies showed little response to traps consisting of sticky-coated panels reflecting light of various wavelengths in the range ultraviolet to red. This result may have been due to the failure of the isolated visual stimuli presented to compete with the complex of attractive stimuli emanating from cattle.

Hargett and Goulding (1962) showed in outdoor experiments using laboratory-reared horn flies that a strong, specific, visually-stimulated orientation occurred towards a (white) steer placed against either a black or a white background. By contrast, little orientation occurred towards a sheep or man placed similarly or to a white rectangle of area similar to the lateral surface area of the steer. In this work, the experimental flies were sealed off from the test animals inside a box with glass panels, eliminating olfactory and probably thermal stimulation. These workers also showed that, in laboratory chambers, horn flies oriented to black areas in preference to white or clear. This result may be related to the observed preference of horn flies for dark over light-coloured animals in the field.

However, the visual stimuli used by horn flies to recognize hosts must apparently be fairly specific, or else the landing response requires additional stimuli, as the artificial "cows" used by Kinzer *et al.* (1978) and Dalton *et al*. (1978) did not elicit significant landing responses without the addition of thermal and olfactory stimuli.

In the absence of other behaviourally significant host-related stimuli, horn flies in the laboratory oriented preferentially to surfaces heated to temperatures in the range 24^0C to 42^0C compared with surfaces at 21^0C; at temperatures above 43^0C the heated surfaces repelled the flies (Hargett and Goulding 1962). Heated surfaces in the favourable temperature range also elicited probing with the mouthparts. Field observations that horn and buffalo flies occur mainly on

shaded surfaces of the host animal in hot weather may be related to an avoidance of excessively hot areas (Handschin 1932; Schreiber and Campbell 1986).

Gamal El-Din (1972) found that in a laboratory choice olfactometer with airstreams containing similar levels of moisture and dung odour on each side, horn flies oriented preferentially towards the side heated to 32 - 35^{0}C rather than the side at 21^{0}C. However, in view of the design of the olfactometer, increased volatilization of attractive compounds from the dung samples on the heated side may have been a factor in producing this result. Chamberlain (1987) showed, again using a laboratory olfactometer, that wild and laboratory-reared horn flies of varying ages oriented preferentially towards the warmer of two odour-free airstreams differing by 5 - 9^{0}C in the range 26 - 36.5^{0}C. Probing behaviour was observed, especially in response to the warmer airstream.

The role of warmth in achieving captures of horn flies in the field by the artificial "cows" used by Kinzer *et al.* (1978) and Dalton *et al*. (1978) is somewhat unclear. Released flies oriented preferentially to devices heated to 37.8^{0}C or 40.6^{0}C rather than those heated to 26.7^{0}C (all in presence of carbon dioxide) at night and at 06.00h. However this preference did not occur at 19.00h, a time which approximated that of sunset (Kinzer *et al.* 1978). The discrepancy may be related to differences in the detectability of infrared radiation from heated objects against background levels, which fluctuate during the day (Kinzer *et al.* 1978). Few flies alighted on devices heated to 43^{0}C or 54^{0}C, probably because of a repellent effect of excessively hot surfaces (Dalton *et al.* 1978). A heated artificial device painted black captured more horn flies than a similar device painted silver (Dalton *et al.* 1978). This result was attributed to the greater infrared emissivity of the black device, in view of the similar (low) effectiveness of the two devices when unheated.

Arrestants

Chemicals with arrestant properties have been reported from a number of Arthropod Orders, including Blattodea, Coleoptera, Hemiptera, Lepidoptera, Hymenoptera and Acarina. Arrestants have been used in a limited number of insect and tick control strategies. In Coleoptera, dry ground baits impregnated with insecticide and containing cucurbitacins as arrestants and feeding stimulants were shown to be effective in controlling adults of *Diabrotica* spp. in laboratory and field studies (Metcalf *et al.* 1987)
Norval *et al.* (1992) examined the responses of *Amblyomma* spp. to aggregation pheromones and their potential use in tick control. Insect parasitoids use a variety of semiochemicals to locate and parasitise their hosts. Tumlinson *et al.* (1992) reviewed these semiochemical-mediated interactions. All long range-kairomones were the sex pheromones of the host while short-range host-produced chemicals often acted as arrestants and/or stimulated more intense searching behaviour. Potential applications for such arrestants have been investigated.

For example, parasitism by the braconid *Apanteles kariyai* was increased in the noctuid *Pseudaletia separata* by the use of a synthetic arrestant (2,5-dihexadecyltetrahydrofuran) in laboratory and greenhouse studies (Takabayashi and Takahashi 1988). The kairomone functioned as an arrestant keeping the braconid in the host habitat.

Diptera: *Plant Associated:* Arrestants have been used in conjunction with other semiochemicals in various trapping systems. Haniotakis *et al.* (1991) reported on the

combination of a food attractant, a phagostimulant, a male sex pheromone, a female aggregation pheromone with additional arrestant and aphrodisiac properties and a hygroscopic substance on an insecticide-treated board to trap *Dacus oleae*. Sharp (1987) demonstrated that the addition of sodium hydroxide or ammonium hydroxide increased the arrestant properties of casein hydrolysate and improved trap catches. Scott and Greenway (1984) used activated charcoal in an attempt to interfere with host-plant seeking in *Delia coartata* by adsorbing arrestant compounds exuded from wheat plants.

Animal Associated: Few reports of arrestants are available. The effect of various pheromone components on male sexual behaviour of the housefly *Musca domestica* was documented by Adams and Holt (1987). An arrestant effect of the methylalkane fraction was shown. Warnes and Finlayson (1985a, b) examined the activation and orientation responses of the stable fly *Stomoxys calcitrans* to carbon dioxide and host odours. Positive behavioural responses were shown to carbon dioxide, human breath and acetone, while acetic acid had an inhibitory effect. Cattle sebum was shown to have an arrestant effect, but also elicited further searching activity. To explain the latter behaviour, it was suggested that gustatory stimuli from the sebum induces a number of short search flights after probing failed to produce further stimuli. Cattle sebum was apparently perceived by the gustatory receptors on the tarsi of this fly. Although host location strategies have been extensively studied in the tsetse fly, *Glossina* spp. (Colvin and Gibson 1992) no references to arrestants have been located for these species.

Byford *et al*. (1987) suggest that horn flies (*Haematobia irritans irritans*) find their host over relatively long distances by contrasting visual cues, at shorter distances by specific visual and chemical cues, while movement is arrested on the host by chemical and thermal cues. Apart from the early horn fly studies of Hargett and Goulding (1962) the only recent report of arrestants for biting flies was that for *Stomoxys calcitrans* by Warnes and Finlayson (1985a). They showed that cattle sebum had an arrestant effect on these flies.

The work of Christensen and Dobson (1979) demonstrated that bulls and testosterone-treated steers carried more horn flies than untreated steers. Both bulls and treated steers had larger sebaceous gland cells indicating likely increased sebum production. Given its known arrestant effects, the role of sebum in influencing the host preferences of *Haematobia* spp. needs to be investigated.

REPELLENTS FOR INSECTS OF VETERINARY IMPORTANCE

Introduction

This section aims to list compounds which may be useful against buffalo fly and to review methodology relevant to repellent/deterrent tests against buffalo fly.

Use of terms such as “repellent”, “deterrent” and “inhibitor” in much of the available literature does not adhere to the strict definitions listed in publications such as Dethier *et al.* (1960). The terms are used much more loosely and somewhat interchangably to describe any stimulus, usually chemical, which interferes with insect behaviour in such a way that a particular outcome eg number of bites, number of flies, number of eggs etc is reduced. Most of the studies in this area, particularly the field trials, cannot distinguish between true repellent action and a range of other effects such as toxicity, locomotor stimulation, feeding deterrents and ovipositional deterrents. Instead they measure an outcome which is the product of a number of behaviours (eg feeding is preceded by host location and landing) without defining which of the relevant behaviours is affected. True repellents act in the vapour phase to reduce landing rate by inducing orientation away from the source of the repellent. These are often referred to as

vapour repellents or vapour-active repellents. Locomotor stimulants reduce the duration of visits/landings, usually through gustation. These are often referred to as contact repellents. The term repellent will be used in this review to include repellents, locomotor stimulants and deterrents unless a more precise identification of the behavioural mode of action can be made.

This section has been confined to literature published since 1940. There was an increase in relevant research during World War II and consequently, 1940 marks the start of the current era in repellents.
However research into repellents appears to have been overtaken by the emergence of the modern synthetic insecticides and as a result their development slowed thereafter and the number of effective repellents remains very small compared to the number of insecticides available.

Active Compounds

Shaw *et al.* (1943) reviewed the very early literature on repellent sprays for cattle and conducted further tests. They tested "Thanite" (fenchyl thiocynyl acetate), pyrethrum, "Yarmor" pine oil, "DHS Activator" (ethylene glycol ether of pinene) and 4 unnamed commercial sprays. They found that all products tested reduced the numbers of horn flies (*Haematobia irritans irritans*, Diptera: Muscidae) on treated cattle but that stable fly (*S. calcitrans,* Diptera: Muscidae) results were more variable. Results for *S. calcitrans* were generally unimpressive with modest reductions in fly numbers lasting at most 7 hours.

Howell and Fenton (1944) tested a spray containing pyrethrum and a mixtue of organic thiocyanates against horn fly and *S. calcitrans.* Efficacy durations of up to 10 hours for horn fly and up to 5 hours for *S. calcitrans* were obtained.

Travis *et al.* (1946) compared a number of compounds for use on human skin against what were then the standard repellents, citronella and butyl carbitol acetate. Compounds were tested in a cage/forearm assay against *Anopheles quadrimaculatus*, *Aedes aegypti* (both Diptera: Culicidae) and *S. calcitrans*. Field tests were conducted against *Aedes taeniorhynchus*, *S. calcitrans*, *Eusimulium pecuarum* (Diptera: Simuliidae) and *Culicoides* spp. (Diptera: Ceratopogonidae). Dimethyl phthalate, 2-ethyl-1,3-hexanediol (Rutgers 612), n-butyl mesityl oxide oxalate (Indalone,I-I-dimethyl-I-carbobutoxy-K-dihydropyrone) and a 3:1:1 mixture of these respectively were found to be the best repellents but results were highly variable. Laboratory and field protection periods were generally comparable. Mean protection periods were generally in the range of several hours and up to 10 hours. Only areas of skin covered by the compounds were protected.

Granett *et al.* (1949) used a bait sandwich/*Musca domestica* laboratory system and a half cow/hornfly and *S. calcitrans* field system to demonstrate the efficacy of 2 butoxypolypropylene glycol compounds (Crag fly repellent). Fly numbers were reduced for up to 2 days and up to several days when the whole cows were treated.

Goodwin *et al.* (1952) found pyrenone (pyrethrins + piperonyl butoxide), butoxypolypropylene glycol (the latter with and without pyrethrum) and a combination of bentonite sulfur and lindane to be effective against hornfly and tabanids for at least 5 days in field trials on cattle. In a continuation of this work Goodwin *et al.* (1954) found that pyrenone and butoxypolypropylene glycol reduced horn fly and tabanid numbers for at least 7 days and sulfoxide pyrexcel gave variable but sometimes good results also in field trials on cattle.

Starnes and Granett (1953) developed a laboratory rabbit/cheesecloth/small cage system for testing compounds against *S. calcitrans* and compared it with a bait method of testing

compounds against *Musca domestica*. The effect of Indalone and butoxypolypropylene glycol on cheesecloth lasted at least 14 days. Thanite, Rutgers 612, citronella and pyrenone lasted 10, 10, 4 and 4 days respectively. They found that repellent activity against *M. domestica* was a poor predictor for activity against *S. calcitrans*. They also found that laboratory activity was a poor predictor of field activity. In the field butoxypolypropylene glycol was best and Indalone was poor. They found that sweat could reduce the efficacy of some repellents eg Indalone but enhance the efficacy of others namely butoxypolypropylene glycol and speculated that this may partly explain the observed differences between laboratory and field.

Goodhue and Stansbury (1953) using a laboratory molasses bait sandwich test found that the most active against *M. domestica* were diethyl isocinchomeronate, di n-propyl isocinchomeronate, tert-dodecylmercaptopolyoxyethylene and butadiene-furfural copolymer. Not all of these were active against *S. calcitrans.* Di n-propyl isocinchomeronate and butadiene-furfural copolymer were effective to some extent. They noted that in most cases, the *S. calcitrans* were continuously landing and leaving, indicating that contact was generally necessary for effect.

Dethier (1956) reviewed the discovery of more effective compounds for human use during and after World War II. The list includes M-2020 a mixture of dimethyl phthalate, Rutgers 612 and dimethyl carbate designed for several hours protection when used on skin. M-1960 was a mixture of benzyl benzoate, n-butylacetanilide, 2-butyl-2-ethyl-1,3-propanediol and "Tween 80" and gave 7 days protection against a variety of pests when used on clothing. Undecylenic acid was also added to some mixtures of M-1960 to increase efficacy against *Aedes* spp.. Compounds active against ticks included n-butyl and n-propyl acetanilide, undecylenic acid and hexyl mandelate. He notes the lack of information on mode of action but cites what little is available. One study found that oxygen improved efficacy eg alcohols, ketones aldehydes and esters were more active than hydrocarbons. Vapour repellency was negatively correlated with boiling point but there were exceptions. Compounds with higher boiling points tended to last longer. He discusses the mode of action of repellents. Compounds could be irritating without necessarily forcing the insect to move away. DDT acted as a locomotor stimulant in mosquitoes. It resulted in the following sequence of behaviours. Restlessness, readjustment of position, change in normal light reaction and finally flight followed by death. However DDT also has the potential to act as an arrestant. He cites a study which found that susceptible *M. domestica* spend more time on DDT-treated surfaces than on untreated surfaces. Some compounds are attractive at low concentrations and repellent at high concentrations eg iso-valeraldehyde. By contrast to the relatively slow activity of DDT some compounds elicit immediate directed avoidance responses. Repellency did not correlate with toxicity for insecticides but rather appears to be a characteristic of some insecticides but not others. Each compound acts on 1 or more of various sites including antennal chemoreceptors, tarsal and oral chemoreceptors and a common chemical sense other than olfactory or gustatory, (eg responses have been obtained using the isolated nerve cord of cockroaches). They do not necessarily act upon the receptors involved in the behaviour targetted for reduction eg host location or feeding. He concluded his mode of action discussion thus: "It is clear that the nature of the response elicited by repellent compounds depends not only upon a variety of intrinsic biological factors such as age, state of nutrition etc, but upon the concentration of the repellent, which sensory system it is stimulating and whether and to what extent other sensory systems are being acted upon simultaneously by other stimuli."

Bruce and Decker (1957) field tested a number of compounds against *S. calcitrans* . Dipropyl isocinchomeronate and dibutyl succinate (Tabutrex) reduced *S. calcitrans* numbers for up to 6 days. The activity of dipropyl isocinchomeronate was prolonged slightly by the addition of the pyrethroid synergist n-octyl bicycloheptenedicarboximide (MGK264). They noted that the compounds generally acted immediately on contact but at higher temperatures appeared to have vapour activity and at lower rates appeared to have a delayed contact effect.

Neel (1957) in a field trial, found that horn fly numbers were not further reduced by the addition of repellents (butoxypolypropylene glycol and dibutyl succinate) to an insecticide (methoxychlor).

Granett (1960), in a laboratory cage/membrane test found that the following were repellent to *S. calcitrans* in decreasing order of efficacy; dipropyl isocinchomeronate, butoxypolypropylene glycol, n-butyl mesityl oxide oxalate and diethyl toluamide. Dibutyl succinate was ineffective. Field tests on cattle were conducted and found that the first 2 of these compounds reduced fly numbers for at least 28 hrs.

Yeoman and Warren (1968) note that most repellents were developed for use on people against mosquitoes and as such depend mainly on vapour activity on the olfactory chemoreceptors. However they contend that *S. calcitrans* is relatively insensitive to vapours (ie to true repellents) but more sensitive to contact via tarsal chemoreceptors (ie to locomotor stimulants). Consequently low volatility compounds should work better and should also have greater persistence. In a mouse assay they found that butyl 3-methylcinchoninate was the most effective of 10 compunds tested including dimethyl phthalate, 2-ethyl-1,3-hexanediol, butoxypolypropylene glycol, di-n-propyl isocinchomeronate, N,N-diethyl m-toluamide, 2,3,4,5-bis-(butylene)-tetrahydrofurfural, dibutyl succinate, 2-hydroxyethyl n-octyl sulphide and N-benzoyl piperidine. In a treated fabric/human hand assay protective periods of up to 34 days were recorded. However generally laboratory tests with aged bags tend to produce results far more flattering than skin tests or field tests on animals. They quote 4 stages in the response of *S. calcitrans* to a host, positive taxis and landing, extend the proboscis, probing and engorgement.
All stages were affected by butyl 3-methylcinchoninate but the later stages were more sensitive so that as repellent concentration decreased more stages were completed until finally normal feeding occurred. They speculate that the high lipid solubility of butyl 3-methyl cinchoninate enables it to penetrate the cuticular waxes of the tarsal chemoreceptors and interfere with the initiation of the feeding reflexes. They also think that it was active on the chemoreceptors on the labellum if contact was made. This group of chemicals is discussed in more detail in Lindberg and Ulff (1968). They examined the relationship between various chemical characteristics (namely molecular and electronic configuration, molecular polarizability, lipid solubility and volatility) and repellency They found that molecular configuration eg the length of particular chains on the molecule was important.

Bodenstein *et al.* (1970) tested a number of compounds against *Musca autumnalis* (Diptera: Muscidae) using the laboratory sandwich bait test. They found that the most active compounds tended to fall into the groups of sulfides (which have the disadvantage of unacceptable odour), sulfoxides (which are unstable) and disubstituted amides which were relatively stable.

Schreck *et al.* (1978) claim that N,N-diethyl m-toluamide (deet) is effective for only short periods against *S. calcitrans* regardless of formulation with 250mg deet/human forearm providing a mean protection period of only a few hours. They tested 75 compounds against *S. calcitrans* in a human forearm/outdoor cage system. 11 of the tested compounds were better than deet, of which the best were 1-(3-cyclohexene-1-yl carbonyl)piperidine and 1-((2-methylcyclohexyl)-carbonyl)-piperidine. They note that their successful compounds share an alicyclic carboxylic acid feature with HECC (2-hydroxy-ethyl cyclohexanecarboxylate) a good repellent for *Chrysops spp*.(Diptera:Tabanidae).

Bartlett (1985) found, in a laboratory olfactometer study with *S. calcitrans,* that pyrethrum was toxic but not repellent at lower rates and displayed a vapour repellency at higher doses.

Matzigkeit (1990) lists 10 plants (including neem) reputed to be useful as fly repellents but cites little supporting literature and does not name any of the active compounds in the plants. Schmutterer (1990) reviewed the potential of neem. Inhibition of settling, ovipostion and feeding were reviewed but except for the study of Rice *et al.* (1985) mentioned below, none of the studies mentioned involved veterinary insects. Feeding reductions could be produced when insects were topically treated or injected, indicating that antifeedant properties do not rely solely on gustatory chemoreceptors. The persistence of neem products is limited to 7 days or less in the field

Mathur *et al.* (1987) compared diethyl phenyl acetamide (DEPA) against dimethyl phthalate in 2 field tests. Overall efficacy (ie reduction of fly numbers on animals) against a variety of stock-associated muscids and tabanids was assessed. DEPA was superior to DMP in both tests. Duration of efficacy was greater when compounds were applied to hessian coats which were then placed on the animals rather than when the compounds were applied directly to the animals. However this latter difference could have been at least partly due to any of many differences between the tests. Parashar *et al.* (1993) found that DEPA was slightly superior to diethyl-m-toluamide and dimethyl phthalate in a laboratory/*S. calcitrans*/rabbit/feeding rate and time to first bite system.

Hogsette and Koehler (1994) found modest reductions in feeding by houseflies on sucrose solutions treated with >2.25% boric acid or >3% polybor (disodium octaborate tetrahydrate). Interestingly, the dose response was not linear. With boric acid feeding increased up to 2.25% and then decreased at higher rates.

Carroll (1994) tested 21 botanical compounds against the northern fowl mite*, Ornithonyssus sylviarum* (northern fowl mite, Acari: Macronyssidae) using an *in vitro* assay in which the compounds were mixed in the blood meal. The methodology and assessment were fairly rudimentary. Consequently its impossible to separate lethal effects from genuine feeding deterrence and impossible to be sure how the compounds were working. They may have been permeating the experimental vials as vapours or they may have been working by ingestion. At 1% and 0.1%,19 and 17 respectively of the compounds reduced feeding and/or caused mortality. The following compounds at 0.1% reduced feeding rates by 90% or more: pulegone, geraniol, terpin 4-ol, nerol, and nerolidol. 100% reduction was produced by citronellal and bay extract and the authors suggest that these may have potential as systemic feeding deterrents although how they could be administered is not covered. However in a rudimentary filter paper assay none of the compounds showed any significant repellency. Given that citronellal is a major component of citronella oil which is generally one of the poorer repellents on cattle, these results do not bode well for the other compounds.

In stark contrast to the limited persistence of repellents, van Gerwen and Barton Browne (1983) found that 1,1-bis-(4-ethoxyphenyl)-2-nitropropane (GH74) applied to sheep greatly reduced oviposition by *Lucilia cuprina* (Diptera: Calliphoridae) up to 28 weeks after application. This compound also has some insecticidal properties which would have also contributed to the effect.

In a laboratory assay using artificial oviposition pads, Rice *et al.* (1985) showed that neem oil, especially when converted to a concentrated azadirachtin preparation, reduced oviposition by *L. cuprina*.

Bentley and Day (1989) reviewed oviposition inhibitors for mosquitoes. Inorganic salts particularly NaCl can have an effect at various concentrations depending on the species. The mode of action appears to be contact. Fatty acids such as acetic, propionic, isobutyric, isovaleric, caproic, nonanoic and octadecenoic acids inhibit oviposition in some species. Given that these fatty acids are likely to be produced in the rumen and consequently be present in the

dung , it seems unlikely that they will inhibit buffalo fly. Perhaps they act as oviposition stimulants for buffalo fly? A variety of phytochemicals inhibit mosquito oviposition eg terpenes such as eucalyptol (1,8-cineole), citronellal and geraniol.

It is difficult to separate oviposition inhibitors from repellents. Since ovipostion involves location, landing etc and is akinetic ie incompatible with locomotion, true repellents and locomotor stimulants could also inhibit oviposition as effectively as true oviposition deterrents.

There are several commercial fly repellents currently registered for use on cattle in Queensland. These are all very similar. "Repel-X", "Ban-Fly" and "Supershield" all contain 6 active ingredients namely oil of citronella, diethyl toluamide, dipropyl isocinchomeronate, N-octyl bicycloheptenedicarboximide, piperonyl butoxide (synergist for pyrethrins) and pyrethrins. N-octyl bicycloheptenedicarboximide is a synergist for pyrethroids but it may have some independent repellent activity because it is included in the current formultion of "Rid" in the absence of any pyrethroid compounds. "Musca-Ban" is similar to the others except that it does not include diethyl toluamide. All are applied as aerosols at least once per day. "Supershield" claims exclude pyrethroid resistant flies so perhaps these products owe most of their activity to the pyrethrins.

Fly repellents registered for other animals are largely similar, consisting of various combinations of the previously listed compounds with 2 exceptions. "Wound Dressing Fly Repellent" contains 2,3,4,5-bis-(2-butylene)-tetrahydrofurfural and dibutyl phthalate, a repellent normally used on clothing. No indication is given of the effective duration. "Flee Fly Bite Healing Balm for Dogs" (there is a similar formulation for horses) contains cajeput oil, cedarwood oil and citronella oil. Application is recommended once or twice daily.

Screening for new repellents could not be recommended as part of the current project. Successful programs in the past have been large and/or long term, neither of which could be supported by the current project. Also due to the lack of information on mode of action for existing repellents, searching for new compounds remains a highly speculative task.

The single biggest problem with current repellents is their short persistence due to evaporation, degradation and absorption. A controlled release system such as an impregnated eartag would be useful. Alternatively a self-treatment system such as a backrubber could be considered.

Testing Methodology

Insecticidal effects are noted in some studies of some compounds and many studies, particularly field tests, can not distinguish between inhibitory action and insecticidal action eg for pyrethrum.

Shaw *et al.* (1943) made up experimental groups of cattle balanced for breed, daily milk production and fly susceptibility. As an additional measure, they repeated the tests while rotating the treatments so that all groups received all treatments. They used numbers of stable and horn flies per animal as the measure of efficacy. They needed to transform fly count data to normalise the distribution in order to otain dependable measurements of mean and variation. These techniques and other early field techniques with cattle are discussed in Fryer *et al.* (1943) and Fryer *et al.* (1948).

Howell and Fenton (1944) used Eltings formula to apply a fixed amount of repellent per unit of cattle surface area. They selected groups of cattle balanced for horn and stable fly burdens and used fly counts as the measure of efficacy with results expressed as a ratio of fly numbers on treated to untreated animals.

Travis *et al.* (1946) used a cage/forearm assay against *Anopheles quadrimaculatus, Aedes aegypti* (both Diptera: Culicidae) and *S. calcitrans*. Field tests were also conducted. Forearms were exposed for 5 minutes at 20 minute intervals until the first bite was received. Time to first bite was the measure of efficacy. They used paired tests where each forearm of a test subject received a different treatment and these were exposed alternately to the same insects. Biting rate of the insects was checked frequently throughout tests by exposure of an untreated forearm. The methods were designed to screen large numbers of compounds rather than make precise comparisons and consequently the results were highly variable.
Variability was greater for some species than others. Some of the variability was attributed to the insects and to perspiration by the subjects which was thought to reduce the efficacy of the repellents.

Granett *et al.* (1949) used the half cow method (treating only one side of an animal and comparing the fly numbers on the treated and untreated sides) to reduce the variability caused by variations in fly burdens between animals.

Goodhue and Stansbury (1953) describe a version of the widely used bait sandwich laboratory testing technique.

Dethier (1956) noted that most studies were designed for rapid assessment of activity with little attention paid to mode of action or the relationship between chemical structure and efficacy. Because of the large number of variables involved, he recommended that field efficacy should be assessed by field tests and mode of action testing should be done in the lab. He discussed the variables involved such as temperature, RH, light intensity, time of day, host attractiveness, etc but also less obvious factors eg biting rate, nutritional state, age. For biting rate, although effective duration decreased in non-linear fashion as control biting rate increased, ratios of effective durations between repellents remained relatively constant. Biting rate was affected by numerous factors eg time of day, species,mating rate. The variability of repellency due to sweating, accelerated breakdown and absorption when applied to skin is noted and used to explain the superior duration of repellents on fabric compared to repellents on skin. Extending the duration of repellent activity depends on reducing these sources of degradation. He also attributed some of the variation in results to poor choice of repellency criteria. For example time to first bite actually tests 2 characteristics, namely inherent efficacy and duration of activity. He supported the findings of Fryer *et al.* (1948) who reviewed the techniques for cattle and concluded that the whole cow method was superior to the half cow if applied properly ie by obtaining a measure of each animal*s attractiveness and using these to set up balanced groups in a latin square design with periodic fly counts. He proposed an approach to repellent tests similar to the dose/mortality tests with insecticides.

He advocated the use of controlled artificial attractants to avoid the problems of host variability, eg indole plugs and simulated wounds for blowflies. He suggested testing locomotor stimulants on inanimate substrates and testing vapour repellents against a controllable attractant such as heat, light or moisture.

Bovingdon (1958) developed a technique for testing potential locomotor stimulants using an inert substrate, namely 6 glass plates (3 treated, 3 untreated) in a relatively complex cage. Insects on the treated and untreated surfaces were counted every minute for 40 minutes. This method used no conflicting attraction such as a host and so was not subject to the variability which comes with the use of an attractant. The method was used as an initial screen for activity.

Roberts *et al.* (1960) describe a method for testing small quantities of compounds against *S. calcitrans* by attaching small cages of flies to the flanks of cattle for 20 minutes and then assessing the proportion of flies which had fed.

Granett (1960) describes a simple laboratory cage technique for testing compounds against *S. calcitrans* by applying the test compounds to an animal membrane ("Silverlight") over a reservoir of blood (other fluids eg sucrose solutions were also tried successfully) thereby producing a standardised attractant. He incorporated a fluorescent dye at 0.5 % in the blood so that partially fed flies could be detected easily without dissection. Repellency was estimated from a feeding rate and corrected repellency was calculated using Abbott's formula and the untreated repellency rate when the untreated feeding rate was not 100%.
The RC50 (concentration which repelled 50% of flies) was estimated from a log/probit plot of the results for 4 concentrations. He found that the membrane technique gave a better correlation with field results than an assay using treated fabric.

Shephard (1960) contains a number of chapters of interest to repellent testing including a chapter on each of repellent and attractant testing. The chapter on repellents provides a broad review (but little critical appraisal) of methods eg paired tests of compounds and early use of olfactometers.

Yeoman and Warren (1968) describe methodology for testing locomotor stimulants against *S. calcitrans* by 2 methods namely a screened mouse system and a fabric/human hand system. They also used the bait sandwich technique of Goodhue and Stansbury (1953) for testing vapour repellents and locomotor stimulants against *Lucilia sericata*. They emphasised the need to produce flies of standardised aggressiveness and developed techniques for this which defined larval rearing, adult age, adult feeding history, temperature, light and humidity. However they did not standardise the sex ratio of the test flies. In the mouse assay they used percentage fed as the measure of efficacy. The *L. sericata* assay used loss of weight of a bait due to feeding as the measure of efficacy.

Bodenstein *et al.* (1970) adapted the bait sandwich test for *M. autumnalis* by using cattle dung as the bait instead of molasses.

Schreck *et al.* (1978) used a large cage in which 4 treatments could be tested simultaneously on human forearms against *S. calcitrans*. Time to first confirmed bite ie 2 bites on successive exposures (exposures were at 30 minute intervals). Tests were conducted over a number of occasions with deet as the standard in each test. Consequently in addition to times, results were expressed as ratio of activity to that of deet, a way of allowing for the differences between different tests eg lack of standardisation of insects and different test subjects.

Bartlett (1985) developed an olfactometer for testing vapour repellency against *S. calcitrans.* Numbers of flies on a target (metal gauze over a pad of clipped cattle hair) were counted every 15 seconds for 2 minutes. A blackened light bulb was used to heat the target to 37^0C. A mixture of humidified air, carbon dioxide and "cattle odour" (produced by placing freshly clipped cattle hair in the flask of water used to humidify the air) were blown through the target. Compounds were supplied on gauze behind the target so that they were introduced into the airstream. Second and subsequent 2 minute exposures were found to be more reproducible than first exposures of each batch of flies so the results from the first exposure were routinely discarded. The responses of 3 and 4 day old flies were more consistent than 2 day old flies.

Carroll (1994) described a method of making "Parafilm" membranes for feeding blood to *Ornithonyssus sylviarum* (northern fowl mite, Acari: Macronyssidae) which may be of some use for buffalo fly. Buffalo fly are generally reluctant to feed through membranes but this method must produce a very thin membrane to be suitable for mites. Percentage fed in 24hrs was used as the measure of efficacy.

POPULATION DYNAMICS OF THE BUFFALO FLY *H. IRRITANS EXIGUA*

A population model of the buffalo fly would be valuable for predicting the effects of traps and lures on fly numbers. Models and equations are available for the closely related horn fly, *H. i. irritans* (Berry *et al.* 1973; Haufe 1985; Kunz and Cunningham 1977; Lysyk 1992; Miller 1977; Palmer and Bay 1984; Palmer *et al.* 1981; Thomas *et al.* 1974) and they have been used for various purposes, including prediction of horn fly numbers (Marley *et al.* 1993).

There is no population model for the buffalo fly but there is extensive field and laboratory data on several parameters that have an important influence on fly numbers (Cook and Spain 1980, 1981, 1982; Doube 1988; Doube *et al.* 1988; MacQueen and Doube 1988; MacQueen *et al.* 1986); Sutherst, Annual Reports, CSIRO Division of Entomology and Reports to the MRC). At the moment, it is reasonable to adopt the population model for the horn fly and fit the available buffalo fly parameters into this model.
Lysyk (1992) and personal communication) studied the development of the horn fly using the equations and models of Palmer *et al.* (1981), Miller (1977), Thomas *et al.* (1974) and fitted the data into a simulation model for control of flies (Weidhaas 1986). The effect of changes in parameters such as the mortality rate, development rate etc, on the fly population could be determined. In the Weidhaas model, seven key population parameters are used to predict the reproductive rate R of the fly.

Adult survival per day	=	SA	
Preoviposition period days		=	D
Survival of larvae in dung/day	=	Si	
Development time of larvae		=	i
Average number of eggs per oviposition cycle	=	M	
Days between cycles of egg laying		=	C
Sex ratio		=	SR

$$\text{Reproductive rate, } R = \frac{Si^{i} \times M \times SA^{D} \times SR}{1 - SA^{C}} = \frac{\text{Number of flies}}{\text{Number of flies in previous generation}}$$

For the buffalo fly the information available, or the best estimates are as follows:

SA = 0.83. This is based on survival of flies on cattle in pen conditions at 26-300C and humidity fluctuating considerably but averaging 50-60% RH. Under these conditions, survival of flies following a single infestation is approximately 40% by day 5, or a survival per day of 0.83. There is evidence that this value can be higher at higher humidity.

D = 3. Preoviposition period is three days under the conditions given above or at 300C for 16 hours and 240C for 8 hours each day which more closely simulates field conditions.

Si = 0.87. This is based on information from Doube *et al*. (1988) on survival of buffalo fly larvae in dung at Rockhampton. Approximately 25% of larvae survived when other dung fauna were competing in the dung. The development time from egg laying to adult emergence (i) is taken as 10 days (see below) therefore survival per day Si = 0.87.

i = 7 days. Development time of the larvae at 28-300C is approximately seven days.

M = 25. The number of eggs in each oviposition cycle is approximately 25.

C = 1. Once oviposition has started there is approximately one day between cycles.

SR = 0.5. Equal sex ratio has been established.

In summary, for the buffalo fly, the values are:

SA	=	0.83
SA^D	=	0.57 = survival of females to day 3, the start of egg laying
D	=	3
Si	=	0.87
i	=	7
si^i	=	0.38 = survival of larvae to seven days
M	=	25
SR	=	0.5
C	=	1
SA^C	=	0.83 = survival of females between egg laying cycles

$$\text{Therefore } R = \frac{Si^i \times M \times SA^D \times SR}{1 - SA^C} = \frac{0.38 \times 25 \times 0.57 \times 0.5}{1 - 0.83} = 16$$

This indicates a 16 fold increase in population per generation. This is higher than the maximum 3 fold increase in the field (Doube 1988) and probably reflects the more favourable conditions for flies in some of the laboratory situations. The equation is nevertheless useful for determining the potential of non-insecticidal control strategies for reducing fly reproductive rate.

If, for example, adult fly populations were reduced by 25% per day by these control measures (survival at day 5 would be 10% not 40%) then $SA^D = 0.185$ $SA^C = 0.58$, and R = 2.0, a large reduction in reproductive rate of the fly.

Under these latter conditions there may be zero growth or a decline in fly populations. The conditions in the field do not support as high a reproductive rate (Doube 1988) as determined, mainly on the basis of laboratory data therefore a 25% reduction in the adult population could lead to zero population growth or reducing fly populations with each generation.
In fact the horn fly field simulation model of Palmer and Bay (1984), partly funded by MRC, showed that a 20% increase in mortality per day would limit flies in the field to very low numbers. Unfortunately the parameters used in this model were not stated.

To improve the predictive powers of the equation, information is needed on several parameters in the fly's development in the field. The more critical deficiencies in our current knowledge are as follows:

. adult survival per day (SA),
. larval survival in dung under a variety of conditions.

The other parameters such as days between oviposition cycles (C), and average number of eggs laid on dung per oviposition cycle (M) are either reasonably well known or can be estimated with reasonable certainty.

Average adult survival per day would have to be determined on a range of animals with different "tolerance" to the fly because there are some animals on which flies do not survive long in the sense that they move to another, more favourable host for reasons not yet understood. Survival on "favoured" animals may be longer but this could be offset by density dependent mortality factors.

Considerable data has been accumulated on the adult survival to day 5 in the laboratory from which an approximate percent survival per day can be calculated.
It would be worth checking however, on the mortality/day curve as the mortality might increase sharply at the time of first oviposition at day 3-4, thus giving a higher number of ovipositing females than expected from averaging the data obtained in day 5 (i.e. incorporate Gomperts function).

The average number of eggs laid per oviposition cycle in the field would require some collection of data from the field. The figure used here (M = 25) is from laboratory data only.

Days between oviposition cycles (C = 1) appears to be a reasonable estimate but could be checked by artificially releasing a synchronous fly population on to "isolated" cattle in the field.

The proportion of emerging flies that find a host is unknown. This is presumed to be high because of the proximity of cattle to dung but confirmation of this in the field would be useful.

CONCLUSIONS

Field and laboratory experiments have indicated that horn (and probably buffalo) flies orient to their hosts from a distance. Host odour appears to be of great importance in this process, as well as in the location of host dung for oviposition. However, the results of some experiments suggest that visual stimuli from hosts are also employed in orientation. Once a host is located, arrestants such as sebum might play a role in maintaining host contact, and in determining host preferences.

Conventional use of existing repellents would appear to offer little potential for improved buffalo fly control. Most of the repellents have been available for many years without gaining widespread use. However this could be attributed to the past success of the synthetic pyrethroids. Repellents may have a role in combination with attractants.

The available population models for buffalo fly would be valuable in predicting the effects of behaviour-modifying systems such as traps and lures, however the current deficiencies in parameter values would have to be addressed.

REFERENCES

Adams TS and Holt GG (1987) Effect of pheromone components when applied to different models on male sexual behaviour in the housefly *Musca domestica*. *J. Insect Physiol.* **33**: 9-18.

Agee HR and Patterson RS (1983) Spectral sensitivity of stable, face, and horn flies and behavioral responses of stable flies to visual traps (Diptera: Muscidae). *Environ. Entomol.* **12**: 1823-1828.

Allan SA, Day JF and Edman JD (1987) Visual ecology of biting flies. *Annu. Rev. Entomol.* **32**: 297-316.

Arundel JH and Sutherland AK (1988) Animal Health in Australia Vol 10 Ectoparasitic Diseases of Sheep, Cattle, Goats and Horses. Department of Primary Industries and Energy. Commonwealth of Australia, Canberra.

Bartlett C (1985) An olfactometer for measuring the repelllent effect of chemicals on the stable fly *Stomoxys calcitrans* (L.). *Pestic. Sci.* **16**: 479-487.

Barton Browne L (1977) Host-related responses and their suppression: Some behavioural considerations. *In:* Chemical Control of Insect Behaviour Theory and Application, Shorey HH and McKelvey JJ (eds.), John Wiley and Sons, New York.

Bentley MD and Day JF (1989) Chemical ecology and behavioural aspects of mosquito oviposition. *Annu. Rev. Entomol.* **34**: 401-421.

Berry IL, Miller JA, Schmidt CD and Eschle JL (1973) Simulation of horn fly populations. *Trans. ASEA (Am. Soc. Agric. Eng.)* **16**: 482-485.

Bodenstein OF, Fales JH and Walker RL (1970) Laboratory evaluations of materials as repellents for the face fly, 1962-69. *J. Econ. Entomol.* **63**: 1753-1755.

Bolton HT, Butler JF and Carlson DA (1980) A mating stimulant pheromone of the horn fly, *Haematobia irritans* (L): Demonstration of biological activity in separated cuticular components. *J. Chem. Ecol.* **6**: 951-964.

Bovingdon HHS (1958) An apparatus for screening compounds for repellency to flies and mosquitoes. *Ann. appl. Biol.* **46**: 47-54.

Bruce WG (1964) The history and biology of the horn fly, *Haematobia irritans* (Linnaeus): with comments on control. N.C. Agric. Exp. Stn. Tech. Bull. 157, 32 pp.

Bruce WN and Decker GC (1957) Experiments with several repellent formulations applied to cattle for the control of stable flies. *J. Econ. Entomol.* **50**: 709-713.

Byford RL, Broce AB, Lockwood JA, Smith SM, Morrison DG and Bagley CP (1987) Horn fly (Diptera: Muscidae) dispersal among cattle herds. *J. Econ. Entomol.* **80**: 421-426.

Byford RL, Craig ME and Crosby BL (1992) A review of ectoparasites and their effect on cattle production. *J. Animal Sci.* **70**: 597-602.

Carde RT and Baker TC (1984) Sexual communication with pheromones. *In:* Chemical ecology of insects, Bell WJ and Carde RT (eds.), Chapman and Hall, London and New York.

Carroll JF (1994) Feeding deterrence of northern fowl mites (Acari: Macronyssidae) by some naturally occurring plant substances. *Pestic. Sci.* **41**: 203-207.

Chamberlain WF (1981) Dispersal of horn flies: I. Effect of host proximity. *Southwest. Entomol.* **6**: 316-325.

Chamberlain WF (1982) Dispersal of horn flies: II. Wild flies. *Southwest. Entomol.* **7**: 230-234.

Chamberlain WF (1984) Dispersal of horn flies: III. Effect of environmental factors. *Southwest. Entomol.* **9**: 73-78.

Chamberlain WF (1985) Dispersal of horn flies: IV. Random non-attractive flight. *Southwest. Entomol.* **10**: 126-129.

Chamberlain WF (1987) Responses of wild and laboratory-reared horn flies (Diptera: Muscidae) to heat, light, and airflow. *Environ. Entomol.* **16**: 1107-1113.

Christensen CM and Dobson RC (1979) Effects of teststerone propionate on the sebaceous glands and subsequent attractiveness of Angus bulls and steers to horn flies, *Haematobia irritans* (Diptera: Muscidae). *J. Kansas Entomol. Soc.* **52**: 386-391.

Colvin J and Gibson G (1992) Host-seeking behaviour and management of tsetse. *Annu. Rev. Entomol.* **37**: 21-40.

Cook IM (1980) Studies on the biology of immature stages of the buffalo fly *Haematobia irritans exigua* de Meijere (Diptera:Muscidae). Ph.D. thesis. Department of Zoology, James Cook University of North Queensland.

Cook IM and Spain AV (1980) The effect of temperature and moisture levels of the larval breeding medium on puparial forms in the buffalo fly, *Haematobia irritans exigua* (Diptera: Muscidae). *Aust. J. Zool.* **28**: 547-552.

Cook IM and Spain AV (1981) Rates of development of immature stages of the buffalo fly, *Haematobia irritans exigua* de Meijere (Diptera: Muscidae) in relation to temperature. *Aust. J. Zool.* **29**: 7-14.

Cook IM and Spain AV (1982) The effects of temperature and moisture on the survival of the immature stages of the buffalo fly *Haematobia irritans exigua* de Meijere (Diptera: Muscidae). *Aust. J. Zool.* **30**: 923-930.

Dalton LW, Kinzer HG, Reeves JM and Atmar JW (1978) Host location in the horn fly: roles of heat, carbon dioxide, water vapor, and cow-produced odors in attraction. *Southwest. Entomol.* **3**: 147-153.

Dethier VG (1956) Repellents. *Annu. Rev. Entomol.* **1**: 181-202.

Dethier VG, Barton Browne L and Smith CN (1960) The designation of chemicals in terms of the responses they elicit from insects. *J. Econ. Entomol.* **53**: 134-136.

Dicke M, Sabelis MW, Takabayashi J, Bruin J and Posthomus MA (1990) Plant strategies of manipulating predator-prey interactions through allelochemicals: Prospects for application in pest control. *J. Chem. Ecol.* **16**: 3091-3118.

Dickens JC and Payne TL (1981) Chemical messengers and insect behaviour. *In:* CRC Handbook of Natural Pesticides: Methods Volume 1 Theory, Practice and Detection, Mandava NB (ed.), CRC Press Inc., Boca Raton, Florida.

Dobson RC, Kutz FW and Sanders DP (1970) Attraction of horn flies to testosterone-treated steers. *J. Econ. Entomol.* **63**: 323-325.

Donald AD (1994) Parasites, animal production and sustainable development. *Vet. Parasitol.* **54**: 27-47.

Doube BM (1984) The effect of breed and coat colour on numbers of the buffalo fly *Haematobia irritans exigua* De Meijere, (Diptera: Muscidae) on bovine hosts. *J. Aust. Entomol. Soc.* **23**: 39-45.

Doube BM (1988) Biological control of buffalo fly in Australia: the potential of southern Africa dung fauna. *Misc. Publ. Entomol. Soc. Amer.* **61**: 16-34.

Doube BM and MacQueen A (1978) Host location by buffalo flies. C.S.I.R.O. Division of Entomology Annual Report 1977-78, p 52.

Doube BM, MacQueen A and Fay HAC (1988) Effects of dung fauna on survival and size of buffalo flies (*Haematobia* spp.) breeding in the field in South Africa and Australia. *J. Appl. Ecology* **25**: 523-536.

Drummond RO, Lambert G, Smalley HE and Terrill CE (1981) Estimated losses of livestock to pests. *In:* CRC Handbook of Pest Management in Agriculture, vol. 1, Pimmentel D (ed.), CRC Press, Boca Raton Florida.

Drummond RO, George JE and Kunz SE (1986) Control of Arthropod Pests of Livestock: A Review of Technology. CRC Press Inc., Boca Raton Florida.

Eddy GW, Roth AR and Plapp FW (1962) Studies on the flight habits of some marked insects. *J. Econ. Entomol.* **55**: 603-607.

Ernst CM and Krafsur ES (1984) Horn fly (Diptera: muscidae): sampling considerations of host breed and color. *Environ. Entomol.* **13**: 892-894.

Ferrar P (1969) Colonisation of an island by the buffalo fly, *Haematobia exigua*. *Aust. Vet. J.* **45**: 290-292.

Ferrar P (1979) The immature stages of dung-breeding muscoid flies in Australia, with notes on the species, and keys to larvae and puparia. *Aust. J. Zool.* **73**: 1-106.

Franks RE, Burns EC and England NC (1964) Color preference of the horn fly, *Haematobia irritans* on beef cattle. *J. Econ. Entomol.* **57**: 371-372.

Fryer HC, Shaw AO, Atkeson FW, Smith RC and Borgmann AR (1943) Techniques for conducting fly-repellency tests on cattle. *J. Econ. Entomol.* **36**: 33.

Fryer HC, Atkeson FW and Smith RC (1948) Comparison of methods for testing repellent-type fly sprays. *J. Econ. Entomol.* **41**: 80-88.

Galun R (1976) The physiology of hematophagous insect/animal host relationships. *Proc. XV Int. Cong. Ent.*: 257-265.

Gamal-Eddin FM (1972) Studies on the behavior of the blood-sucking fly *Siphona irritans*, Lin. toward some environmental factors, to pave the way for proper control (Diptera: Muscidae). *J. Egypt. Vet. Med. Assoc.* **32**: 229-246.

Goodhue LD and Stansbury RE (1953) Some new fly repellents from laboratory screening tests. *J. Econ. Entomol.* **46**: 982-985.

Goodwin WJ, Sloan MJ and Schwardt HH (1952) Repellency test for horse flies and horn flies in New York state. *J. Econ. Entomol.* **45**: 121-122.

Goodwin WJ, Moore S and Schwardt HH (1954) Horse fly and horn fly repellent tests on cattle in New York. *J. Econ. Entomol.* **46**: 1088-1089.

Granett P (1960) Use of an animal membrane in the evaluation of chemical repellents against the stable fly. *J. Econ. Entomol.* **53**: 432-435.

Granett P, Haynes HL, Connola DP, Bowery TG and Barber GW (1949) Two butoxypolypropylene glycol compounds as fly repellents for livestock. *J. Econ. Entomol.* **42**: 281-286.

Guillot FS, Miller JA and Kunz SE (1988) The physiological age of female horn flies (Diptera: Muscidae) emigrating form a natural population. *J. Econ. Entomol.* **81**: 555-561.

Hammer O (1942) Biological and ecological investigations on flies associated with pasturing cattle and their excrement. *Vidensk. Medd. Dansk. naturf. For. Kobenhavn* **105**: 141-393.

Handschin E (1932) A preliminary report on investigations on the buffalo fly (*Lyperosia exigua* DeMeij.) and its parasites in Java and Northern Australia. Counc. Sci. Ind. Res. Aust. Pamp. 31, pp. 1-24.

Haniotakis G, Kozyrakis M, Fitsakis T and Antonidaki A (1991) An effective mass trapping method for the control of *Dacus oleae* (Diptera: Tephritidae). *J. Econ. Entomol.* **84**: 564-569.

Hargett LT and Goulding RL (1962) Studies on the behavior of the horn fly, *Haematobia irritans* (Linn.). Oreg. State Univ. Agric. Exp. Stn. Tech. Bull. 61, pp. 1-27.

Harris RL, Frazar ED and Schmidt CD (1968) Notes on the mating habits of the horn fly. *J. Econ. Entomol.* **61**: 1639-1640.

Harris RL, Miller JA and Frazar ED (1974) Horn flies and stable flies: feeding activity. *Ann. Entomol. Soc. Am.* **67**: 891-894.

Haufe WO (1985) A modelling system for horn flies on cattle. *In:* Proceedings of a research workshop on modelling of arthropods affecting animals and man, Purdue University, West Lafayette, Science and Education Administration Agriculture Research (ed.), United States Department of Agriculture, Washington.

Hoelscher CE, Combs RL and Brazzel JR (1968) Horn fly dispersal. *J. Econ. Entomol.* **61**: 370-373.

Hogsette JA and Koehler PG (1994) Repellency of aqueous solutions of boric acid and polybor 3 to house flies. *J. Econ. Entomol.* **87**: 1033-1037.

Holloway MTP and Phelps RJ (1991) The response of *Stomoxys* spp. (Diptera: Muscidae) to traps and artificial host odours in the field. *Bull. Entomol. Res.* **81**: 51-55.

Howell DE and Fenton FA (1944) The repellency of a pyrethrum-thiocyanate oil spray to flies attacking cattle. *J. Econ. Entomol.* **37**: 677-680.

Hummel HE and Miller TA, eds. (1987) Techniques in Pheromone Research. Springer-Verlag, New York.

Kennedy JS (1977) Behaviourally discriminating assays of attractants and repellents. *In:* Chemical control of insect behaviour - Theory and application, Shorey HH and McKelvey JJ (eds.), John Wiley & Sons, New York.

Kennedy JS (1978) The concepts of olfactory "arrestment" and "attraction". *Physiol. Entomol.* **3**: 91-98.

Kettle DS (1984) Medical and Veterinary Entomology. Croom Helm, London and Sydney.

Kinzer HG and Reeves JM (1974) Dispersal and host location of the horn fly. *Environ. Entomol.* **3**: 107-111.

Kinzer HG, Burns IW and Auclair JL (1970) An olfactometer for measuring host attraction in the horn fly. *J. Econ. Entomol.* **63**: 1335-1337.

Kinzer HG, Reeves JM and Atmar JW (1978) Host location by the horn fly: field evaluation of an artificial device for measuring attraction to various stimuli. *Environ. Entomol.* **7**: 375-378.

Krijgsman BJ and Windred GL (1930) Reizphysiologische Untersuchungen an blutsaugenden Arthropoden im Zusammenhang mit ihrer Nahrungswahl. *Z. Vgl. Physiol.* **13**: 61-73.

Krijgsman BJ and Windred GL (1933) Investigations on the buffalo fly, "Lyperosia exigua" De Meij. *C.S.I.R. Pamphlet* **43**: 1-40.

Kunz SE (1986) Integrated pest management and Dipteran pests in the new world. *In:* Parasitology - Quo Vadit, pp. 659-664, Howell MJ (ed.), Australian Academy of Science, Canberra.

Kunz SE and Cunningham JR (1977) A population prediction equation with notes on the biology of the horn fly in Texas. *Southwest. Entomol.* **2**: 79-87.

Kunz SE, Blume RR, Hogan BF and Matter JJ (1970) Biological and ecological investigations of horn flies in central Texas: Influence of time of manure deposition on oviposition. *J. Econ. Entomol.* **63**: 920-923.

Kunz SE, Kinzer HG and Miller JA (1983) Areawide cattle treatments on populations of horn flies (Diptera: Mucidae). *J. Econ. Entomol.* **76**: 525-528.

Lehmann T (1993) Ectoparasites: Direct impact on host fitness. *Parasitol. Today* **9**: 8-12.

Letts GA (1962) Early livestock introductions to the "top-end" of the northern territory. *Aust. Vet. J.* **38**: 282-287.

Lewis T, ed. (1984) Insect Communication. Academic Press, London.

Lindberg UH and Ulff B (1968) Cinchoninic and 3-alkylcinchoninic acid alkyl esters. A new class of insect repellents. *Acta. Pharm. Suecica.* **5**: 441-448.

Lysyk TJ (1992) Simulating development of immature horn flies, *Haematobia irritans irritans* (L.) (Diptera: Muscidae), in Alberta. *Can. Entomol.* **124**: 841-851.

Mackley JW, Carlson DA and Butler JF (1981) Identification of the cuticular hydrocarbons of the horn fly and assays for attraction. *J. Chem. Ecol.* **7**: 669-683.

MacQueen A and Beirne BP (1975) Influence of other insects on production of horn fly, *Haematobia irritans* (Diptera: Muscidae) from cattle dung in south-central British Columbia. *Can. Entomol.* **107**: 1255-1264.

MacQueen A and Doube BM (1988) Emergence, host finding and longevity of adult *Haematobia irritans exigua* de Meijere (Diptera: Muscidae). *J. Aust. Entomol. Soc.* **27**: 167-174.

MacQueen A, Doube BM and Skilling L (1980) Natural oviposition by buffalo fly females. C.S.I.R.O. Division of Entomology Annual Report 1979-80, p75.

MacQueen A, Wallace MMH and Doube BM (1986) Seasonal changes in favourability of cattle dung in central Queensland for three species of dung-breeding insects. *J. Aust. Entomol. Soc.* **25**: 23-29.

Marley SE, Lockwood JA, Byford RL and Luther DG (1991) Temporal, climatic and physiological mediation of dispersal in the horn fly, *Haematobia irritans* (L.) (Diptera: Muscidae). *Environ. Entomol.* **20**: 1612-1618.

Marley SE, Hall RD and Corwin RM (1993) Ivermectin Cattle Pour-on - Duration of a Single Late Spring Treatment Against Horn Flies, *Haematobia irritans* (L) (Diptera, Muscidae) in Missouri, USA. *Vet. Parasitol.* **51**: 167-172.

Mathur KK, Parashar BD, Gupta GP, Pani BP and Rao KM (1987) Repellency of N,N-diethylphenyl acetamide and dimethyl phthalate against muscids and tabanidae attacking cattle and equines. *Indian. Vet. J.* **64**: 742-746.

Matzigkeit U (1990) Natural veterinary medicine. Ectoparasites in the tropics and subtropics. Verlag Josef Margraf, Weikersheim.

McLintock J and Depner KR (1954) A review of the life-history and habits of the horn fly,*Siphona irritans* (L.) (Diptera:Muscidae). *Can. Entomol.* **86**: 20-38.

Metcalf RL, Ferguson JE, Lampman R and Andersen JF (1987) Dry cucurbitacin-containing baits for controlling diabroticite beetles (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* **80**: 870-875.

Miller JA (1977) A computer simulation of populations of the horn fly, *Haematobia irritans* (L.). Ph.D. thesis. Texas A & M University, College Station, Texas.

Moore J (1993) Parasites and the behaviour of biting flies. *J. Parasitol.* **79**: 1-16.

Morgan NO (1966) Ultra-violet radiation as an attractant for adult horn flies. *J. Econ. Entomol.* **59**: 1416-1419.

Muirhead-Thomson RC (1991) Trap responses of flying insects. The influence of trap design on capture efficiency. Academic Press Limited, London.

Mustaparta H (1984) Olfaction. *In:* Chemical Ecology of Insects, pp. 37-70, Bell WJ and Carde RT (eds.), Chapman Hall Ltd, London.

Neel WW (1957) The effectiveness of new repellents for the control of horn flies on dairy cattle. *J. Econ. Entomol.* **50**: 502-503.

Norval RAI, Peter T, Soneshine DE and Burridge MJ (1992) Responses of the ticks *Amblyomma hebraeum* and *A.variegatum* to known or potential components of the aggregation-attachment pheromone. 111. Aggregation. *Exp. Appl. Acarol.* **16**: 237-245.

Palmer WA and Bay DE (1984) A computer simulation for describing the abundance of horn fly under various ecological and pest management regimes. *Protection Ecology* **7**: 27-35.

Palmer WA, Bay DE and Sharpe PJH (1981) Influence of temperature on the development and survival of the immature stages of the horn fly, *Haematobia irritans irritans* (L.). *Protection Ecology* **3**: 299-309.

Parashar BD, Gupta GP, Sikder GN and Rao KM (1993) Diethylphenylacetamide: a new insect repellent against stable fly, *Stomoxys calcitrans*. *Med. Vet. Entomol.* **7**: 259-262.

Plimmer JR, Inscoe MN and McGovern TP (1982) Insect Attractants. *Ann. Rev. Pharmacol. Toxicol.* **22**: 297-320.

Pont AC (1973) Studies on Australian Muscidae (Diptera). IV. A revision of the subfamilies Muscinae and Stomoxyinae. *Aust. J. Zool.* **21**: 129-296.

Rice MJ, Sexton S and Esmail AM (1985) Antifeedant phytochemical blocks oviposition by sheep blowfly. *J. Aust. Entomol. Soc.* **24**: 16.

Ridgway RL, Silverstein RM and Inscoe MN, eds. (1990) Behaviour-modifying chemicals for insect management. Marcel Dekker, New York.

Roberts RH, Jones CM and Gless EE (1960) Methods for the evaluation of stable fly toxicants and repellents. *J. Econ. Entomol.* **53**: 301-303.

Schmutterer H (1990) Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. *Annu. Rev. Entomol.* **35**: 271-297.

Schreck CE, McGovern TP and Smith N (1978) Repellency of selected esters and amides of four alicyclic acids against the stable fly, *Stomoxys calcitrans* (Diptera Muscidae). *J. Med. Entomol.* **14**: 589-591.

Schreiber ET and Campbell JB (1986) Horn fly (Diptera: Muscidae) distribution on cattle as influenced by host color and time of day. *Environ. Entomol.* **15**: 1307-1309.

Scott GC and Greenway AR (1984) Effects of activated charcoal on attack by larvae of the wheat bulb fly, *Delia coarctata*. *Ann. appl. Biol.* **104**: 27-30.

Seddon HR (1967) Diseases of domestic animals in Australia. Part 2: Arthropod infestations (flies, lice and fleas), Revised by H.E. Albiston 2nd edn. Department of Health, Commonwealth of Australia, Canberra.

Sharp JL (1987) Laboratory and field experiments to improve enxymatic casein hydrolysata as an arrestant and attractant for Caribbean fruit fly, *Anastrepha suspensa* (Diptera: Tephritidae). *Florida Entomologist* **70**: 225-233.

Shaw AO, Smith RC, Atkeson FW, Fryer HC, Borgmann AR and Holmes FJ (1943) Tests of fly repellents of known ingredients and of selected commercial sprays on dairy cattle. *J. Econ. Entomol.* **36**: 23-32.

Shephard HH, ed. (1960) Methods of testing chemicals on insects, vol. II. Burgess Publishing Company, Minneapolis.

Sheppard DC (1994) Dispersal of Wild-Captured, Marked Horn Flies (Diptera, Muscidae). *Environ. Entomol.* **23**: 29-34.

Simeone RM and Siverstein JB, eds. (1990) Semiochemicals and pest control - prospects for new applications, Journal of Chemical Ecology edn., vol. 16. Plenum Press, New York.

Skidmore P (1985) The biology of the muscidae of the world. Dr W Junk, Dordrecht.

Stadler E (1984) Contact chemoreception. *In:* Chemical Ecology of Insects, pp. 3-35, Bell WJ and Carde RT (eds.), Chapman and Hall, London.

Starnes EB and Granett P (1953) A laboratory method for testing repellents against biting flies. *J. Econ. Entomol.* **46**: 420-423.

Steelman CD, Brown AH, Gbur EE and Tolley G (1991) Interactive Response of the Horn Fly (Diptera, Muscidae) and Selected Breeds of Beef Cattle. *J. Econ. Entomol.* **84**: 1275-1282.

Steelman CD, Gbur EE, Tolley G and Brown AH (1993) Individual variation within breeds of beef cattle in resistance to horn fly (Diptera: Muscidae). *J. Med. Entomol.* **30**: 414-420.

Sutcliffe JF (1987) Distance orientation of biting flies to their hosts. *Insect. Sci. Applic.* **8**: 611-616.

Takabayashi J and Takahashi S (1988) Effect of kairomone on the parasitization rates of *Apanteles kariyai* to *Pseudaletia separata*. *J. Pest. Sci.* **13**: 283-286.

Thomas GD, Berry IL and Morgan CE (1974) Field developmental time of non-diapausing horn flies in Missouri. *Environ. Entomol.* **3**: 151-155.

Tillyard RJ (1931) The buffalo-fly in Australia. *J. Coun. Sci. Industr. Res. Aust.* **4**: 234-243.

Travis BV, Morton FA and Cochran JH (1946) Insect repellents used as skin treatments by the armed forces. *J. Econ. Entomol.* **39**: 627-630.

Tugwell NP, Burns EC and Witherspoon B (1966) Notes on the flight behavior of the horn fly, *Haematobia irritans* (L.) (Diptera: Muscidae). *J. Kansas Entomol. Soc.* **39**: 561-565.

Tugwell NP, Burns EC and Turner JW (1969) Brahman breeding as a factor affecting the attractiveness or repellency of cattle to the horn fly. *J. Econ. Entomol.* **62**: 56-57.

Tumlinson JH, Turlings TCJ and Lewis WJ (1992) The semiochemical complexes that mediate insect parasitoid foraging. *Agric. Zool. Rev.* **5**: 221-252.

Vale GA (1993) Development of Baits for Tsetse Flies (Diptera, Glossinidae) in Zimbabwe. *J. Med. Entomol.* **30**: 831-842.

van Gerwen ACM and Barton Browne L (1983) Oviposition deterrency of 1,1-bis-(4-ethoxyphenyl)-2-nitropropane against the Australian sheep blowfly, *Lucilia cuprina*, in relation to concentration and method of application. *Aust. Vet. J.* **60**: 248-249.

Vite JP and Baader F (1990) Present and future use of semiochemicals in pest management of bark beetles. *J. Chem. Ecol.* **16**: 3031-3041.

Warnes ML and Finlayson LH (1985a) Responses of the stable fly, *Stomoxys calcitrans* (L.) (Diptera: Muscidae), to carbon dioxide and host odours. II. Orientation. *Bull. Entomol. Res.* **75**: 717-727.

Warnes ML and Finlayson LH (1985b) Responses of the stable fly, *Stomoxys calcitrans* (L.) (Diptera: Muscidae), to carbon dioxide and host odours. I. Activation. *Bull. Entomol. Res.* **75**: 519-527.

Weidhaas DE (1986) Biological control of muscoid flies. Models and computer simulation for biological control of flies. *Misc. Publ. Entomol. Soc. Amer.* **61**: 57-68.

Williams JD, Sutherst RW, Maywald GF and Petherbridge CT (1985) The southward spread of buffalo fly (*Haematobia irritans exigua*) in eastern Australia and it survival through a severe winter. *Aust. Vet. J.* **62**: 367-369.

Yeoman GH and Warren BC (1968) Repellents for *Stomoxys calcitrans* (L.), the stable fly: techniques and a comparative laboratory assessment of butyl methylcinchoninate. *Bull. Entomol. Res.* **59**: 563-577.

Zorka TJ and Bay DE (1980) The courtship behaviour of the horn fly. *Southwest. Entomol.* **5**: 196-201.

Zumpt F (1973) The Stomoxyine Biting Flies of the World. Diptera: Muscidae. Taxonomy, biology, economic importance and control measures. Gustav Fischer Verlag, Stuttgart.

MILESTONE REPORTS (EXCLUDING FINANCIAL)

NON-INSECTICIDAL CONTROL OF BUFFALO FLY USING BEHAVIOUR-MODIFYING SYSTEMS DAQ.101 - MILESTONE REPORT

Milestone 1: Literature review

Literature review on studies relating to the use of behaviour-modifying systems in the control of buffalo and horn flies is attached. We ask that the review be treated as a confidential document.

Milestone 2: Laboratory and field trials on identification of sensory cues

Mr Evan James Harris (Assoc. Dip. Appl. Science) has been appointed as a technician (TO2) to work fulltime on project DAQ.101.

Testing of responses of buffalo flies to natural host odours (faeces) in an olfactometer has begun. Initial tests are aimed at standardisation of the experimental procedure.

First stage of modifications in behavioural observation facility for flies (boff) has been carried out: floor sealed, post and pens installed. The room is now ready for the introduction of cattle.

NON-INSECTICIDAL CONTROL OF BUFFALO FLY USING BEHAVIOUR-MODIFYING SYSTEMS DAQ.101 - MILESTONE REPORT

Milestone 3: Modifications to existing facilities at ARI.

The existing behavioural observation facility for flies (boff) at the Animal Research Institute (ARI) was previously used for testing the responses of sheep blowflies. Modifications were required to make the facility suitable for testing of buffalo flies. Primary modifications, such as animal handling capability and exclusion of day light have been completed. Testing of buffalo fly responses to animals has begun in the modified boff.

Further planned alterations to facilitate the separation of chemical, visual and other stimuli originating from an animal for subsequent fly response testing, have been postponed to allow a proper evaluation of the currently existing facility.

NON-INSECTICIDAL CONTROL OF BUFFALO FLY USING BEHAVIOUR-MODIFYING SYSTEMS DAQ.101

MILESTONE REPORT

Milestone 5: Sensory cues identified.

Research over the last 3 months focussed on establishing the importance of various sensory cues for host location and on determining certain aspects of buffalo fly behaviour relevant to any life cycle interruption.

It was evident from the literature on buffalo and horn flies, that olfaction and vision seemed to be the two most important cues in host location. We have engaged in work to demonstrate that these two cues are important in host location for the buffalo fly. At a later stage we will try to establish their relative contributions to the host finding process.

Studies were undertaken to determine if changes in attractancy of buffalo flies to a host occurred, and whether these changes, if any, were related to the time post-eclosion and to starvation time of the flies. This information is needed to standardise responses in experiments and to determine the window of opportunity for attracting flies in the field.

Olfaction

The responses of buffalo flies to various cattle-derived odours have been measured in an olfactometer. The first task was to establish an assay which gives a positive, distinct and consistent response when a test odour is presented. Many parameters which are known to have an impact on such experiments were altered in this search, eg temperature, light conditions, orientation of olfactometer, age and reproductive development of flies, odour, and time of starvation. We discovered that there was a great variability in the flies responses between experiments, even when all the parameters were kept constant. Consequently, we aimed at maximising the response of the flies hoping that variability would decrease.

Various components from cattle (faeces, blood, urine, skin washes and scrapings) gave generally (but not always) poor responses. Odour from an entire animal gave stronger and more consistent responses. However, it should be noted that in these experiments temperature and humidity differences between the control and odour streams existed and may have contributed to the responses. For a search and assessment of animal attractancy components these differences will have to be minimised.

Vision

A choice cage, similar to the one used by Hargett and Goulding (1962, *Oreg. Stae Univ. Agric. Exp. St. Tech. Bull.* **61:** 1-27) for their horn fly vision work, was made and initial tests have been conducted. The visual response of buffalo flies to a red shorthorn steer was weak or absent, possibly due to visual disruption (fences etc) in the yards. Further tests will be conducted with tighter methodology.

Preliminary field trials with traps developed for catching other bloodsucking flies were conducted. Only a small percentage (0-3) of released (10 m from target), unfed flies were recovered in a modified Williams trap (designed for stable flies) and the Manitoba trap (designed for Tabanids). The recovery of flies was somewhat higher (2-13%) on a black target (2 perpendicular plywood sheets 90x90 cm) coated with a sticky substance. All targets and traps were used to present a visual stimulus without any olfactory component.

Starvation and eclosion experiments

The orientation of buffalo flies towards a steer, confined in the centre of a pen with a ceiling light, was measured by making counts of the total number of flies on the animal at intervals of five minutes.

Flies that had eclosed during the previous night were fed on blood for 1 h and then held at 33^{o}C with access only to water. Fly response to the animal increased up to 6 h after the blood meal at which time 80-90% of released flies settled on the animal within 30 to 35 minutes of their release. This was similar to the response level reached by non-blood fed 12-18h old flies 45 min after their release in the pen, indicating that flies can be used 6 hours after a blood meal and will have a strong appetance.

Flies were also held at 30^{o}C with access to water for varying times after eclosion before being released in the room. Settling of flies on a animal increased with time up to 6-7 h after eclosion. However, the maximal response at 45 min was only 20-30%; reasons for the discrepancy with flies of this age between this and the maximal responses reached in earlier experiment are being investigated. The time flies are capable of being attracted to the host or a "trap" has still to be determined.

NON-INSECTICIDAL CONTROL OF BUFFALO FLY USING BEHAVIOUR-MODIFYING SYSTEMS DAQ.101

MILESTONE REPORT

Milestone 7: Sensory cues analysed.

The search for and analysis of sensory cues used by the buffalo fly for host location was continued. The work concentrated on potential attractants for the buffalo fly. Vision and olfaction are believed to play major roles in this area (cf Milestone report No 3, MSR3).

Laboratory experiments were undertaken with an olfactometer and in a fly cage to test the responses of buffalo flies to whole animal and to synthetic odours. Experiments to determine the competitive attractancy between animals and a light source were carried out in holding pens in North Queensland.

Olfaction

The responses of buffalo flies to various odours have been measured in a choice type olfactometer. Previously we had demonstrated that a strong and consistent response of buffalo flies to odours from a steer could be obtained when the control stream was purified, dry air (MSR5). For an evaluation of the flies' response to chemicals rather than water, the olfactometer was modified, so that the control air stream could be matched in terms of temperature and humidity, with the air stream originating from the animal. This was achieved by passing the control air stream through a chamber containing heated water. At the same time, some modifications were made to the control system allocating the air streams to the four replicate chambers, in order to achieve a more consistent air flow.

With the modified olfactometer satisfactory responses of buffalo flies to odour from a steer were obtained when tested against a control air stream of equal temperature and relative humidity (see Figure 1). This experiment showed a consistently high response to the steer odour (60-90%) and only a low response to the control (<15%). The discrimination between odour and control streams was high.

During the winter months the responses of buffalo flies in the olfactometer diminished to a level which made running meaningful experiments difficult. This was in spite of the flies being reared and held, and the experiments being run, under controlled temperature and humidity conditions. Such seasonal fluctuations in behaviour have been observed with other insects, but no explanation is at hand. Satisfactory responses were again being obtained with a change in seasons.

An alternative behavioural bioassay, in which flies orient to an odourous airstream directed against the inner wall of a small pot, or against a flat target, has also been developed. This assay has given clear responses to human breath and cattle odours at times when the olfactometer did not, but doe not allow the percentage response of flies to an odour to be estimated readily. At present, this method is being held in reserve for possible future use in applications where it may have an advantage over the existing olfactometer.

Initial olfactometer tests of synthetic odours (similar to those used as tsetse fly attractants) resulted in increased responses to control rather than the odour stream, suggesting a repellent effect. Dilution of the chemical mixture gave a satisfactory attractant response to these odours but there were some inconsistencies. These results with synthetic chemicals are encouraging and further work in this area is being undertaken.

Chemical identification of odour components

Analysis of cattle odours has been undertaken by overseas groups working on tsetse fly attractants. A wide range of chemical components have been detected by gas chromatography/ mass spectrometry and some of these components elicited behavioural responses in tsetse flies.

Our gas chromatography/ mass spectrometry system has a thermal desorption device for transfer of odour components, collected off the animal, onto the gas chromatography column. This device should improve our chances of detecting additional volatile odour components when compared to solvent desorption as used in the tsetse fly work. Collections of steer odours have been carried out and the analyses revealed many of the previously detected components, but no novel components have yet been identified.

Vision

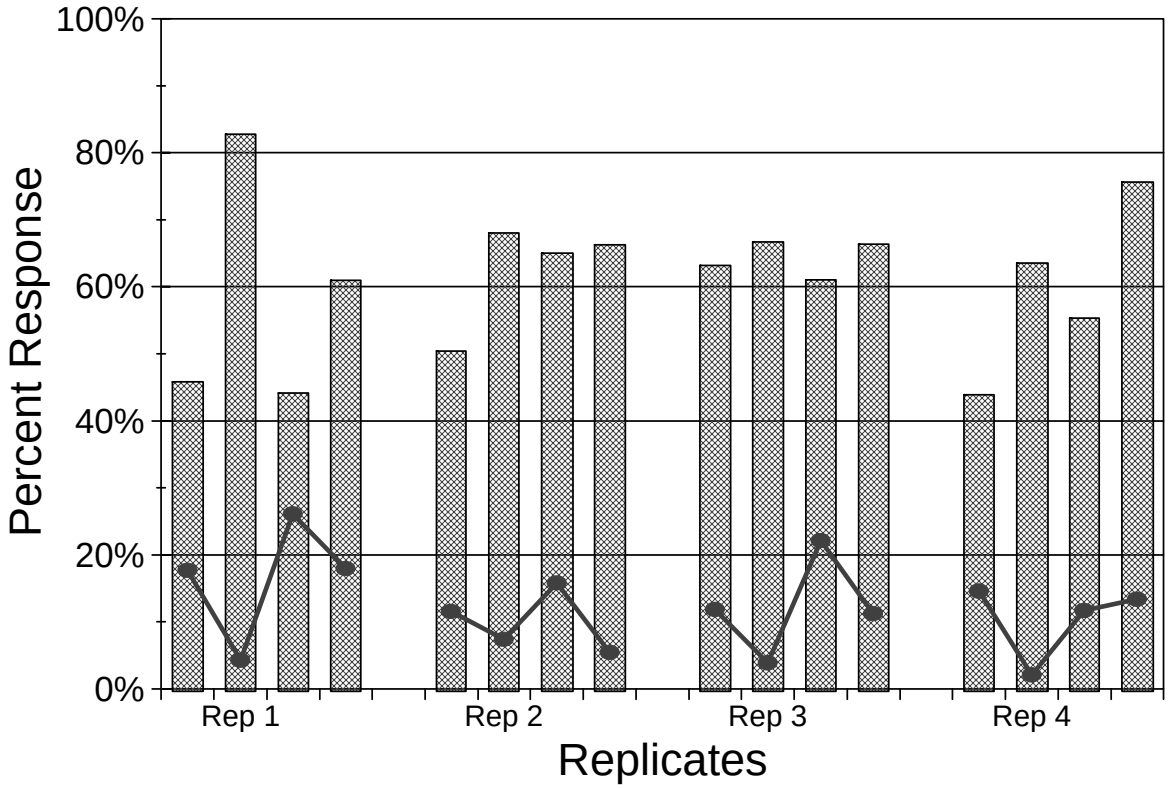
Cage experiments have continued to be problematic but some success has been achieved with field experiments.

In the absence of cattle, a simple black target presenting only visual stimulus attracted up to 32% of flies released 10m upwind of the target. The target was less attractive to newly emerged flies than to older flies which had already been exposed to cattle. The target was less successful at night (when newly-emerged flies are normally seeking hosts) than in the afternoon. The target attracted few flies away from a host animal when tested in a pen which kept the animal within 17m of the target. Nevertheless, a visual component would appear to be desirable in future traps.

Field trials were undertaken to test the potential of UV + visible light as the attractant in a trap. Cattle were penned overnight with or without the light trap. Constant light throughout the night caught enough flies to make a slight reduction in the fly population on a group of 14 cattle. However in a further trial using a lower trap emitting light intermittently throughout the night, fly populations on the cattle were repeatedly reduced. The largest of these reductions was 70% over a single night. This trap will be pursued further to investigate its potential for use with unpenned cattle.

A tsetse fly trap as currently used in Zimbabwe was obtained and has been erected in the field for trials with buffalo flies. This trap relies on a combination of olfactory and visual cues for the attraction of tsetse flies.

Figure 1: Percentage response of buffalo flies to whole cattle odour (hatched bars) or humidified control air stream (solid squares) in a choice type olfactometer (4 replicates with 4 chambers each).



NON-INSECTICIDAL CONTROL OF BUFFALO FLY USING BEHAVIOUR-MODIFYING SYSTEMS DAQ.101

MILESTONE REPORT

Milestone 8: Sheep blowfly trap evaluated for capture of buffalo flies.

Lucitrap is a commercially available trap used for the suppression of Australian sheep blowfly populations. The trap and the synthetic attractant have been developed by us to provide wool producers with an additional tool in the control of blowfly strike. The results from a preliminary investigation into the potential of Lucitrap for attracting buffalo flies are reported here.

The responses of buffalo flies to the Lucitrap were compared with those to a synthetic cow (SCOVOS). Scovos, which is covered with sticky panels to trap flies, provides visual and olfactory stimuli. The olfactory stimuli are provided via a synthetic cow odour (polyethylene sachet with octenol, 4-methylphenol and 3-propylphenol; vial of acetone) developed for attracting tsetse flies. A known number of laboratory-reared buffalo flies were released 10 metres up- and downwind from the target (alternatively Scovos and trap) in a open paddock devoid of animals. The flies which landed on Scovos and got stuck, or which were caught in Lucitrap, were counted after 1.5 hours and the percentage recovery from released flies counted. Lucitrap was used with its original attractant, Lucilure and synthetic steer odour in two separate experiments.

The percentage recovery of buffalo flies were:

Target	% Recovery
Scovos	41
Lucitrap with Lucilure	0
Lucitrap with synthetic steer odour	0

These preliminary trials indicate that the Lucitrap with Lucilure or synthetic steer odours did not catch buffalo flies. The reason no buffalo flies were caught in the Lucitrap, could be attributed to either no or little response by the buffalo flies to the chemicals, or to the flies not entering the trap. At this time, it is not known how large the contribution from the synthetic cow odour is towards the response observed with Scovos.

These preliminary findings should however be confirmed with field populations of buffalo flies. We intend to conduct broader comparisons early next year on cattle properties in Queensland.

NON-INSECTICIDAL CONTROL OF BUFFALO FLY USING BEHAVIOUR-MODIFYING SYSTEMS DAQ.101

MILESTONE REPORT

Milestone 10: Sensory cues analysed.

The search for and analysis of sensory cues used by the buffalo fly for host and ovipositional site location was continued. The work concentrated on potential attractants for the buffalo fly. Olfaction and vision are believed to play major roles in this area (cf Milestone report No 3).

Progress was made in both the search for attractants and the investigation on the role of vision in attractancy. In the search for attractants, improvements to the system for measuring fly responses to odours were implemented and a screening program of potential, chemical attractants is in progress. So far, some of the tested candidate chemicals have elicited a good response in buffalo flies. Preliminary results from electroretinography established that buffalo flies' eyes respond over most of the ultraviolet and visible range, with two maxima, one in the UV and one in blue/green. The potential for using UV light as an attractant in field situations has also been investigated.

Olfaction

For the evaluation of responses of buffalo flies to odours, a choice-type olfactometer had been used. Inconsistencies in fly responses to animal odours were again experienced with the olfactometer. The initially promising results with synthetic attractants used for tsetse flies (cf Milestone report 7) could not be reproduced and even responses to whole cattle odours were erratic. With these observations, a decision was made to carry out the chemical screening program with a modification of the previously developed "flat target" assay (cf Milestone report 7). In this assay, two odour streams are introduced into a fly cage through glass tubes and continuously dispensed onto two opposite perspex sides of the cage. The odours are ventilated from the cage, which is placed in a room with a constant, directional airflow, through the other metal screen walls of the cage. Through the use of test and control (usually air of same temperature and humidity but no chemicals) odour streams a behavioural response of the flies is observed. The flies will move to, and remain in the area where the attractive test odour (eg whole cattle odour) meets the cage wall. When the odour flow is stopped, the flies redistribute in the cage. The quantitative output from the assay is the number of buffalo flies within defined areas on the glass wall on both sides of the cage (test and control) at various times after the start of the odour stream.

The cage type assay was optimised and standardised using odour from a steer. There were consistently high responses of buffalo flies towards the side with steer odour, with 60-90% of all flies present in the cage located within the specified area on the wall. Within the wall area facing the control stream (charcoal-filtered air of matched temperature and humidity), typically 0-5% of the flies were observed. Thus, there was a high response to steer odours and good discrimination between odour and control. It was further established that there was no inherent bias towards one cage side and that no or very few (< 5%) flies moved to the specified areas when clean air was presented to both sides.

Various cattle related odours were then tested in the cage assay for their capacity to entice buffalo flies to move into the specified area. Odours from bovine faeces, urine and hide and cattle breath were all attractive to the flies, although at a somewhat lower level than whole animal odour. This indicates that buffalo flies will respond in the laboratory to odours emanating from a wide variety of cattle related sources.

The flies also responded well to the odours from culture cylinders which held several thousand buffalo flies and associated excreta for six days. Again it was shown that the flies alone (transferred to new container prior to experiment) and the container lining contaminated with excreta elicited a response when presented separately. This indicates that attractive chemicals are emitted from fly excreta and from flies themselves, possibly pheromones in the latter case.

Synthetic chemicals which had been detected in "cattle odour" were also tested in the cage assay. The selected chemicals were presented to the buffalo flies over a wide concentration range, typically 10^{-9} to neat, by serially diluting them in a high boiling carrier (paraffin, olive oil or glycerol). The resulting solutions were applied to filter paper strips which were suspended in the test air stream. Initial tests were carried out with synthetic mixtures which had been found attractive to other cattle seeking flies, eg. Swormlure (screwworm fly attractant), tsetse fly attractant. No responses of buffalo flies to these mixtures were observed in the cage assay.

Screening of single chemicals has also started, with a selection of representatives from the various groups of chemicals associated with cattle odours such as alcohols, aldehydes, short chain fatty acids, phenols, amines etc. The majority of these chemicals did not elicit any responses in our cage assay. However, two chemically closely related representatives of one group gave good responses by the buffalo flies. Further screening of individual candidate components is currently in progress.

In order to be able to collect odours from whole animals in a controlled and reproducible manner, modifications to the experimental fly facility were completed. An airtight housing capable of holding one animal was added to the outside of the existing building, with a large window separating it from the experimental room. Filtered, airconditioned air can be directed to the experimental room and/or the animal housing from where it can be routed back to the experimental room or vented to the outside. This arrangement will allow us to collect animal odours for the cage experiments and to carry out experiments in the larger facility on responses of buffalo flies with and without olfactory and visual stimuli.

Vision

Electroretinograms of buffalo flies have been recorded. This technique measures the electric potential differences in the flies' visual system as a result of the incoming "light". By providing defined wavelengths, it can be established whether a wavelength can be detected by the fly and how it is processed by the central nervous system. These findings have implication in any control method involving vision (during daylight) or light sources (during dark).

In the experiments, a light beam from a photospectrometer was directed through a fibre optic cable to a dark adapted buffalo fly which had microelectrodes attached to eye and head. The electric potential changes occurring when "light" was switched on and off, were amplified and recorded at different wavelengths. It has been established that buffalo flies can detect light in the ultraviolet, from approximately 275 nm, through the entire visible range to 650 nm. There are two potential maxima, one in the ultraviolet (approx. 350 nm) and one in the visible range, 500 nm (blue/green). These are preliminary results because the power of the light source has yet to be calibrated and the potential differences corrected accordingly and more replicates need to be recorded.

Further experiments were conducted to assess the efficacy of various light sources for attracting buffalo flies under pen and field conditions (cf Milestone report No. 7). The effect of the type of light (UV blacklight, white fluorescent) and light/dark cycle (continuous, 1 min light/1 min dark, 15 min light/15 min dark, no light) on the recovery of flies onto a white, sticky target from a penned steer was investigated. With a UV blacklight all cycles (except no light) caught more than 90% of the flies observed on the steer prior to the start.

The recoveries of the flies on the target with the white fluorescent light were 36, 33, 38 and 7% for the above treatments respectively. The intermittent white light did not improve the catch compared to the continuous light, which is in contrast to results of earlier experiments with a UV blacklight (Milestone report No. 7).

A sticky target with a UV blacklight (15 min cycle light/dark) was placed in a 100 x 250 m paddock, containing buffalo fly infested cattle, as a follow on from an earlier, successful experiment in a smaller area (cf Milestone report No 7). Very few buffalo flies were caught, indicating that the cattle had to be held in proximity of the light source for it to be effective.

Experiments were carried out to determine the distance buffalo flies could detect a UV blacklight. Caged flies were exposed to the light at various distances from the light source and the percentage which responded to the light at each distance were recorded. The response was consistently high and decreased only slightly with increasing distance from >90% at 20 m to around 80% at 80 m. On this basis, quite good results could have been expected in the paddock trial described above. However, the results suggest that there is a large difference in behaviour between caged flies and flies on cattle.

Field experiments

Preliminary field experiments using a model cow and laboratory reared (Brisbane) and wild (Brian Pastures Research Station, Gayndah) buffalo flies were carried out. The purpose of these experiments was to investigate the relative importance of visual and olfactory cues used by buffalo flies for orientation towards a target. A model cow with sticky sides to trap flies was used with and without odours. In the Brisbane trials, buffalo flies were released at fixed distances up- and down-wind from the model cow, and the number of flies stuck on the model counted some time after fly release. At the Brian Pastures Research Station, all flies stuck to the target were removed at predetermined intervals, identified and counted.

In a series of Brisbane experiments a mean of 26% of buffalo flies, released 20 metres from the model cow, were caught. This is far more than expected on a random dispersion of the released flies and indicates that the flies are orienting positively to the model cow. A higher percentage of flies released upwind of the model cow were caught compared to the downwind released flies. The addition of tsetse fly attractants or cattle dung did not markedly increase the catch rate of the model cow. In some trials there seemed to be an increase in the catch of downwind released flies which would be expected because only they are exposed to the odour plume travelling with the wind. To a degree these observations agree with the findings from the cage assay, that the tsetse attractant is not very attractive to buffalo flies. In a further experiment it was found that a black rectangle (60 x 120 cm) of approximately the same size as the model cow caught as many buffalo flies as the model cow. This rectangular target was then used in the Brian Pastures Research Station experiments.

At Brian Pastures Research Station the rectangular target with and without tsetse attractants and the Lucitrap with Lucilure and tsetse attractant were tested for their attractivity for wild buffalo flies. The targets/traps were placed in similar locations in cattle paddocks and rotated at 24 hour intervals according to a random 4x4 Latin square design. The flies were removed from the sticky surface or trap at each rotation, identified and counted. The rectangular targets caught a mean of 7.4 and 11.4 buffalo flies per 24 h with and without tsetse attractants respectively, which was significantly more than the Lucitrap which with either lure did not catch any buffalo fly during the trial. This is not unexpected, as the behaviour of the buffalo fly may prevent it entering a trap like Lucitrap even if it is attracted by the odour. This experiment has confirmed that Lucitrap is not suitable for use with buffalo flies. It should be noted that the targets are not very effective either, as 50 to 350 buffalo flies per side were counted on cattle at the station during the experiment.

NON-INSECTICIDAL CONTROL OF BUFFALO FLY USING BEHAVIOUR-MODIFYING SYSTEMS DAQ.101

MILESTONE REPORT

Milestone 12: Effectiveness of identified components in disrupting fly life cycle tested under laboratory and field conditions.

Summary

The responses of buffalo flies to many single chemicals, mixtures of chemicals and bovine derived natural odours enhanced with synthetic components have been assessed in the cage olfactometer. Good responses were obtained for some mixtures and some enhanced bovine odours. It was shown that cuticular hydrocarbons (fly extracts) also elicit a behavioural response in buffalo flies and the chemical nature of these compounds has been determined. Electroretinography established that buffalo flies' eyes respond over most of the ultraviolet and visible range, with two maxima, one in the UV and one in blue/green. Preliminary field trials have established that mixtures used to lure tsetse flies are not a suitable attractant for buffalo flies.

Introduction

Olfaction and vision have been identified as important components in the host cattle and cattle dung location in buffalo flies. Current work aims at modifying the behaviour of buffalo flies with chemical (olfactory) or visual means, so that the flies will not be able to locate cattle or the egg laying site. This would result in a disruption of the flies' life cycle, leading to lower fly populations, and thus assist in control. The search for and the assessment of the effectiveness of chemical and visual components for this purpose are ongoing. Progress has been achieved in both areas, but further improvements in synthetic attractants and in the understanding of the role of visual targets is still required.

Olfaction

The evaluation of responses of buffalo flies to chemical stimuli was continued with the cage olfactory assay. A large number of single chemicals, some synthetic mixtures, cattle derived odours and buffalo fly related components were assessed for their potency to attract the buffalo flies to the designated area in the insect cage.

Single chemical screening

The screening of single chemicals for potential buffalo fly attractants included representatives from various groups of chemicals associated with cattle odours such as alcohols, aldehydes, ketones, short chain fatty acids, phenols, amines and sulfur compounds. These were tested over a wide concentration range, typically from 10^{-6} to undiluted compound. The majority of the approximately 35 individual chemicals tested so far gave no measurable response over the tested concentration range. A few gave weak responses at selected concentrations. Chemicals which showed any response in the initial screening were then incorporated into multicomponent mixture testing as described below.

It was noted that the concentration range of single chemicals for which a positive response was observed, was often quite narrow. In addition, the responses of buffalo flies to single

chemical stimuli were also not always reproducible. This is in contrast to the regular and repeatable responses of the flies to odours sourced from an entire steer. One possible explanation is that the entire animal odour provides a multiple and thus robust stimulus to the fly, whereas single chemicals only stimulate a narrower range of receptors, and whether or not a behavioural response is observed depends on other nervous system "switches" in the fly.

The reasons for the observed in- and between-run inconsistencies with single chemicals are not known at this stage. Moreover, during the winter months there was a general drop in the responsiveness of the flies even to animal odour. The buffalo flies are bred and kept in, and their responses tested under controlled conditions (temperature, humidity, light, diet) and a reasonable uniformity in their behaviour could be expected under these circumstances. An attempt was made to overcome the flies' "winter blues" by placing a negative ion generator in the air stream (as suggested by Prof Jerry Butler at the University of Florida) but no substantial improvement in the response was obtained.

It was also noted that with single chemicals the arrestment of responding flies in the target area which was continuously supplied with odour, was not as persistent as with whole animal odour. With animal odour attractant, approximately 80% of the responding flies were still in the target area after 140 minutes, whereas with single chemicals the maximum response was often obtained before 10 minutes with a noticeable decline of flies at 15 minutes.

Screening of mixtures

Mixtures made up from single chemicals which had provided some responses were also tested for their potency to elicit a response in the cage assay. From previous work with other insects, it is expected that multicomponent attractants will elicit at least behaviour which is additive in relation to the single component responses. Often these effects are synergistic, that is, the overall effect is much bigger than the sum of the individual component responses. With a choice of 6 to 8 chemicals and their relative and absolute concentrations, there is a large number of combinations of potential attractants. The initial concentration of the components used in the mixtures was selected at the maximum response in single chemical screening. However, this may not necessarily be the optimal concentration in the mixture. Thus there is a need to test mixtures with variable concentrations of single components.

Several 2, 3, 4 and 5 component mixtures have been tested so far. In general, mixtures containing components from different chemical groups (amines, alcohols etc) have performed better than the individual components (cf Figure 1). Some mixtures gave no gain in fly response over the individual chemicals. Again there were some problems with reproducibility, thus at this stage these comments are only of a preliminary nature. We are currently investigating ways of increasing consistency by changes to the odour introduction system, which was originally set up for single chemicals. It appears that the omission of certain components from the tested mixtures results in a reduced response of the flies. Virtually no work has been carried out on the variation of relative or absolute concentrations. None of the mixtures tested to date achieved the attractancy of bovine derived material enhanced by synthetic chemicals as discussed below. Thus, it appears that there is ample scope to improve the attractancy of the synthetic mixtures and work with this objective is currently being undertaken.

Animal derived attractants

Odours emanating from bovine faeces, blood and rumen fluid are also slightly to moderately attractive to buffalo flies. The response to these materials was enhanced by the addition of one or several of the chemicals which were found to elicit a response in buffalo flies as illustrated in Figure 1.

This indicates that the augmentation (if component already present in material) or addition of selected synthetic chemicals increases the response of the flies to these materials. This observation suggests that there is a potential for synthetic attractants to compete with or overcome naturally occurring olfactory attractants from cattle.

Fly derived attractants

We had previously shown that flies responded to filter paper lining from cages where thousand of buffalo flies had been kept. We have now demonstrated that odours from soiled cage linings enhance the attractancy of whole animal odour. It is suspected that hydrocarbons contained in the flies' excretions could be sex or aggregation pheromones. An investigation of the chemical nature of these components is described below.

The olfactory attractancy for buffalo flies to fly cage paper lining and live flies led us to investigate the chemical nature of this stimulus. Other groups had reported the importance of insect produced hydrocarbons in their communication and mating. The fly cage paper lining which contained excreta from a thousand flies kept in the cage for a week was sequentially extracted with hexane and methanol. Both extracts attracted buffalo flies, with the combination being most potent. The chemicals contained in the hexane were mainly saturated and unsaturated hydrocarbons. Buffalo flies of different sex and age were also extracted with hexane to obtain the cuticular hydrocarbons, which have been identified in related flies as sex and/or aggregation pheromones. The hydrocarbons were analysed by gas chromatography/ mass spectrometry and it was shown that there were fundamental differences in the chemical nature and the amounts of hydrocarbons present in flies of different sex and age. It is possible that these hydrocarbons could be used to alter the behaviour of buffalo flies.

Influences of physiological parameters

No difference in response to whole animal odour was found between 3 to 4 and 7 days old flies (OVL strain) and between flies reared at Oonoonba Veterinary Laboratory (QDPI), Townsville, and at the Tropical Animal Production facilities (CSIRO) at Indooroopilly.

The responses of the laboratory strain were also compared to a wild strain (F1) from Landsdown research station. The freshly collected wild strain did not show a better response to a synthetic mixture than the OVL strain in the cage assay. There was also no difference found in general behavioural activity tests (time spent flying, walking, grooming; escape, recapture) between laboratories and wild flies, except that the wild flies spent more time grooming and less time doing apparently nothing than did the laboratory reared flies. Thus it appears that the behaviour of the laboratory flies is similar to wild flies, which would suggest that the results obtained with lab flies would be valid for wild strains.

Vision

Electroretinograms

Further electroretinograms of buffalo flies have been recorded. This technique measures the electric potential differences in the flies' visual system as a result of the incoming "light". By providing defined wavelengths, it can be established what wavelengths can be detected by the fly and how they are initially processed by the central nervous system. These findings have

implication in any control method involving vision (during daylight) or light sources (during darkness).

Replicates of the original runs were recorded with more data points at crucial wavelengths. The power of the light source at different wavelength was determined and the effect of power variation on the size of the output signal has been established. From this we were able to produce a graph of the potential differences against the wavelength used for stimulation (Figure 2). Light detection in buffalo flies ranged from 300 to 675 nm, with major maxima at 350,375 and 475 nm. It should now be assessed whether material with maximal reflection at these wavelengths will show an increased attractancy or landing rate for buffalo flies.

Pen trials

Experiments to help establish the relative importance of vision and odour were also carried out. A small number of buffalo flies (eg 50) were released into a large experimental room (8x5x3 m) which had a small annexe capable of presenting a steer visually and/or olfactorily. The annexe and experimental room were separated by a glass partition, but were connected through a closeable air duct. The flies in the room could thus be presented with nothing (blank), with vision of steer only, or odour of steer (pumped through connective duct) with or without vision of steer (a curtain was used in latter case). A grid of clear, sticky strips were attached to the glass partition in the room containing the flies and the number of flies attracted and thereby caught were counted.

With no stimulus present (blank) only a few flies were caught on the sticky strips. The combined vision and odour stimulus was always the best inducement for the flies to land on the glass partition with a typical catch of 50 to 70% within 20 to 30 minutes. Vision and odour alone still caught considerably more flies than the blank, but less than their combination. Vision alone was in these experiments more powerful in inducing the flies to land on the glass than the odour alone. When the steer was replaced by a 2-dimensional model of a steer the number of responding flies dropped markedly with and without the presence of steer odour. Adding movement to the model did not improve the outcome.

Field experiments

Tsetse attractants

Two preliminary field experiments evaluating tsetse fly lures against buffalo flies were conducted at Brian Pasture Research Station, Gayndah. Pairwise comparisons between black, vertical sticky targets (120x60 cm rectangle, 80 cm above ground) with and without tsetse lure were carried out in paddocks carrying cattle with buffalo flies. In accordance with previous results, it was found that the tsetse lure did not increase the catch of buffalo flies. In the first trial, the sticky target caught 10.3 and 6.6 buffalo flies per 24 hours without and with tsetse lure respectively (not significantly different, $P > 0.05$). The number of buffalo flies on cattle during the experiment were between 20 and 500 per side per beast. In the second trial the corresponding fly numbers were 2.0 and 3.0 (not significantly different) on the targets without and with lure, and 20 to 200 flies per side per beast. These figures clearly illustrate the point that a strong attractant combined with appropriate application technology is required if an impact on fly numbers in the field is to be achieved.

Behaviour of flies on animals

During light trap experiments on yarded cattle, it was noticed that the numbers of flies on cattle appeared to decrease at night. Significant numbers of flies were collected away from the hosts on the surrounding fences and grass. Preliminary quantitative work has confirmed that the fly populations do often decrease at night, especially in the first few hours after dusk.

Addtionally, the flies which remained on the cattle at night tended to move to the lower parts of the host ie fewer on the head, upper neck and back and more on the belly and legs. This work was conducted under relatively cool conditions and will be repeated under warmer temperatures. If it proves to be consistent, it may have implications for trapping as it is likely to be much easier to attract flies which have left the host than those already on a host.

Development of female flies on cattle

Mark-recapture work showed that in controlled air temperature of 29-31C, female flies on cattle generally mated at 24-36hrs old and were gravid after 48hrs. Once gravid, subsequent egg batches were matured rapidly so that the females were almost constantly ready to oviposit and may consequently be susceptible to oviposition-based disruptants.

Conclusions

Progress has been made in the identification and assessment of sensory cues for interrupting the life cycle of buffalo flies. Synthetic mixtures which elicit a positive orientation of buffalo flies have been formulated. The spectral characteristics of the flies' vision has been defined. It has been demonstrated that a combination of olfactory and visual cues is better than either alone. However, the current status of the synthetic attractants and the knowledge and understanding of visual component do not enable us to achieve an effective disruption of the buffalo fly's life cycle. For this purpose both these components need to be improved before they are evaluated for their field effectiveness. Thus, future work will concentrate on the optimisation of the synthetic attractants, on the manipulation of behaviour with respect to visual cues and on some preliminary field assessments of these components.

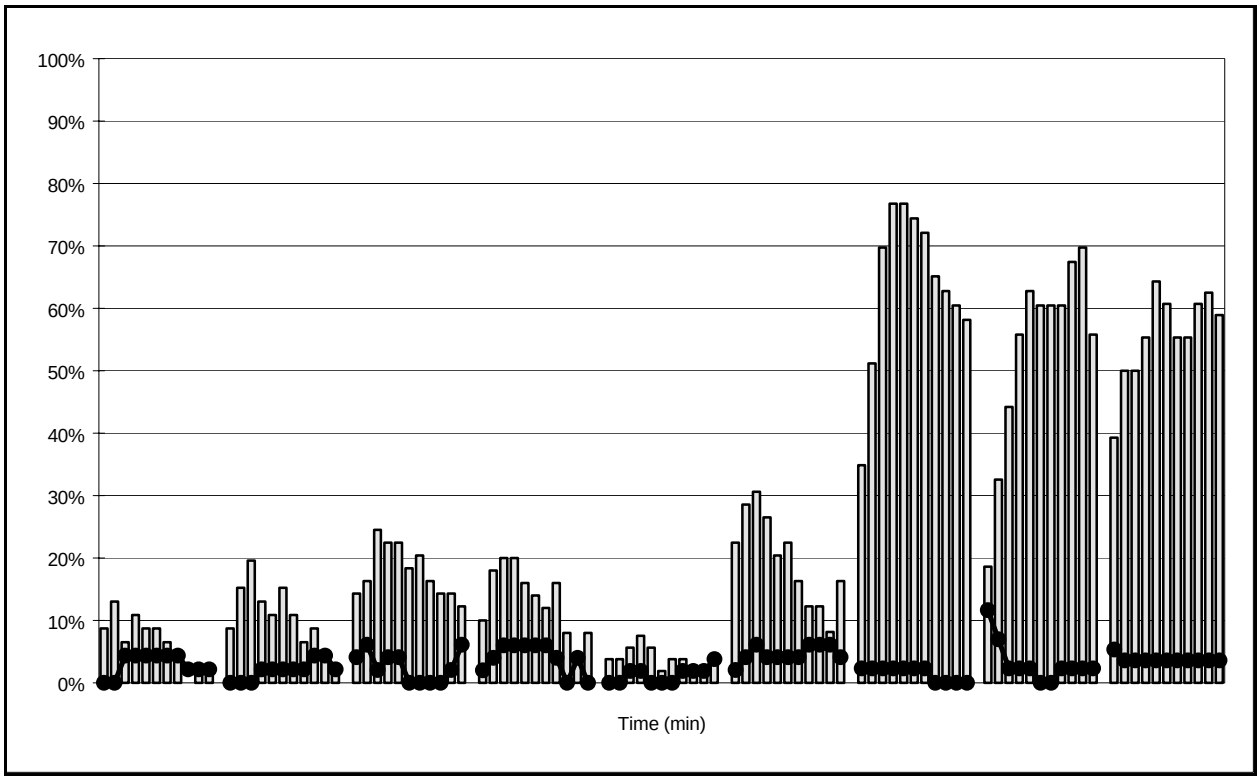


Figure 1: Percentage of buffalo flies responding to treatment (grey bars) and control (black line) at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 15 minutes in cage assay. Treatments were (from left to right): 1) Single chemical A; 2) two chemicals A+B; 3) two chemicals A+C; 4) three chemicals A+B+C; 5) cattle dung; 6) dung + chemical A; 7) dung + chemicals A+B; 8) as 7) but chemical B at higher concentration; 9) dung + chemicals A+B+C

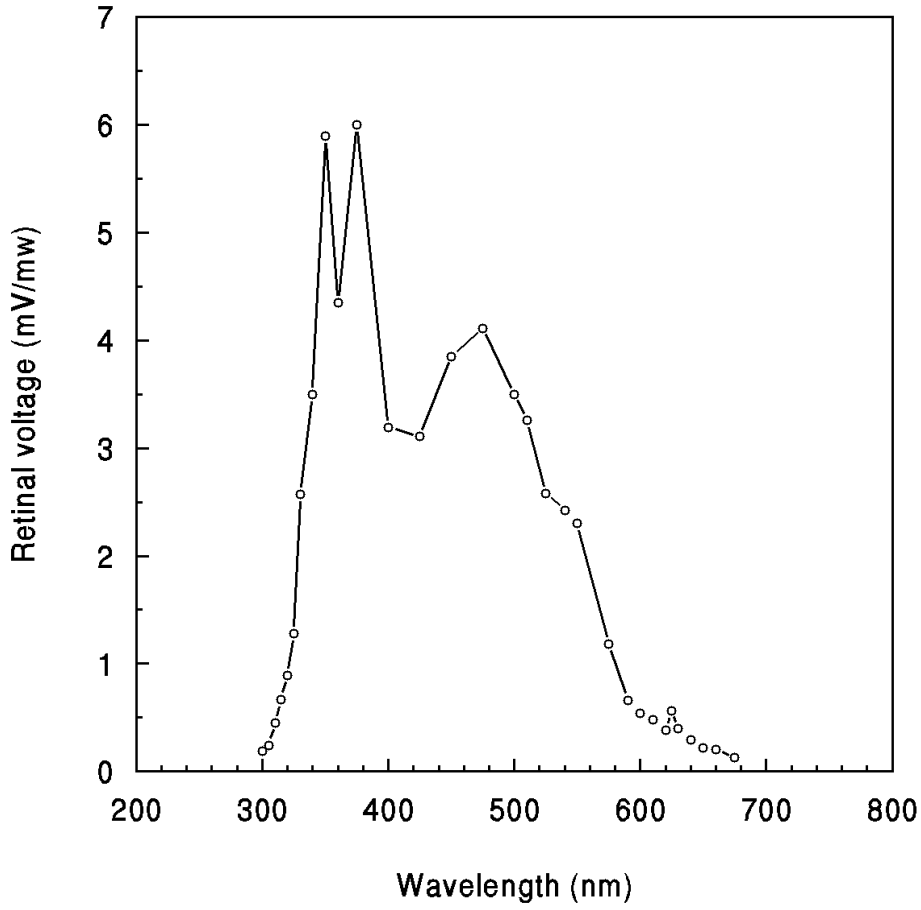


Figure 2: Buffalo fly electroretinogram: Retinal voltage (corrected for power of light) against wavelength of incoming light.