Natural Resources Institute

AERIAL SPRAYING FOR TSETSE FLY CONTROL

A handbook of aerial spray calibration and monitoring for the Sequential Aerosol Technique

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GLOSSARY

Active ingredient	This is the actual poisonous substance of the pesticide. It is normally abbreviated as 'a.i.'.
Aerosol	Used here to describe small drops around 20 µm diameter. These are much smaller than those drops used in most agricultural spraying. However, engineers regard the term aerosol as referring to drops under 5 µm.
Area dosage	This is the amount of active ingredient in grams applied to a given area, usually one hectare.
Atomization	This is the process of making spray, or breaking up the liquid formulation into drops.
Atomizer	The tool which breaks up the spray. The rotary atomizers used in tsetse spraying spin as the air rotates the propellers, and depend on air speed, and therefore the aircraft speed. A faster rotation can be achieved by altering the propeller pitch or blade angle. Rotation speeds used in tsetse work are faster than those used in agriculture to make smaller drops. 10 500 rpm is a typical rotation speed.
Block	An area from which tsetse are being cleared. There may be parts which have no flies, but it is not practical to find and spray each patch of infestation. Aerial spraying is not economic unless the blocks are several kilometres across.
Calibration	This is checking the settings of equipment to give the necessary amount of insecticide and the required drop size.
Correction factor	Some drops deviate around an object, rather than impacting it. When the relationship between deviating drops and those that impact is known, a true estimate of the number of airborne drops can be produced by multiplying the 'catch' of drops by the correction factor. For example if half the drops deviate around the object, then the correction factor would be two.
Deposition	This is the term used to describe a drop hitting any surface, vertical or horizontal, by impaction or sedimentation.
Drop size	Drop (sometimes called droplet) diameter is measured in microns, written µm. A micron, or micrometre is one millionth of a metre. A 100 µm diameter drop can be seen by the naked eye; smaller drops such as those used in the sequential aerosol technique are invisible unless viewed in the beam of a lamp in darkness. The volume of a drop is proportional to the cube of the diameter, so one 100 µm drop will make 64 drops of 25 µm diameter. This shows that larger drops are wasteful in the sequential aerosol technique.
Drop spectrum	All sprayers produce drops in a range of sizes. This is called the drop spectrum. It is often shown as a histogram giving the proportion of drops in different size classes. Measuring the drop size of emitted spray is not easy. In this type of spraying the drops have become smaller due

	to loss of solvent before they are measured using rotary drop samplers. Thus the sampled spectrum is different to the emitted spectrum
Flow rate	This is the rate of delivery (litres per minute) of insecticide formulation from the atomizer. It is sensed in flight by a flow turbine in the pipework, but must be checked for accuracy before spraying.
Fly collection efficiency	Small drops tend to go around flies, although the flies' hairiness helps to collect drops. Laboratory work has determined the proportion which impact under given drop size and windspeed conditions. This is the fly collection efficiency.
Fly dose	This is the total amount of insecticide active ingredient which a fly receives, or is calculated to be likely to receive from a given condition. The dose can be compared with the LD50 for that insecticide. Three times the LD50 is considered to be a dose to achieve eradication, bearing in mind that there will be large variation in amount received by individual flies in practice.
Impaction	This occurs when a drop carried sideways by the wind lands on a vertical surface such as a plant or insect. The drop hits the surface because of the horizontal momentum it has been given by the wind. Smaller drops tend to go around large obstructions and are said to have a low impaction (or collection) efficiency.
LD50	This stands for lethal dose (50%), and is the result of toxicological testing of insecticides against the species. It is the mass of active ingredient (usually nanograms per insect) which kills half the insects, leaving the other half alive. Lethal dose has been shown to vary with species and physiological stage of the insect. Pregnant flies or well fed flies need a higher dose to kill them.
Mature drops	These are drops which have lost their solvent and attained a diameter which is small enough for their fall to earth to be slow, so that they are carried by the wind.
Meteorological conditions	This term relates to the weather patterns. The overriding factor in the sequential aerosol technique is air movement. The reason is that the drops are so small that they are carried by breezes or turbulence. Some wind is needed to carry drops through the vegetation to the flies at their resting sites, but convective turbulence which occurs in sunny conditions, would carry drops upwards. The spraying is usually carried out at night or around dawn or dusk when conditions are more suitable.
Monitoring	Measuring how well the spraying is being carried out, and identifying where possible problems such as fly survival might be encountered.
Navigation equipment	The system fitted to the aircraft to tell the pilot the direction he should fly. Great accuracy is now possible and this should allow each spray pass to be in the correct place.
Number Median Diameter (NMD)	The diameter such that half of the total number of drops are bigger, and half are smaller.
Rotary sampler	This is the basic spray sampling tool which catches airborne spray as it passes by. It consists of a battery-powered slide holder which spins at

	around 420 rpm. Glass slides 6 mm wide are coated with magnesium oxide and fitted to the sampler. Drops hitting the soft oxide surface cause craters which can be counted and measured later, giving both number and size of spray drops. The data is processed to give spray flux.
Sedimentation	This occurs when a drop falls downwards and lands on a horizontal surface under the influence of gravity.
Spray concentration	The amount of active ingredient (a.i.) in a given amount of the commercial formulation. This is normallly given as number of grams in a given volume of formulation. For example 30% endosulfan contains 300 grams of active ingredient in each litre. Note that because endosulfan is heavy (density 1.7) it is not the same as 300 ml in one litre.
Spray flux	The amount of spray per square centimetre being carried, more or less horizontally, by the wind.
Swath width	This is the width of the strip at right angles to the sprayers path where there is 'significant' deposit.
Sequential aerosol technique (SAT)	This technique of tsetse fly control, sometimes called the sequential drift spraying technique, uses small drops, which we call aerosols, applied several times (in a sequence) at intervals to kill existing flies before they breed, and hence before they can produce a new generation. Because the sequence is timed to coincide with the tsetse life cycle, based on daily temperature (which controls their rate of development), it is less damaging to other insect species.
Track spacing	The distance between parallel passes of the sprayer. Because spray is carried further than this distance, any point is exposed to spray from several different passes.
Volume Median Diameter (VMD)	The diameter such that half of the spray volume is in smaller drops, and half in larger drops.

INTRODUCTION

This handbook is written for tsetse control officers who are not spraying specialists, but who may be called on to carry out quality control in aerial spraying operations against tsetse flies (*Glossina* spp., the vectors for trypanosomiasis). It describes safety aspects, the adjustment of spraying equipment to produce the correct spray quality, and the measurement of the quantity of spray in the treated area. A method for predicting the dose on the flies and making an estimate of mortality is described.

BACKGROUND

The Sequential Aerosol Technique (SAT) is a method of tsetse fly control which uses aerial application to apply several very low doses of insecticide as tiny drops. Aerial spraying is one of several techniques currently used to control tsetse fly. Other methods include the use of insecticide-treated, odour-baited targets, ground spraying in which selected vegetation is treated with insecticide, direct spraying of cattle with insecticide, or dosing cattle with prophylactic drugs to suppress the disease (*nagana*) carried by the flies. Methods based on large-scale clearing of vegetation which may provide tsetse habitat, or culling of wild animals which may be reservoir hosts, are no longer considered acceptable.

The selection of a control method involves many factors. Cost is clearly an important element, but if human epidemics of sleeping sickness occur, the urgency of the problem may override financial considerations. The choice is also dependent on topographic factors and the local infrastructure; reasonably level terrain is required for aerial spraying, whereas a network of suitable roads is required for ground-based techniques.

This handbook does not attempt to compare the merits of different methods of tsetse fly control, but some factors relating to aerial spraying are summarized below.

Cost benefits of aerial spraying

Although aerial spraying is one of the more expensive control methods due to the high cost of flying hours and insecticide, it has several key advantages:

- it can clear flies from large areas in a short period of time (less than three months from the start of the first spray);
- it is effective in areas where access is difficult, dangerous, or undesirable, for ground-based methods;
- it is not labour intensive. The technique relies on a small number of staff using a technological solution, and does not require a large control organization;
- there are no significant environmental side effects;
- there are no recurrent costs.

The purpose of aerial spray calibration and monitoring

The SAT is a specialized ultra low volume (ULV) control method which requires careful attention to detail at all stages. Spray calibration and monitoring ensure that the operation is given the best chance of success. The objectives are specifically to:

- ensure the spray drop spectrum emitted during the control operation is the correct size;
- ensure the flow rate will provide the correct dosage for the ground speed and track spacing;
- monitor the meteorological conditions to make certain that spraying only takes place under suitable conditions;
- monitor the flying height and track spacing of the aircraft by making observations in the spray block as spraying takes place;
- check that spray is reaching different parts of the spray block by taking a series of samples during the operation;
- check that sufficient numbers of insecticidal spray drops are present to kill tsetse flies; and
- make an estimate of the likely fly dose and mortality based on sampler data.

The fly dose and mortality estimates involve a considerable amount of data processing. Non-specialists may consider spray flux measurement adequate. This is the quantity of spray carried horizontally by the wind. Estimates of the fly dose and mortality predictions are based on the spray flux, but incorporate fly collection efficiency corrections, and adjustments for insecticide toxicity and concentration.

SAFETY

The safety of the local population, spray operators, support staff, and the environment is of crucial importance during an aerial spraying operation. Staff involved with monitoring spray must pay attention to the method of transporting, storing and handling the insecticide, and particularly loading it into the aircraft.

The local population

It is important that people who live in and around the spray block are informed of the options for tsetse control and participate in the planning of these schemes. This will prevent resentment or fear, and usually result in their co-operation and support. Everyone should be informed when spraying will take place, so that they can stay indoors during this period, to minimize the risk of exposure to insecticide. Those who are exposed are very unlikely to suffer any harm because of the small amount of insecticide involved, but contamination should always be avoided wherever possible.

Spray operators and support staff

Because staff handle insecticide during storage, transportation, and loading the aircraft, as well as maintaining the application equipment, they are most at risk of accidental contamination. Suitable protective clothing *must* be provided and worn. This should include cotton overalls, nitrile gloves, mask, visor or safety goggles, rubber apron and boots. A closed system for transferring insecticide directly from the drums into the aircraft spray tank is highly desirable. Pouring from drums inevitably produces splashing, and is a potential source of contamination.

Accidents

As accidental contamination of operators occurs, despite precautions, soap and water must be available immediately adjacent to the pesticide handling area so that washing can take place without delay. There are no specific antidotes to endosulfan and deltamethrin, the insecticides most used in SAT, but general First Aid principles apply:

• **Skin contact**	Wash immediately with soap and water
• **Eye contact**	Wash immediately with clean water for at least 15 minutes
• **Inhalation** (breathing insecticide fumes)	Move to fresh air. Rest and keep warm until medical help is available
• **Swallowing insecticide**	Drink lots of water. Get medical help urgently. Do not induce vomiting

In all cases get a doctor or take the person to a hospital. Take details of the insecticide, and if possible a sample label for the medical personnel.

If breathing stops, begin resuscitation immediately. Lie the person on their back. Open the airway by lifting the chin and pressing forehead back. If breathing does not resume, clear the airway by turning the casualty's head to one side and opening his mouth. Make sure there is no pesticide contamination around the mouth and no obstructions inside the mouth. Start artificial ventilation by pinching the patient's nose and blowing air into his lungs through the mouth. The chest will rise as air enters the lungs, and fall when the mouth is removed. The ventilation should be maintained until breathing re-starts or medical help arrives.

It is recommended that operators be trained in First Aid.

The environment

If an area is overdosed due to miscalculation or a calibration or navigational error, it may receive a dose which produces serious effects on other organisms, such as fish. This underlines the importance of correct calibration and accurate track spacing.

PROCEDURES FOR SEQUENTIAL AEROSOL TECHNIQUE (SAT)

Some information on SAT will help understanding of the need for spray calibration and monitoring.

Timing

Time of year

Control operations are best carried out in the cold dry season, the period of the year when the flies are least abundant, most stressed, and less widely distributed. A further advantage of this period is the reduced leaf cover which allows the spray to penetrate the canopy and minimises 'filtration' by the foliage. The dry weather means that unmetalled airstrips are not muddy, and can be used by the aircraft for landing and take off.

Spray timing and the tsetse fly life cycle

Tsetse flies are unusual insects because the females give birth to single larvae, which burrow immediately into the soil. They pupate and emerge after several days. While underground they are safe from spray, so the SAT must target the adult flies. For successful control two criteria must be met:

- no fly reproduction takes place during the operation; and
- the spraying period extends for a longer period than the pupal period.

Usually four or five sprays over a period of weeks are needed to get rid of all individuals, including pupae, as they emerge. Appendix 3 describes how spray timing is calculated.

Meteorological conditions and drop movement

Transportation of the drops to the flies is controlled by air movement so the meteorological conditions are crucial to the success of spraying. A strong wind will mix and disperse the spray, whereas calm conditions will allow it to settle vertically. Useful windspeeds for aerial spraying are in the range of 0.5-5 m/sec. Convection (warm air rising from the ground), which occurs in sunny conditions, would carry the very small drops upwards, away from the places where the tsetse flies are resting, so the technique cannot be used in these conditions.

At night or around sunrise or sunset, winds are typically light, and the sun is not generating convective turbulence. Atmospheric conditions are said to be 'stable', and there could be a temperature 'inversion', which occurs when the air temperature is cooler near the ground than higher up. Under these conditions there is little mixing of lower level air with higher layers, and hence most of the spray is retained in the useful lower region of the atmosphere. These criteria are more frequent during the evening, night, or early morning, which is why SAT is most often carried out during these periods.

Flying pattern

The aircraft emit spray while flying a parallel pattern across the tsetse-infested spray block. One aeroplane can fly alone or, to increase the workrate, several can fly in formation. A cloud of insecticide drops, so small that they fall very slowly to the ground, are released from the aircraft and are carried by air movement almost horizontally, through the vegetation. Drops reach tsetse fly resting sites, and some will deposit on the flies.

Where trees and topography allow, the flying height should be 10-15 m above the ground, to give an acceptable swath width. Too low, and spray will not be evenly distributed across the inter-track spacing, and too high would allow much of the spray to drift out of the target area.

Although the leading aircraft is normally equipped with sophisticated navigation equipment, the pilots usually fly between two vehicle-mounted marker lights which are fixed to tall telescopic masts enabling them to be seen above trees near the edges of the area. These lights are used to check the navigation equipment when necessary. The aircraft compass alone is not sufficiently accurate to guide the heading, because even a small directional error could bring the aircraft several hundred metres off track, at the end of a run of several kilometres. After reaching the edge of the spray block, the aircraft pass over the marker vehicle light and switch off the spray. They then leave the spray block, execute a procedure turn, and re-enter to pass again over the marker light, which has been moved while the aircraft was turning. The light is moved upwind along the edge of the spray block by the distance of one track spacing, which is usually 200-350 m. In this way they move across the spray block until the whole area has been treated. Future guidance systems may not require the navigational update provided by the marker lights, but it will still be necessary to verify the accuracy of the flight path by ground checks.

Spraying a large block may take several nights. It is desirable to slightly overlap the areas sprayed on consecutive nights because flies could move the short distance from an unsprayed area to a treated area during the daytime and thus avoid the following night's spray. Each night, spraying should start at the downwind edge of the area to be treated, so that the cloud of insecticide moves away from the region which will be sprayed later. This avoids flying through insecticide spray.

Spraying equipment

The spraying equipment most commonly used to produce the aerosol is the wind-driven Micronair AU4000 rotating cage atomizer (Figure 1). One or two of these are usually

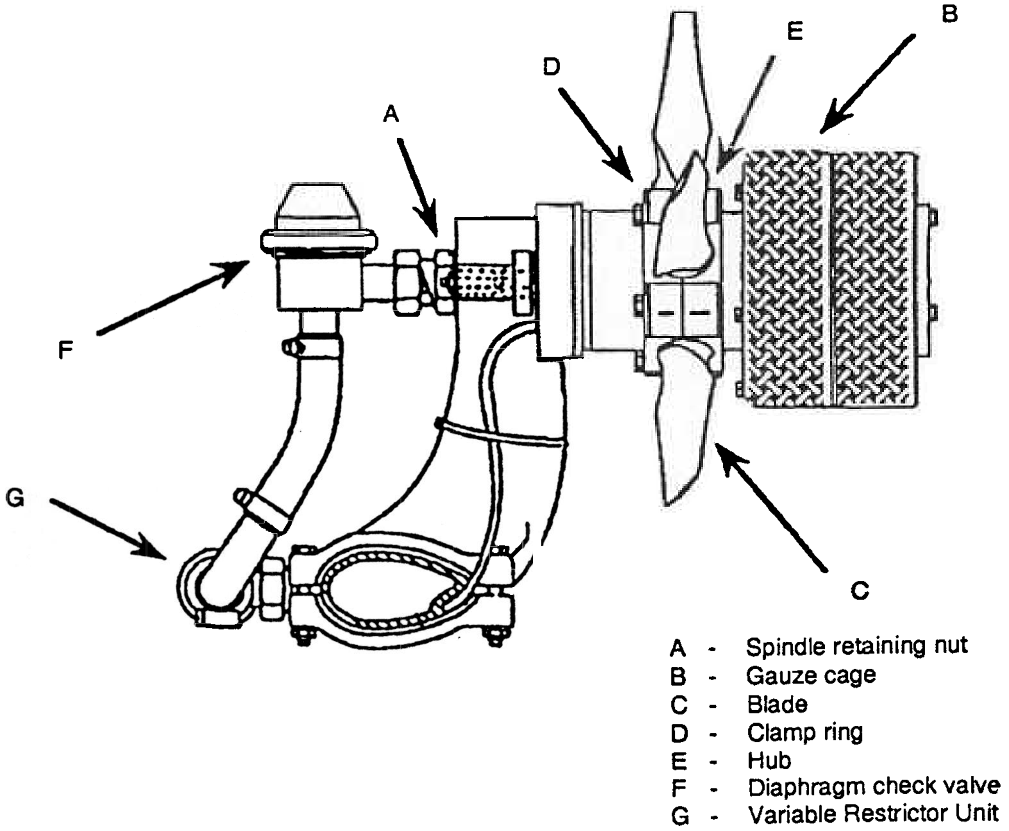

Figure 1 Aircraft-mounted Micronair AU4000. The gauze cage is rotated by air acting on the propellor blades.

mounted on the aircraft fuselage for tsetse control work. Cockpit monitors for flow rate and atomizer rotation speed allow the pilot or aircrew to make sure the required values are being maintained during flight. Atomizer rotation speed is dependent on the aircraft speed and the angle of the propeller blades. These blades are adjusted by slackening the five bolts on the clamp ring and rotating each blade (Micronair, 1991). Drops are produced by two processes; by being thrown off the atomizer by the rotation, and by being broken up by the air shear at the surface of the cage. An in-flight rotation speed of around 10 500 to 11 000 rpm will produce a drop spectrum at the ground level suitable for tsetse control, provided the correct insecticide formulation has been used. Drops do not leave the atomiser at the correct size, but become smaller as the volatile part of the spray evaporates. When they leave the atomizer drops are around 60-70 um, but quickly reach 20-25 µm. The chemical company supplying the insecticide will have formulated the product to take account of this requirement. Standard ultra-low volume (ULV) formulations used in agriculture are not suitable, due to an insufficient volatile component. Without evaporation of the solvent, drops would remain too large and fall to the ground, rather than drifting through the vegetation canopy. If the drops could be produced nearer to the required final size the formulation would not need the volatile component. The size of aircraft spray tank to treat a given area would be greatly reduced (although the quantity of active ingredient of insecticide would be the same). However, there is not a suitable atomizer capable of producing 20-25 µm drops at the present time.

Drop size

Most pesticide from conventional sprayers, such as a knapsack sprayer, a tractor-mounted boom, or an agricultural aircraft, is in large drops which fall to the ground or onto the crop in a few seconds. The drops used in the drift spraying technique are much smaller. They are referred to as aerosols, and fall very slowly to the ground (sedimentation). Because of their size they tend to be carried around by breezes like dust or smoke.

AIRCRAFT CALIBRATION

This is the process of checking and adjusting spraying equipment on the aircraft to give the correct flow rate and drop size.

The flow rate, which is the volume (l/min) delivered by the insecticide pump to the atomizer, is measured in flight by an electronic flow meter. This device must be checked prior to use as follows: with the aircraft on the ground, partly fill the tanks, then switch on the pump and collect the liquid from the stationary atomizer in a bucket, over a period of several minutes. Water can be used for this initial flow rate calibration. While making this flow check, the agreement between the cockpit display and the volume collected in the bucket is checked. The flow meter must give a true indication of liquid emission rate.

Required flow rate

The flow rate needed to give the correct area application rate can be calculated from first principles using the speed of the aircraft, the track spacing, and the volume application rate, or by using the following formula.

In litres per minute the flow rate is:

$$\frac{\text{track spacing (m)} \times \text{application rate (l/ha)} \times \text{speed (kph)}}{600}$$

For example, using a track spacing of 250 m, a volume application rate of 0.12 l/ha, and a flying speed of 180 kph would require a flow rate of:

$$\frac{250 \times 0.12 \times 180}{600} = 9\ \text{l/min}$$

This flow rate is high for a single atomizer, and two would be preferable.

Drop size calibration

Drop size can only be checked during flight because the forward speed of the aircraft is required to spin the atomizer. To calibrate the drop size the aircraft is flown over an array of rotary samplers (Figure 2) laid out on the ground, which collect the spray drops. This is done prior to the actual spraying operation.

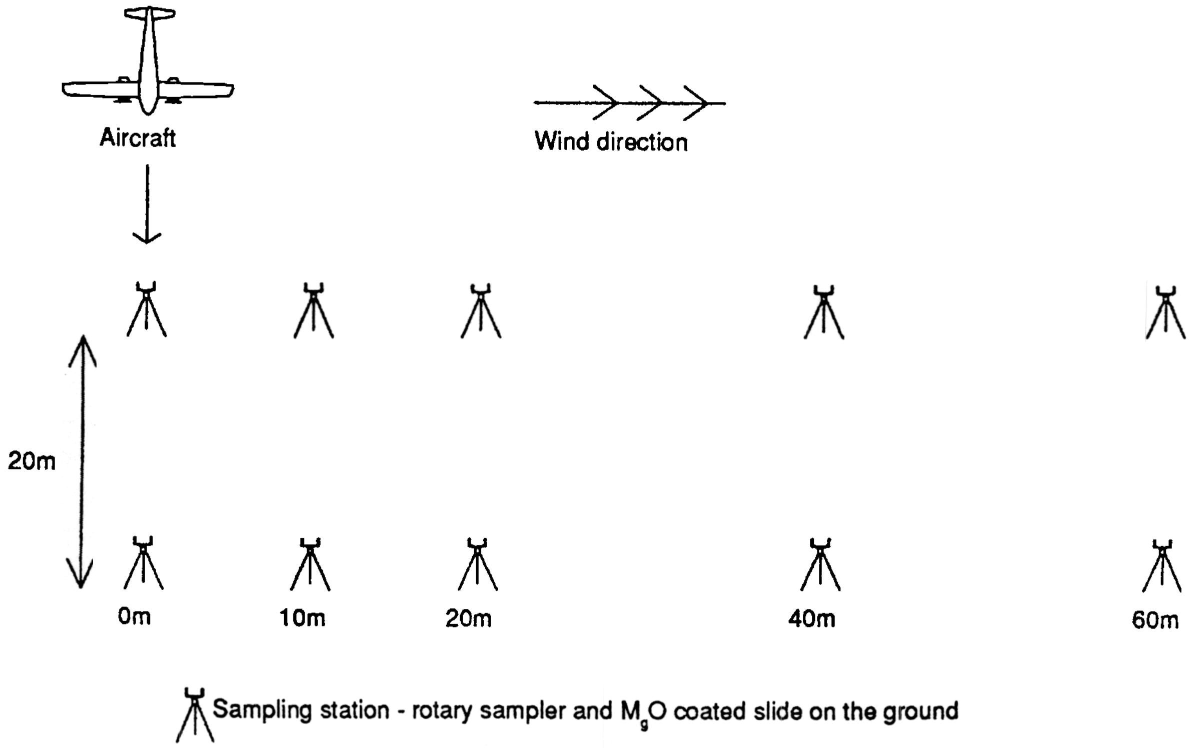

Figure 2 Layout of rotary and ground samplers for drop size calibration

Collecting drops

The spray sampling equipment used in drop size calibration and spray monitoring during the control operation is the rotary sampler. This (Figure 3) consists of a slide holder which is 13 cm across, mounted on the spindle of an electric motor, which rotates the assembly at around 420 rpm when connected to a six volt battery. Two drop sampling slides are placed in the holder. Before fitting, these slides (0.6 cm wide) are coated with a thin layer (approx 0.25 mm) of magnesium oxide (MgO). This is done by burning magnesium ribbon underneath them as they are held on a stand. The ribbon can be lighted with a cigarette lighter or two matches burning together. The burning magnesium should be held about five centimetres below the slide, so that the smoke, which contains the oxide, deposits on the glass to form a layer. Magnesium burns very intensely, and protective gloves and goggles should be worn whilst preparing the slides, and the ribbon must be held using pliers or forceps. The MgO-coated slides are prepared beforehand and transported to and from the field in a modified microscope slide box, which has been padded to prevent damage to the delicate oxide layer. Slides are placed in the sampler so that the MgO layer faces forward as the sampler rotates in a clockwise direction.

The grid (Figure 2) of rotary samplers is laid out on the ground in an open area in the same direction as the wind. The slides are then inserted into the holders, and the motors are switched on when the aircraft are ready. The aircraft flies past along the upwind edge of the sampling array. After allowing several minutes for all the airborne spray to be carried past the samplers, they are switched off and the slides are labelled and returned to the slide box. All slides are examined later by microscope in a darkened room.

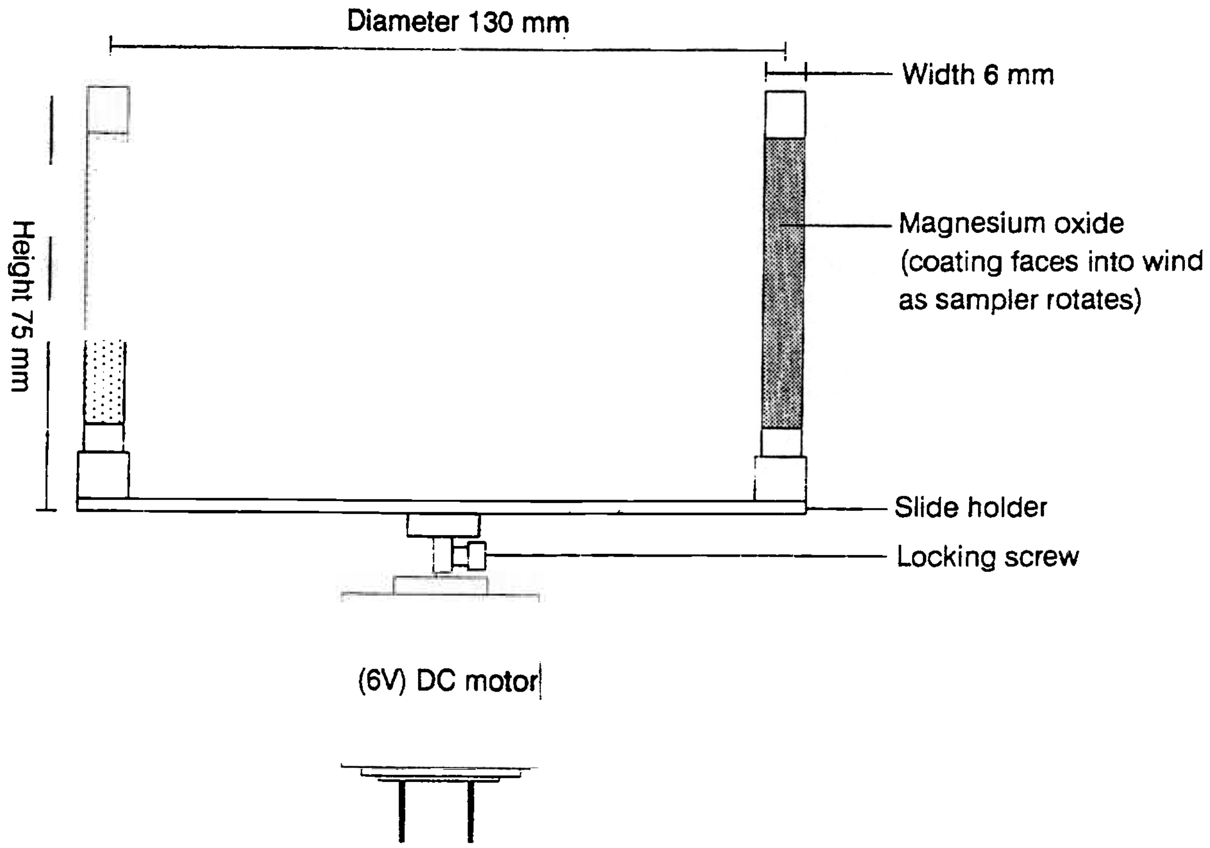

Figure 3 Diagram of electrically-powered rotary sampler

Counting and sizing of drops

Using a microscope with a magnification of x100, the spray drop craters can be clearly seen when illuminated from below with transmitted light. If a fluorescent dye has been put in the spray tank, viewing under ultra-violet light allows spray to be distinguished from other drops, such as water mists. White light should then be used when measuring the size of the drops, as fluorescing drops may appear larger than the crater they are in.

The microscope eyepiece should be fitted with a Porton G12 graticule, printed with a series of different sized lines and circles. Before using the graticule, the circle sizes are determined by measuring the largest circle using a stage micrometer. This is a very small linear 'ruler' etched onto a glass slide (Appendix 5). Each circle is smaller than the adjacent one by a factor of 0.707 (1 / square root of 2) on the Porton graticule.

The eye sees the graticule image super-imposed on the drops on the slide. This allows the drops to be compared with the measure on the graticule, so that individual drops can be sorted into size classes, rather than accurately measuring the diameter of each drop. A drop larger than circle four on the graticule, but smaller than circle five, is placed into class five by convention. All drops in that class are between the diameter of circles four and five, and the actual diameter depends on the magnification. The geometric mean diameter of each class is used in subsequent calculations. The best way to read this data is to have one person using the microscope and calling out the size class of drops as the slide is examined, while a colleague writes the results on a prepared scoresheet (Appendix 6). A mark is made in the size class column for each drop called. Deposition of drops is not uniform across the slides from the rotary sampler because of the aerodynamic characteristics of the sampler. Smaller drops tend to impact nearer the edge of the slide, so each slide is examined from edge to edge to obtain an unbiased sample of drops. The presence of very large drops indicates an atomization problem or a leak in the pipework.

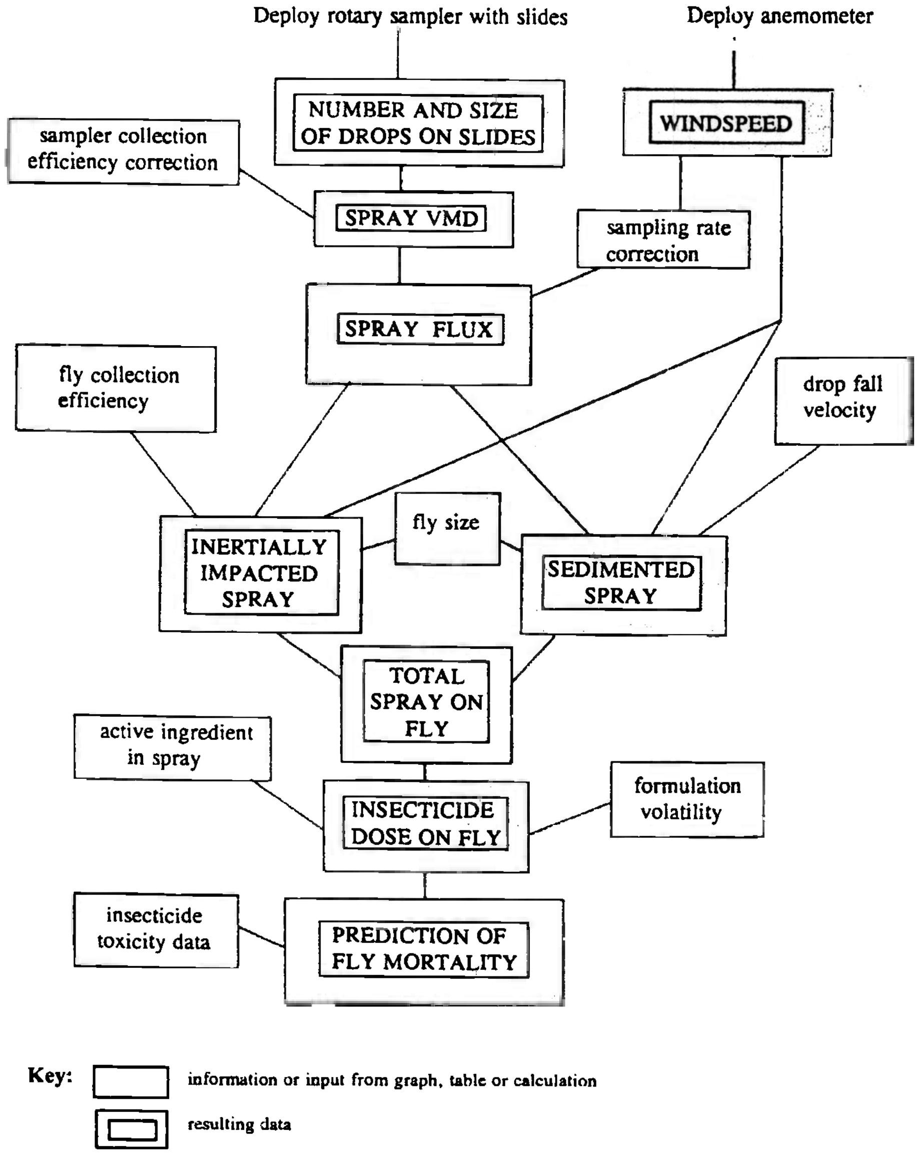

Figure 4 Flow diagram for determination of VMD, spray flux, number of drops/dose on the tsetse fly and predicated mortality

Between 50 and 100 drops can give a good estimate of the drop size distribution. This data from the samplers is sufficient for drop size calibration, when corrected for the differential collection efficiency of each drop size class (needed because bigger drops collect better). Further calculations such as the determination of spray flux require windspeed (Figure 4).

The raw data from the samplers, that is the number of drops in each size class, do not give a true picture of airborne drops because, despite spinning, the sampler collects large drops more readily than small ones. A proportion of the smaller drops go round the rotating slides, with the airstream, rather than impacting, so a correction factor is required for smaller drops.

Figure 5 shows the relation between drop diameter and collection efficiency of the rotary sampler. This was determined empirically in a wind tunnel. Using the graph in Figure 5 the respective correction for each size class can be obtained. For example if eight drops of mean diameter 20 µm were counted on a slide, from Figure 5 the collection efficiency is 36% (an average of 64% did not impact, but passed around the slide with the slip-stream), so the size class correction factor is 100/36 = 2.78, and the number of drops used in the size distribution calculation would be 8 x 2.78, ie. 22.2. The effect of windspeed on collection efficiency relating to different drop sizes is small enough to be ignored in this sampler drop size correction.

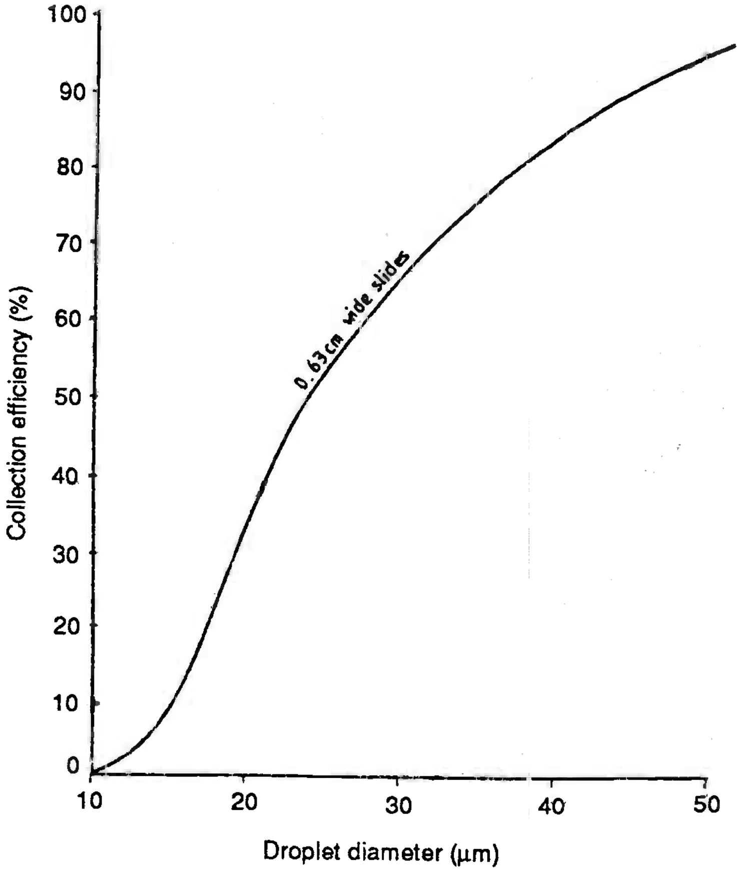

Figure 5 Variation of droplet collection efficiencies of droplet samplers over windspeed range 0.25-1.5 m s^{-1}

After making these corrections to the raw data (i.e. numbers of drops in each size class) the corrected data is used to calculate spray size spectrum. The term used to describe spray size is Volume Median Diameter (VMD). This is the diameter at which half the volume of spray is contained in larger drops, and half in smaller drops. The VMD can be derived graphically, by plotting the cumulative volume percentage against drop diameter, on log probit paper, then reading off the diameter at the 50% point. There is an example of this in Appendix 1. The same method can be used to calculate Number Median Diameter (NMD) which is the diameter at which half of the spray drops are larger and half smaller (using cumulative number instead of volume on the graph).

Alternatively VMD and NMD can be obtained by using a computer which is programmed to carry out the calculation, *see* Appendix 2.

MONITORING SPRAY DURING THE CONTROL OPERATION

Meteorological monitoring

Windspeed and direction are important to drift spraying. When meteorological conditions, particularly windspeed, are close to the acceptable limits, it is useful to have equipment (Appendix 5) to record these conditions so that any trends can be seen. This will help in deciding whether or not to proceed with spraying. Meteorological stations situated in different regions of the spray block, record windspeed and direction and will determine how uniform conditions are over the area. Different wind directions are a potential source of problems because there may be areas getting little or no drifted spray, allowing pockets of fly survival.

Deployment of samplers

The same rotary samplers are used to monitor the spray as are used to calibrate drop size. They are set out in different parts of the spray block before spraying starts. Windspeed must be known to calculate drop flux, so a cup anemometer (Appendix 5) is positioned close to each sampler to measure speed directly or to measure wind run. The mean windspeed is calculated using wind run and the length of time the anemometer was operating. Both sampler and anemometer can be switched on and off by a timer unit (Appendix 5). All equipment is powered by a six-volt battery, and it is convenient to have timers to switch on the equipment to coincide with spraying because distances between samplers make manual switching difficult. The use of timers pre-supposes that the scheduled spraying takes place as planned, and this can be a problem if spraying is delayed because they will switch on the samplers at the wrong time. Problems can be minimized by setting timers to run the equipment for a period before and after spraying is expected, but this reduces the precision of the windspeed measurement when spraying takes place.

As the oxide-coated slides rotate, they sweep spray drops from the air. They may also collect water drops from mists if the humidity is high, so to help distinguish between insecticide and water drops a small quantity of fluorescent dye (0.02-0.05% Uvitex OB or similar, Appendix 5) can be mixed into the spray formulation before it is loaded into the aircraft spray tanks. Viewed under ultra-violet light the fluorescing insecticide drops can be distinguished from mist drops.

After spraying, the samplers are collected and examined in the same way as those used to calibrate drop size.

If the windspeed has also been measured at each sampler site, the spray flux i.e. the quantity of spray passing through the unit vertical area (or a 1 cm square imaginary window) can be determined. The spray flux is a parameter which can be used to compare the amounts of insecticide reaching different parts of the spray block, and give an indication of how much variation in fly dose rate can be expected.

Estimation of spray flux

Number flux (q_n) is the number of drops moving horizontally through a vertical 'window' of one square centimetre. Volume flux (q_v) is the volume contained in those drops.

Sampling rate correction

As the sampler rotates it may not sweep all the air which passes it. The faster the windspeed, the more air will pass through without being swept by the slides. This means data from the sampler must be corrected to take account of this non-sampled proportion, which varies with windspeed (Cooper, 1987).

A factor of 0.44 x windspeed x (corrected) number of drops per square cm on the slide gives the horizontal number flux (q_n) for the dimensions and speed of the sampler described here. Hence if the windspeed is two metres per second, and the slides have 15 drops per square centimetre (corrected):

$$q_n = 0.44 \times 2 \times 15 = 13.2 \text{ drops}$$

Volume flux (q_v) depends on drop size as well, and is number flux x volume of the drops:

$$q_v = \text{volume} \times q_n$$

Estimating fly dose

A summation model involving volume flux (q_v), windspeed (u), fly size and collection efficiency is used to estimate the volume of spray contacting the fly, and hence the dose of insecticide which it receives can then be calculated from the concentration of active ingredient in the original formulation and the proportion of the original drop which has evaporated.

Fly collection efficiency

Impaction of a drop on a resting tsetse fly is influenced by the diameter of the drop and the windspeed. Larger drops are more likely to impact (higher collection efficiency) in a manner analagous to their impaction on the samplers, and the chance of impaction on the fly is increased at higher windspeeds. Collection efficiencies (Figure 6) for particular drop diameter and windspeed combinations have been determined empirically in a low speed wind tunnel using pinned flies (Johnstone *et al* ,1989). Extrapolation is necessary with field data outside the range of drop sizes and windspeeds.

Fly size

The size of flies varies with species. A measure of projected vertical area (a_v), and projected horizontal area (a_h), can be made holding the fly against graph paper marked in square centimetres.

Volume of spray reaching the fly

(a) Inertial deposition (impaction) (q_i)

Volume from impaction (q_i) = volume flux (q_v) x collection efficiency (CE) x projected vertical fly area (a_v)

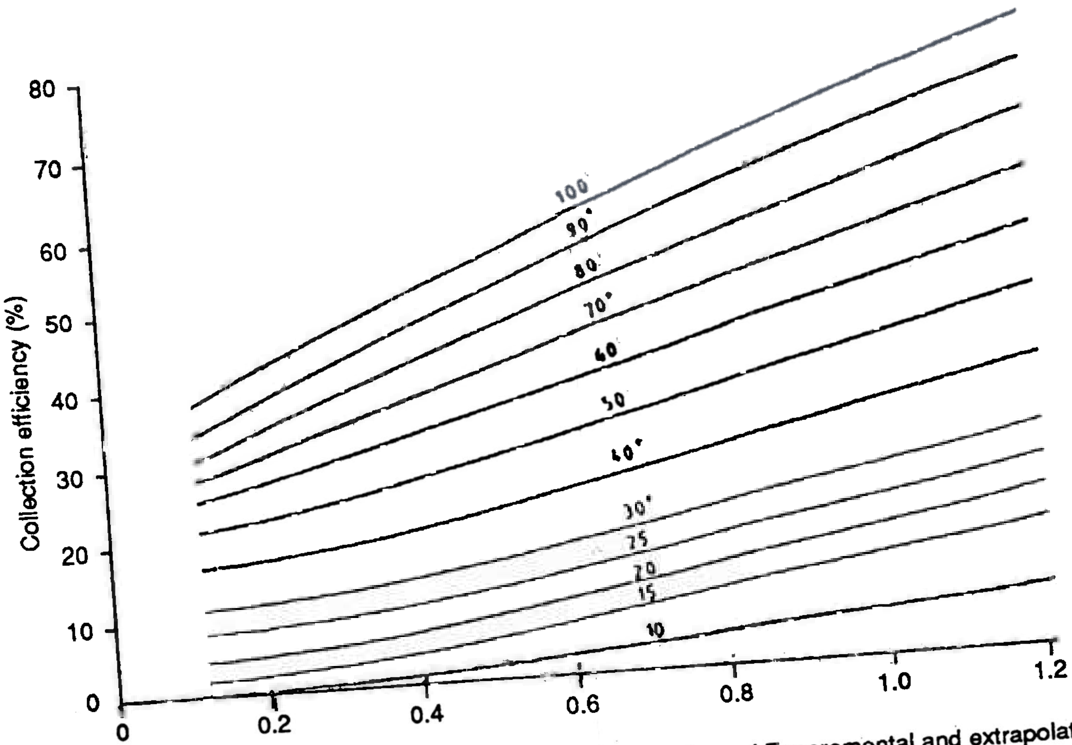

Figure 6 Drop collection efficency for different drop sizes and windspeed Experemental and extrapolated*

(b) Sedimentary deposition of drops (q_h) onto flies

The quantity falling through the unit horizontal area q_h, can be calculated from volumetric flux (q_v) windspeed (u) and drop sedimentation velocity (v_s) using the ratio of windspeed to sedimentation velocity (the latter can be looked up in Appendix 4)

$$q_h/q_v = v_s/u$$

so that sedimentary deposition

$$(q_h) = q_v(v_s/u)$$

volume which sediments on the fly

$$(q_s) = q_h \times \text{projected horizontal fly area } (a_h)$$

Total volume received (q_t) is made up of inertially collected volume (q_i) and sedimented volume (q_s).

$$q_t = q_i + q_s$$

Proportion of insecticide in spray drop

When the spray reaches the sampler (or insect) it has lost much of the volatile solvent in the formulation, and so the active ingredient (insecticide) has become more concentrated than in the original mixture.

When using the insecticide endosulfan, which is usually supplied as a 20% or 30% formulation for tsetse spraying, the volatile components will have evaporated after a few seconds. The 'mature' drop will then be mainly insecticide e.g. 90%, (proportion P = 0.9) with a certain amount of involatile co-formulants. This figure can be used in calculating insect dose.

With deltamethrin, the original formulation contains a much smaller proportion, typically 0.35%, of active ingredient, because of the high activity of the insecticide. So that the drop does not rapidly evaporate to produce a deltamethrin dust, non volatile components are included in the formulation. The final 'mature' drops, after evaporation of the volatile component, will consist of the active ingredient dissolved in this involatile component.

The proportion (P) of insecticide in the 'mature' drop, to which the fly is exposed, can be calculated from the diameter of the drop and ratio of involatile/volatile components in the original formulation.

For example if the original formulation ratio were 25:75, the sampled spray drop would have a volume near to a quarter of the original drop volume at emission. This drop would contain all the original active ingredient because the insecticide deltamethrin is not very volatile, so if the formulation was supplied at 0.35%:

$$P = 0.35 \times \frac{1}{0.25} = 1.4\% \text{ (P or 0.014)}$$

An alternative estimation of the relationship between drop size and evaporation which was derived empirically, is given by Johnstone (1987).

PREDICTION OF MORTALITY

Insecticide dose and variation

The final estimate of insecticide dose per fly is calculated from:

total volume (q_t) x the proportion of insecticide in the 'mature' drop (P)

This dose variability between sampling sites would be approximately equivalent to the variation in spray flux unless marked differences in drop size or windspeed occur between sites. Both markedly affect impaction efficiency of drops on the fly.

After making the dose estimates from all the field data, each estimate of the mass of insecticide on the fly can be compared with toxicological data for that particular insecticide, to give a picture of the likely fly mortality. There may be areas where fly underdosing is indicated, allowing survival.

Insecticide toxicity

Both the insecticide toxicity and the amount reaching the insect contribute to mortality. Temperature may also have a bearing on the toxicology. The toxicity of endosulfan is positively correlated with temperature, while deltamethrin has a negative correlation. This is demonstrated in laboratory results (Smith, personal communication) using laboratory-reared *Glossina morsitans* and *G. pallidipes*. At 18°C the LD50 value for endosulfan against *G. morsitans* was 6.9 ng decreasing to 2.77 ng at 25°C and 1.9 ng at 30°C, which is a factor of four. Corresponding values for deltamethrin are 0.003 ng at 18°C, 0.06 ng at 25°C, and 0.18 ng at 30°C.

Glossina pallidipes showed a similar pattern when dosed with deltamethrin, although the LD50 value was around ten times higher. At 18°C the LD50 value was 0.31 ng, rising to 0.57 ng at 25°C and 1.16 ng at 30°C. Wild *G. pallidipes*, and especially pregnant females, showed lower susceptibility to endosulfan, with a mean at 25°C of 7.5 ng and 15.2 ng for males and females respectively (Fenn, in press).

The final stage of the data processing is to compare the estimated field dose of insecticide with toxicity data for the particular species in the spray block. Johnstone (1990) has used a value of three times the insecticide LD50 as a yardstick to define potential underdosed areas which are likely sites of surviving flies.

The survival prediction

In practice, when the calculated dose prediction is compared with the LD50 of the insecticide, the estimate of mortality described above can be lower than the results achieved. This may indicate that the model contains an under-estimation of one of the collection parameters, or it may have omitted a component of the process which would increase the dose of insecticide received by the fly . Secondary factors may increase mortality. It is assumed for example, that the tsetse flies do not fly when the cloud of insecticide passes. If they did, collection would be considerably greater. It is also possible that sub-lethal doses of insecticide impairs behavioural instincts such as the ability to avoid predation, and may also suppress the urge to find shade during the day, resulting in death through overheating or dehydration.

Survival

If fly mortality predictions indicate possible survival, fly surveys must be carried out in the area in question. If surveys confirm survival, it is important to determine the reason, and repeat spraying without delay to prevent surviving females from ovipositing.

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APPENDIX 1

GRAPHICAL DETERMINATION OF THE VOLUME MEDIAN DIAMETER (VMD) AND NUMBER MEDIAN DIAMETER (NMD)

After the magnesium oxide-coated glass slides have been removed from the rotary sampler they are examined under a microscope fitted with a Porton G12 graticule. This enables the drops to be individually sized into the respective classes, by reference to the circles on the graticule. Prior to the drop count, the circles, which define the upper and lower size class limits, are measured using a stage micrometer, which is a microscopic ruler etched onto a glass slide. It is easier to measure the largest circle, then multiply by 0.707 (1/square root of 2) to calculate the rest of the class size limits. A drop larger than circle six, but smaller than circle seven would be scored as class seven etc. After 50 - 100 drops have been sized in this way the number in each size class is summed to give a total. This is the basic raw data which, after correction for the different collection efficiencies (see handbook text), will be used to calculate NMD and VMD. The crater made by the drop in the oxide coating is larger than the drop, and a factor of 0.86 is used to find the true drop size, hence a 55 µm crater was formed by a 47.3 µm drop (55 x 0.86).

The geometric mean diameter (D) of each class is used in the calculation. This mean, weighted towards the upper size limit is the square root of (lower limit x upper limit).

The number in each size class (N) is then corrected (NC) for the sampler collection efficiency to allow for the fact that smaller drops are not collected efficiently (*see* Figure 4).

Number Median Diameter (NMD

The number in each size class divided by the total number of drops is the proportion, or percentage of the drops in that class. This is added for each increasing size class to determine the accumulated number (NCA). This is plotted against the mean drop size, see below. At the 0.5, or 50% point, the NMD is read off the graph.

Volume Median Diameter (VMD)

The volume of a drop is proportional to the cube of the diameter. Each drop geometric mean size class diameter is cubed to determine the relative volume (D3). For convenience in making the graph, this is multiplied divided by 1000 to render all the numbers smaller, and hence easier to handle. This does not affect the result.

To determine the volume of all the drops in a particular class, the relative volume of a single drop is multiplied by the number in that class (NC x D3). The total volume (the sum of all size classes) is derived in the same way as the NMD above. The accumulated proportion or percentage (VA) is then determined. As with the NMD this accumulated proportion is plotted against the mean drop size, and the VMD read off at the 0.5 (50%) point. Log probit paper has a suitable scale to enable the values to be taken from the graph.

Table A1.1 Data table for graphical determination of VMD and NMD

Class upper limits MgO (µm)	Actual size MgO x 0.86 (µm)	No. in class N	Corrected no. in class NC	Accum. % no. in NCA	Geometric mean D	Vol D3 x10-3	NCxD3 vol	Accum. % vol. VA
11.3	9.7	1	100.0	16.8	8.1	0.53	53.0	2.2
15.9	13.7	8	200.0	50.4	11.5	1.52	304.0	13.6
22.5	19.3	32	176.0	80.0	16.3	4.33	762.0	42.7
31.8	27.3	61	116.0	99.5	23.0	12.10	1403.0	96.3
45.0	38.7	2	2.8	100.0	32.5	34.20	95.8	100.0
63.6	54.7	0			46.0	97.30		
90.0	77.4	0			65.1	276.00		
	Total	104	595.0				2617.8	

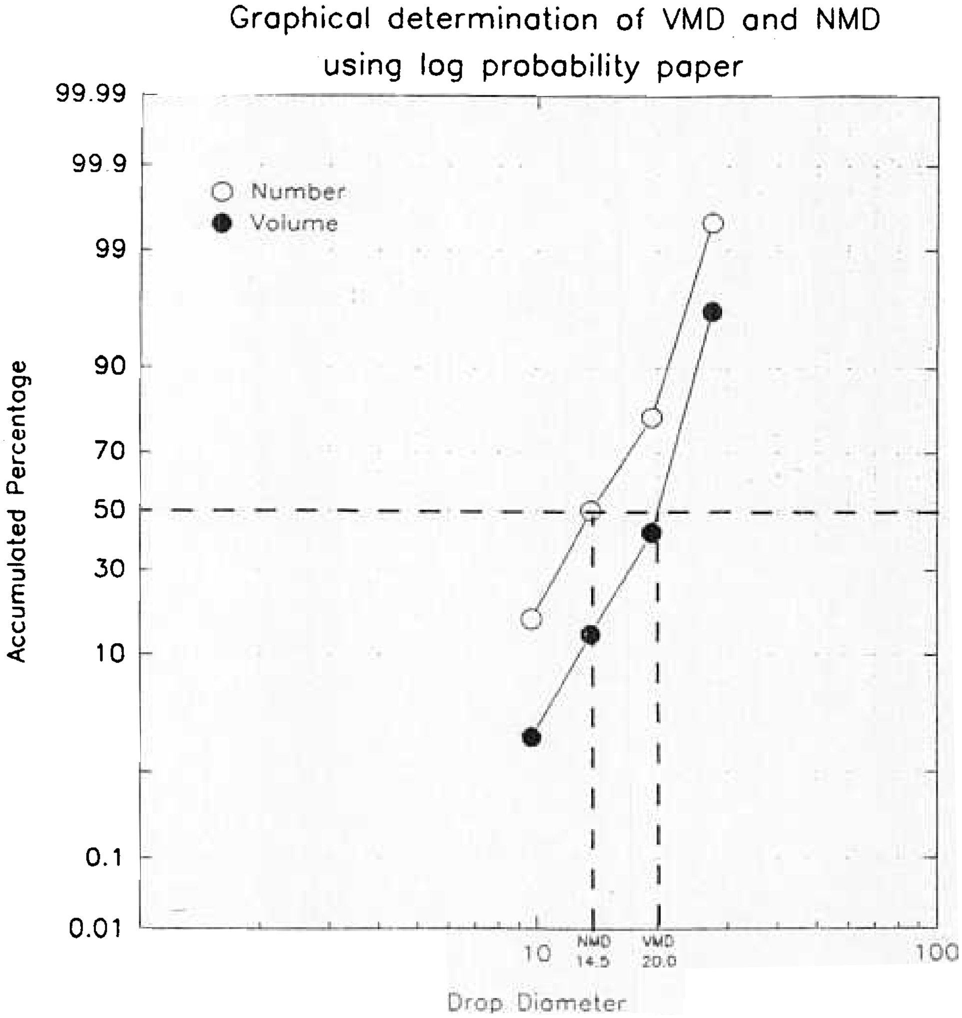

Figure A1.1 Graphical determination of VMD and NMD

APPENDIX 2

BASIC PROGRAM FOR COMPUTER DETERMINATION OF THE NMD AND VMD OF A SPRAY SAMPLE

```
10 REM NRI PROGRAM FOR SPRAY SIZE ANALYSIS
20 INPUT "HOW MANY SIZE CLASSES ARE THERE"; C
30 DIM N(C): DIM S(C): DIM P(C): DIM V(C): DIM R(C)
40 INPUT "ENTER LOWER SIZE LIMIT OF SMALLEST SIZE CLASS"; S(0)
50 FOR M=1 TO C
60 S(M)=(2^0.5)*S(M-1)
70 NEXT M
80 PRINT "UPPER SIZE LIMIT OF LARGEST DROP IS ";INT(S(C)+0.5)
90 LET T=0:LET X=0
100 FOR M=1 TO C
110 INPUT N(M)
120 LET T=T+N(M)
130 NEXT M
140 PRINT "TOTAL DROPS ENTERED ";T
150 FOR M=1 TO C
160 LET P(M)=(N(M))/T+P(M-1)
170 IF P(M)=0.5 THEN PRINT "NMD = ";INT(S(M)+.5): LET M=C
180 IF P(M)>0.5 THEN LET X=S(M-1)+((S(M)-S(M-1))*(.5-(P(M-1)))/(P(M)-P(M-1)))
190 IF P(M)>0.5 THEN LET M=C
200 NEXT M
210 IF X>0 THEN PRINT "NMD IS "; INT(X+.5)
220 LET T=0
230 LET F=4/3(pi)
240 FOR M=1 TO C
250 V(M)=(((S(M)*S(M-1))^.5)*.5)^S*F
260 NEXT M
270 FOR M=1 TO C
280 R(M)=N(M)*V(M)
290 LET T=T+R(M)
300 NEXT M
310 FOR M=1 TO C
320 Q(M)=R(M)/T+Q(M-1)
330 IF Q(M)=0.5 THEN PRINT "VMD IS ";INT(Q(M)+.5):LET M=C
340 IF Q(M)>0.5 THEN LET X=S(M-1)+((S(M)-S(M-1))*(.5-(Q(M-1)))/(Q(M)-Q(M-1))): LET
M=C
350 NEXT M
360 IF X>0 THEN PRINT "VMD IS ";INT(X+.5)
370 PRINT "TOTAL VOLUME IS ";INT(T/1000+.5)
380 GOTO 80
390 END
```

Notes:
Volume is in picolitres if size units are µm.

To add a calculation of number and volume per centimetre on the slide (useful for ground samples), add the following extra lines. First measure the area of the microscope field, using the stage micrometer, and calculate how many fields are in one square centimetre. For example if the field of view were 0.27 cm^2 then there are 1/0.27 (=3.703) fields in a square centimetre. Put 0.3703 in lines 145 and 375 in place of [FOV/CM].

```
135 INPUT "HOW MANY FIELDS WERE EXAMINED ";B
145 PRINT "NUMBER OF DROPS PER CM IS ";T/B*[FOV/CM]
375 PRINT "VOLUME (PL) PER CM IS ";T/1000/B*[FOV/CM]
```

The program uses FOR:NEXT loops (starting with the counter, M, at 1, and increasing M each circuit, until M=C (C is the number of size classes). Data is input or processed as the loop repeats, and when it has gone through C loops it progresses to the next line of the program.

At the start of the program, on entering the lower size limit of the smallest class [line 40], the loop uses the size progression from the eyepiece graticule, which is the square root of 2 (1.414), to define all the class limits (upper and lower).

In calculating the NMD (and VMD) the program examines the accumulated percentage number and volume. When this is 50% the NMD and VMD have been reached. If the 50% point does not coincide with a class limit the program calculates the NMD and VMD based on the class limits above and below the 50% point, on a proportional basis.

Before entering data from rotary samplers (but not ground samplers) the numbers for each size class must be corrected for sampler collection efficiency because of the tendency for smaller drops to go round the slides.

When the correction for each size class has been looked up from the graph in Figure 5 (and this depends on the size classes, and hence the microscope/graticule combination) the program could be modified by inserting the respective multiplier for each size class S(M) to do the correction automatically, but this has not been written into the program because it will depend on the graticule/microscope combination.

Computer spreadsheet software such as Supercalc can be used as an alternative to this program, to calculate these spray parameters, and also additional information such as the fly dose estimation. A copy of the 'template' for users of Supercalc can be obtained from the authors.

APPENDIX 3

CALCULATING THE TIME INTERVAL BETWEEN SPRAYS AND PUPAL PERIOD

First larval period

The first spray should kill all the flies. Newly emerged flies start to appear soon after spraying and the second application must be timed to kill them before they have a chance to reproduce, that is to deposit new pupae in the soil (larviposition). This is called the first larval period (FLP), (or days from emergence to oviposition) (Allsopp, 1990). The reproductive rate is governed by temperature, so spray timing depends on temperature. Warmer conditions speed up development, and the time for the female to reach sexual maturity is shorter when the temperature is higher. Hence the interval between the two sprays must be shorter so that females emerging after one treatment have insufficient time to deposit larvae before the next. The formula used to calculate the FLP, and hence spray interval is given below:

$$\text{First larval period} = \frac{1}{0.0661 + 0.0035(t-24)}$$

where t is accumulated mean air temperature in degrees centigrade.

If the mean temperature was 18°C, the days from emergence to larviposition, referred to as first larval period by Allsopp, comes out as 22 days in the formula. For each day at that temperature, a proportion (1/22 or 0.045) of the development takes place. This daily development can be accumulated and extrapolated to determine approximately when larviposition is expected. The planning and logistics require advance notice in order to synchronise preparations for spraying, so it is necessary to make a guess on likely temperatures, anticipate the date of larviposition, and spray beforehand. Clearly spraying must take place before any larviposition occurs, so spraying takes place two to three days before the calculated time to larviposition.

Pupal period

Pupal period, which is the duration of the life cycle spent in the soil undergoing metamorphosis into an adult fly, is also shorter in higher temperatures. The entire spraying operation must continue for a sufficient period to ensure that any adults emerging from the soil are sprayed. If the pupal period was, for example, seven weeks it would be pointess to stop spraying after six weeks because flies would then emerge and re-infest the area. In practice five sprays (with intervals calculated as described above) cover a span of time which is long enough to give a safety margin and ensure that there is no residual reservoir of soil-borne pupae at the end of the control operation. The formula used to calculate pupal period is:

$$\text{Pupal period (days)} = \frac{1}{0.0323 + 0.0028(t-24)}$$

For each of these two calculations the average daily air temperature (t) is required, so it is necessary to set up a recording thermometer, or at least a maximum-minimum thermometer at the spray site. The accumulated average temperature is used in the formulae. For example if the temperature on the first day was 15°C, followed by two days at 16°C, the accumulated mean temperature would be:

$$\frac{15 + 16 + 16}{3} = 15.66°\text{C}$$

If this mean temperature is used to calculate pupal period in the above formula, as follows

$$\text{Pupal period (days)} = \frac{1}{0.0323 + 0.0028(15.66\text{-}24)} = 112$$

At the higher temperature of 25°C, the pupal period is 34 days, so it can be seen that temperature has a marked effect on the development of the flies.

APPENDIX 4

SEDIMENTATION VELOCITY (M/S) OF DIFFERENT SIZE DROPS IN AIR (SPECIFIC GRAVITY 1)

Diameter (µm)	Sedimentation rate (m/s)
8	0.002
10	0.003
12	0.004
14	0.006
16	0.008
18	0.010
20	0.012
22	0.015
24	0.017
26	0.020
28	0.024
30	0.027
32	0.030
34	0.034
36	0.038
38	0.040
40	0.048
42	0.053
44	0.058
46	0.063
48	0.068
50	0.070
52	0.075
54	0.080

APPENDIX 5

EQUIPMENT: MANUFACTURERS AND SUPPLIERS

Spraying equipment

Micronair Limited, Bembridge Fort, Sandown, PO36 8QS, UK.
Tel:0983 406111

Meteorological equipment

Vector Instruments Limited, 113 Marsh Rd., Rhyl, Clwyd, LL18 2AB, Wales.
Tel:0745 350700

Wessex Power Technology Limited, 189 Ashley Rd., Poole, BH14 9DL, Dorset, UK.
Tel:0202 723000

Graticule and stage micrometer

Graticules Limited, Morley Road, Tonbridge, Kent, TN9 1RN, UK.
Tel:0732 359061

Rotary sampler motor

Radio Spares Limited, P.O.Box 99, Corby, Northants, NN17 9RS, UK.
Tel:0536 201234

Timer (6 volt)

Controlplan, 11 High St., Dronsfield, Chesterfield, S18 6PX, UK.
Tel:0246 411154

APPENDIX 6

Sheet No.
Date:

Formulation: % a.i.
Location:

Pass area (sq cm)

	Slide ref.	C4	C5	C6	C7	C8	C9	C10	C11	No. of passes	Total drops	NMD µm	VMD µm	Mean wind speed m/s	Spray flux
Tally															
Total															
Corr.															
Tally															
Total															
Corr.															
Tally															
Total															
Corr.															
Tally															
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