FIELD EXPERIMENTATION

INSECT PATHOLOGY MANUAL

Section V

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INTRODUCTION

To develop new pesticidal formulations you must do bioassays in the laboratory. Insect pathogens which can kill insects in the laboratory must than be tested extensively in the field before they can be recommended for use as control agents. A micro-applicator is often used in tests on locusts in the laboratory, where drops (usually in the range of 0.5-2 μl) are applied to test insects; at ULV field rates a locust might receive 10nl of formulation at most (less than 1/100th of the volume in a bioassay).

Depending on the scale of the trial, you may be able to grow fungus up in bottles before embarking on full scale mass production. Field trials can be very difficult and may use up resources without yielding worthwhile results. In this section we discuss a "step by step" approach, with an increase in the scale of trials at each stage (*Table 5.1*). Be clear about the context and objectives of trials and ensure success at each level before moving on to the next.

Table 5.1
FIELD TESTING STAGES

Preliminary	from bioassay to the field, arena and cage trials on station
Small scale	typically < 1 ha plots
Medium scale	typically 1 - 50 ha plots
Simulated operations or large scale trials	aerial trials could consist of blocks 100 km^2(e.g. desert locust swarms) or consist of a number of small-medium scale plots.
At each stage verify:	**What is the objective of the trial?** **Is the trial a logical progression from previous work?** **Is the trial over-ambitious?**

1. PREPARING MYCOINSECTICIDE FORMULATIONS IN THE FIELD

(See also Section 4, Section 7, and the Green Muscle User's Handbook)

A key to the success of field trials using entomopathogens is the availability of adequate, carefully prepared formulation. Fungal preparations are generally supplied either as concentrates or powders. Normally the concentration and application rates should be marked on the bottle label, in which case follow the manufacturer's recommendations.

THE STANDARD 'GREEN MUSCLE' TECHNIQUE

A useful application rate for initial trials is $5x10^{12}$ spores per ha in 2 l.

1 Pure dry powder of *Metarhizium* spores contains $5x10^{12}$ spores in 100 g, so simply suspend 100 g Metarhizium spores in 2 litres of formulating oil to treat 1ha.

2 Two formulations, according to the availability of the ingredients are: 50% Shellsol T: 50% Ondina EL oil - standard (mineral oil) technique 70% "paraffin": 30% peanut (groundnut) oil - adapted (vegetable oil) technique

3 For many experiments it is useful to include a UV fluorescent tracer such as Lumogen at a final concentration of 0.5-1%, depending on the spore concentration.

N.B. ALWAYS do a germination test on spores before application and retain a sample from the sprayer reservoir for checking after the trial.

TECHNIQUES WITH "EXPERIMENTAL" FORMULATIONS AND STRAINS

How to develop "experimental" formulations, usually consisting of novel formulating ingredients or fungus strains.

Making a spore concentrate:

1 Culture the fungus under sterile conditions using an appropriate agar medium in Petri dishes or "sloped" in medical flat - or whisky bottles. Conidiation usually takes place in 10-15 days (less for *Beauveria).*

2 Wash spores off the substrate surface using the light paraffin component. If necessary, use a spatula or brush to carefully agitate the surface of the medium.

3 If the fungus is *Metarhizium* the resulting suspension should be nearly black in appearance. The formulation must be filtered using a muslin cloth or a 150 µm (100 mesh) sieve. Any particles larger than this may cause blockages of sprayer restrictors.

4 If the formulation is to be sprayed in the field it may need to be concentrated.

a) leave the filtrate to settle for 12 hours (e.g. overnight);

b) decant the clear liquid. You should now have a "concentrate" containing approximately 10^{10} conidia/ml.;

c) Check the concentration then dilute as necessary; follow the 'Spore counting using a haemocytometer' procedure described in Section 2 (Laboratory techniques).

5 To store this concentrate for more than a few days, add 100 g of silica gel per litre and place in a refrigerator.

PREPARING A FIELD FORMULATION

Make up the formulation the day before you intend to carry out the application, so that you have time to do a germination test (Section 2 or Green Muscle User's Handbook).

1 If the concentrate contains silica separate the concentrate from the silica gel. Shake the bottle thoroughly (2-3 minutes) until clean silica gel crystals can be seen. Filter the formulation, through muslin cloth or a 150 µm (100 mesh) sieve, into a mixing vessel.

2 If the concentrate does not contain silica gel, shake the bottle thoroughly (> 1 minute) until totally dispersed and decant into the mixing vessel through the filter. Use some blank oil formulation to rinse out the filter and the bottle.

N.B. Cooled formulations should be allowed to warm up (in the shade) for 2 hours before use in the field.

3 Bring the concentrate to a field concentration of $2.5x1\ 0^{12}$ conidia/l by adding appropriate quantities of formulating oils.

To make a formulation of 2.5×10^9 conidia/ml

Dilute 250 ml the concentrate containing 10^{10} conidia/ml with

- 250 ml Shellsol T and 500 ml Ondina EL oil or
- 450 ml kerosene and 300 ml peanut oil.

This gives a field application rate of $5x10^{12}$conidia/ha when applied at 2 l/ha.

4 Dilute a small sample of formulation 1:99 with paraffin and check the concentration (see Section 2).

An original formulation containing 2.5×10^9 conidia/ml (2.5×10^{12} conidia/l), should have a conidial density similar to that in *Figure 4.5.*

5 Check the flow rate of the product: flow rates and conidial concentrations should correspond to the 'Green Muscle' label (e.g. 60 ml/min with a Micro Ulva fitted with a red restrictor).

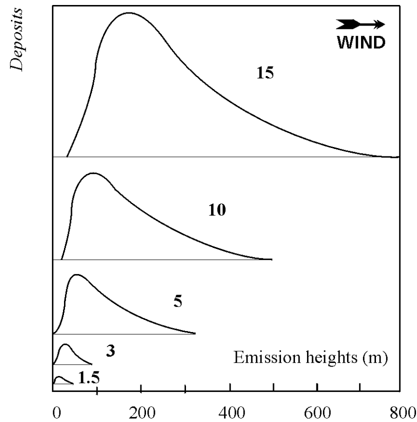
6 Use the formulation within 2 to 3 days.

For extra reliability store in a refrigerator (<10) and check viability regularly (at least once a week).

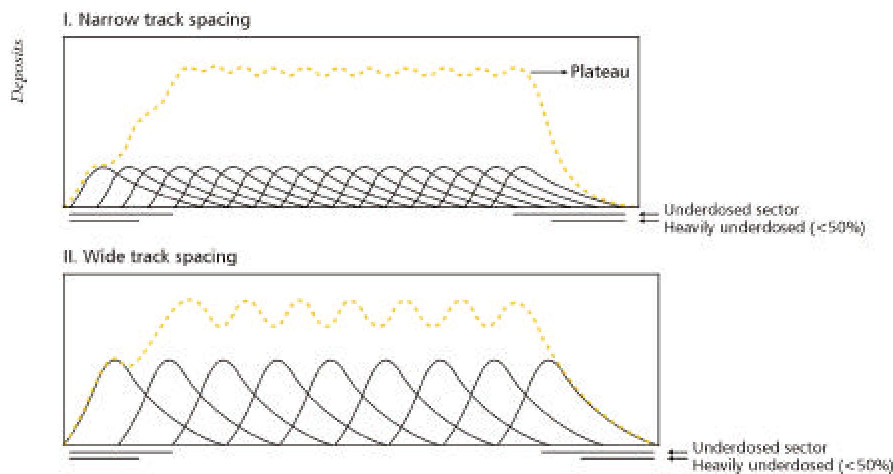
2. PRELIMINARY FIELD TESTS

UNDERSTANDING DRIFT SPRAYING AND DROPLET TRACING

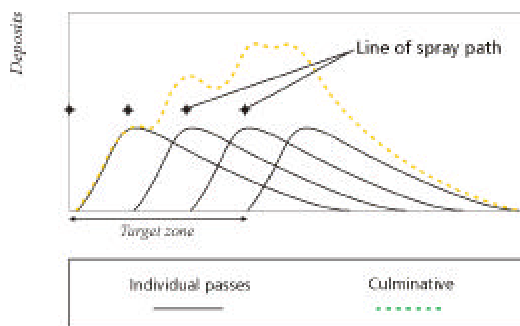
Figure 5.1
DOWNWIND DROPLET DEPOSIT PROFILES
a) effect with various heghts of emission



b) cumulative deposits with track spacings



c) possible effect when incorrectly spraying a small block (target zone less than swath width)



With ULV drift spraying, a lethal dose must be delivered to the target by a relatively small number of spray droplets containing a high concentration of active ingredient. For successful trials using these techniques, it is important to determine the pattern of down-wind droplet deposition. Such tests should be carried out with all unfamiliar machinery, formulations, crops or climatic conditions. Similar tests can be used for demonstrating the mechanism of ULV spraying during training courses.

The "typical" patterns of droplet deposition are illustrated in *Figure 5.1*. The position and magnitude of the initial peak is dependent on droplet size, emission height, flow rate, wind-speed, air turbulence and formulation characteristics. After the "peak" there is a progressive diminution of deposited droplets, at increasing distances downwind of the spray line. Analysis of this swath pattern data (*Figure 5.2.*) can be used for determining practical swath widths with different application techniques. The appropriate positioning of sampling posts is a matter for experience: a last down-wind post at 100 m may be needed for spraying with hand-held equipment in moderately strong winds; a 200 m post may be required for vehicle mounted sprayers or 500-1000 m for aerial tests.

It is especially important to understand the way drift spray deposits are accumulated over several swaths, and note the possible effect of incorrectly spraying a small plot (*Figures 5.2*). One possible solution is to spray twice or more along the upwind track line (*Figure 4.22*).

A step-by-step method for estimating spray recovery has been described by Picken (in Heinrichs *et al.*,1981). In the absence of sophisticated analytical equipment, spray deposition must be estimated from droplet numbers. This can be done using tracer pigments or formulation sensitive cards. There may only be time for very approximate assessments of droplet size. The droplet size spectra produced by a particular combination nozzle, operating conditions and formulation should be measured in advance in the laboratory.

"ARENA" TEST

The "typical" pattern shown in *Figure 5.1.* can be used to obtain deposits with droplet numbers similar to those encountered following normal field applications. These tests are sometimes called "field bioassays". They can be carried out on a 0.25 ha (50 m x 50 m) plot on a research station, see *Figure 5.3.* This trial

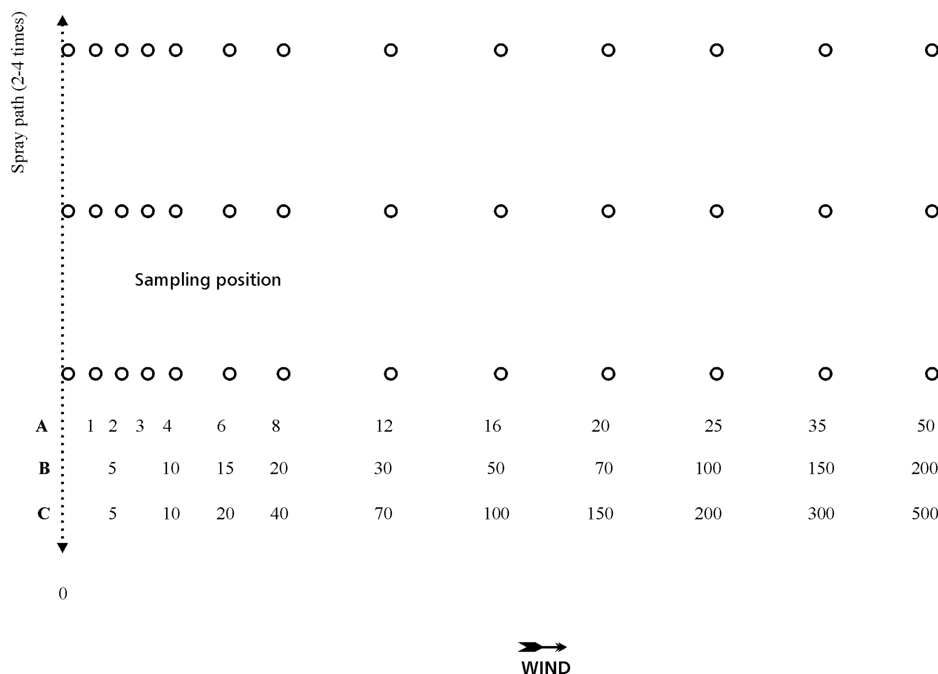
format is the simplest way of applying a pathogen to insects using a sprayer instead of a pipette. As well as gaining confidence and familiarity with sprayers, this trial format is useful for testing new formulations and comparing strains.

The technique relies on:

- winds travelling in a consistent direction
- the target insect being caged, sedentary or "tethered" in some way, without causing excessive mortality

The experimental layout is designed to provide a variety of mean droplet numbers at sampling stations, from which it should be possible to correlate mortality with numbers of droplet impactions. The distances from the spray line have no particular significance in themselves, apart from providing an ordinal ranking of probability of spray deposit levels. In order to assess secondary pickup, plants in pots sprayed, and then untreated insects are released on them. The droplet coverage on treated insects and plants can be evaluated with a tracer pigment.

Figure 5.2
SWATH WIDTH DETERMINATION



SUGGESTED SAMPLING POST POSITION FOR ASSESSING DRIFT FROM:
A: hand-held spinning disc sprayers
B: vehicle mounted sprayers
C: aerial sprayers

Illustration is not to scale and is for hand-held sprayers. Further down-wind posts may be required if wind speed is > 5 m/s.

ASSEMBLE ALL THE EQUIPMENT YOU WILL NEED

1 Place a grid of stakes in a suitable area of the research station; the figure shows arenas situated at 5, 10, 20 and 30 m downwind of a marked spray path.

2 Hoe $1m^2$ arenas at each stake.

3 Place wooden poles, maize plants or cassava sticks less than 1 m tall in each arena.

4 Attach artificial targets to the poles; these may include: oil-sensitive paper, black glossy cards (that clearly show the u.v. tracer), agar plates, slides.

5 You must do two (2) treatments: the control (without fungus) and the test formulation (with fungus). You can use the same site for both treatments if you treat the control first.

6 Prepare at least 10 grasshoppers for each arena for each of the two treatments.

7 Prepare the sprayer and formulation.

8 Check

- walking speed;
- flow rate;
- disc speed, etc. (use the form in *Table 5.1*).

Work out how many passes with the sprayer will give you the required dose.

9 Check:

- fungus formulation (spore concentration, germination test);
- sprayer calibration (flow rate, walking speed, battery condition, disc speed);
- wind speed, temperature, humidity.

10 Place 10 grasshoppers or locusts at each arena.

11 Spray along the spray line the required number of times, using a blank formulation for the control. Make sure that the wind direction is at right angles to the spray line, and that droplets are drifting onto the stakes.

12 Repeat the above procedure for spraying with the test formulation.

13 After spraying, catch as many grasshoppers as you can.

14 Place the grasshoppers in labelled cages for laboratory incubation, using a different cage for each arena.

15 Collect the papers, slides and agar plates; label each one correctly.

16 Count droplets on a sample of the insects.

17 Count the droplet density on the papers.

18 Incubate the agar plates for 20 hours, check for spore germination.

19 Incubate the insects for 21 days.

20 Feed and check grasshoppers each day to see how many have died.

21 Dying insects should be put into petri dishes with moist tissue paper to look for sporulation.

22 Analyse the data; plot the percentage of grasshoppers remaining alive and the percentage sporulating against time for both treatments. Kaplan-Meir survival analysis in SPSS is a recommended technique, but any logisitc analysis can be used.

POSSIBLE PROBLEMS

Although nymphs and adult *Zonocerus* can be released directly onto the plants, adult *Schistocerca* need to be tethered or they will fly away. We have used paper clips on the wings, but this has been criticised as the wings no longer protect the abdomen. Cooling the insects has been recommended, but this may cause condensation on the insect body under humid conditions and help spore germination

TESTS WITH CAGES AND OTHER ENCLOSURES

It may be difficult to carry out arena tests where winds are inconsistent or target pests are highly active. Cages of various sizes are often used in entomological tests, especially where highly mobile insects must be contained or sprayed areas must be evaluated over prolonged periods.

There are several practical problems in using cages

1 obtaining a "typical" spray deposit in the cage for representative dose transfer to the target pest

2 high background mortality due to restricted movement of insects (e.g. inability to find shade, depletion of food source) and "handling" mortality

3 change of micro-climate: increased temperature and humidity can cause artificially high insect mortality and increase the mortality due to the pathogen

4 excessive predation and removal of cadavers by ants and other predators

5 theft, vandalism and damage by animals

6 decontamination of cages after use must be carried out thoroughly with biopesticides - prolonged exposure to sunlight usually may be effective, but micro-organisms will persist in the soil. The use of certain chemical pesticides may necessitate replacement of the mesh, especially if made of absorbent cloth.

Proper controls are essential and if mortality is high in these cages, confidence in the results will be severely reduced.

Even when control mortality is low, the effects of pathogens may be enhanced under cage conditions which stress the insects. Certain species (especially immature stages) are highly susceptible to "handling" mortality. Insects can be captured for placement in cages, but a change of sweep netting technique may be necessary. For example, rather than beating vigorously for grasshopper nymphs, nets are trailed above the ground while walking briskly or driving through the vegetation. If foliage has been sprayed do not use this technique since the nets become heavily contaminated. If there is no alternative a different net must be used for each treatment. Never collect nymphs when the foliage is very wet - after rainfall or heavy dew.

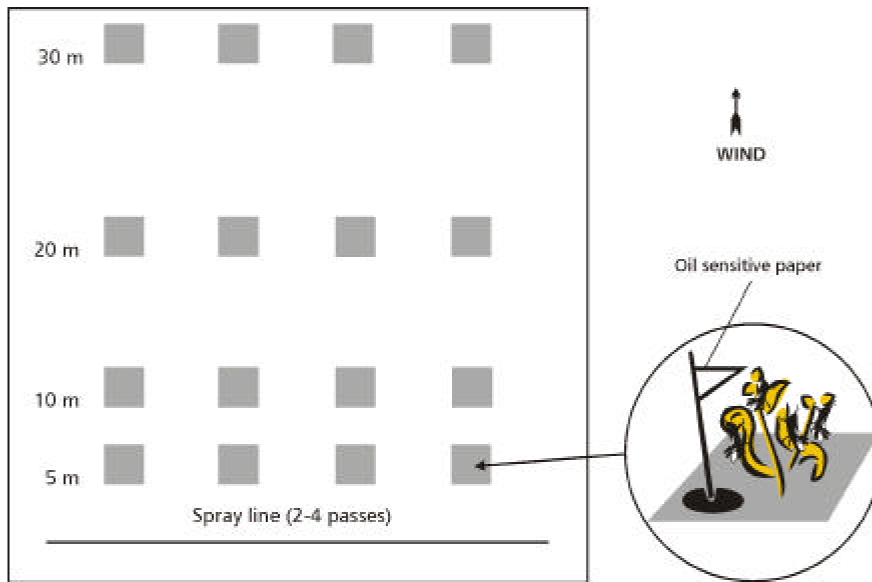
Large-Mesh Treatment Cages:

Locusts and other insects can be contained in cages with very large mesh (at least 5 mm) and treated as in the arena test. It is essential to check that the spray has adequately penetrated the mesh and impacted on the insects.

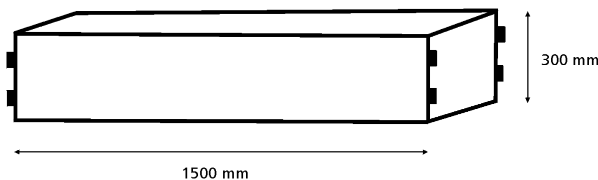
Small Field Cages:

Small portable cages are especially useful for assessing trials with large numbers of treatments and replicates. Can be used for keeping treated insects out in the field or for enclosing captured specimens over an area of vegetation previously sprayed in a realistic manner.

Figure 5.3
TESTING IN "ARENAS"
plan of four row design

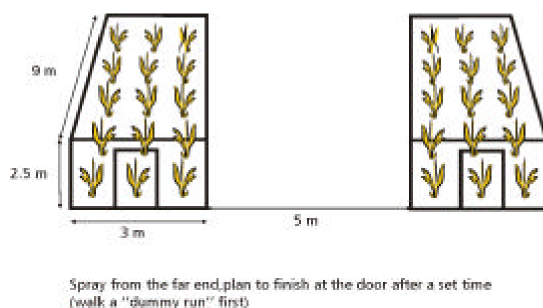


field enclosure ("boma") for hopper samples



Four walls of equal dimesion: constructed from smooth flooring plastic riveted to metal frames and joined by two clamps at each corner.

Figure 5.4
TEST IN LARGE CAGES
paired of 3 way comparision



Field Enclosures ("Bomas"):

Developed in South Africa for trials on brown locust nymphs. Immediately after spraying, a portion of each band is corralled by placing an open-topped 1.5 x.5 m enclosure ("boma") consisting of four 0.3 m high, smooth-sided, plastic walls mounted on metal frames (*Figure 5.3*). Samples of hoppers can subsequently be transferred from each enclosure into cages for further evaluation; the cages used for this purpose are 250 x 250 x 440 mm and constructed from metal gauze and galvanised steel. A cover of plastic netting may be required to exclude birds and enclose locusts that have fledged into adulthood.

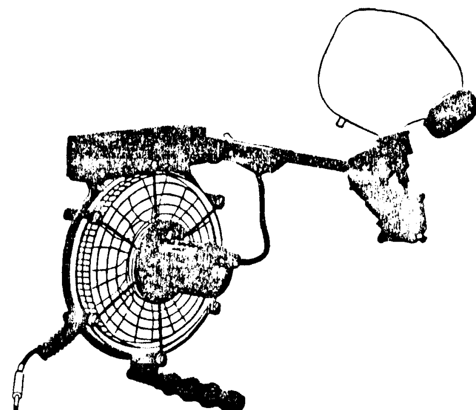
3.

Large Permanent Cages:

Although large cages are expensive to build, they can be useful for testing fungal formulations on adult insects which would otherwise fly away and allow field tests to continue under "near field conditions" near experimental stations and outside the normal field season.

The LUBILOSA Programme has used groups of three cages measuring 9x3x2.5 metres tall, separated by >5 m. They have been constructed in order to compare two formulations with a control treatment. Application of test formulations is carried out using an 'UlvaFan' or similar low-velocity air-assisted sprayer to overcome the problem of low wind speed inside the cage (*Figure 5.4*). If there is a cross wind, tarpaulins or rush mats must be hung on the sides of cages to enclose the spray.

THE MICRON "ULVAFAN" (MK II)



3. SMALL SCALE FIELD TESTS

Small-scale field tests are half-way between an arena trial and a full field trial. The objective is to test the infectivity of the mycopesticide on a field population of grasshoppers or locusts, but usually on a scale too small to have an effect on population levels. The trial consists essentially of spraying a field population and taking samples to incubate in cages.

Make sure you have all the equipment you need.

1 Mark out treatment and control plots of about 0.25 (50 m x 50 m) – 1 ha. Control plots should be up-wind of treatment plots.

2 Estimate the insect population density (see sampling).

3 Check droplet deposits with artificial targets (e.g. black glossy cards) on wooden posts within the plots (see arena tests).

4 You must do two (2) treatments: the control (without fungus) and the test formulation (with fungus).

5 Take a pre-treatment sample of 25 insects per plot. Make sure that they are not contaminated with the fungus formulation.

6 Prepare the sprayer and formulation.

7 Check

- forward walking speed;
- flow rate;
- disc speed, etc. (use the form in *Table 5.1*).

Work out how many passes with the sprayer will give you the required dose.

8 Check:

- the fungus formulation (spore concentration, germination test);
- sprayer calibration (flow rate, walking speed, battery condition, disc speed);
- wind speed, temperature, humidity.

9 Spray the plots, starting with blank formulation for the control. Check the wind direction throughout the spray operation. Don't forget to spray twice along the upwind track line (see *Figure 4.22*).

10 Repeat the above procedure for spraying with the test formulation.

11 After spraying, catch a further 25-50 insects per plot for the post-treatment sample. *

12 Place the grasshoppers in labelled cages for laboratory incubation, using a different cage for each plot. *

13 Collect the papers, slides and agar plates; label each one correctly.

14 Count droplets on a sample of the insects.

15 Count the droplet density on the papers.

16 Incubate the agar plates for 20 hours, check for spore germination.

17 Incubate the insects samples for 21 days in cages.

18 Feed and check grasshoppers each day to see how many have died.

19 Remove dead insects from cages, and place into petri dishes with moist tissue paper to look for sporulation.

20 Count survivors at end point of the experiment (usually 21 days after treatment)

21 Analyse the data; plot both the percentage of grasshoppers dying and the percentage sporulating against time for both treatments.

22 Return to the treatment area on days 3, 7, 10 and 14 post treatment and collect more samples. Look for cadavers and repeat the population counts on each occasion.

N.B. Check the degree of cross-contamination in the sweep net and in the cage by catching and caging insects individually.

POSSIBLE PROBLEMS

Contamination of control plots and insects can cause problems. You are unlikely to see any effect on population counts in an experiment as small as this. Such problems may be solved by progressing to larger experiments, the planning of which will be partially based on data collected in these smaller trials.

4. MEDIUM AND OPERATIONAL SCALE FIELD TRIALS

Each field trial will be different depending on the terrain, the target insect, the resources available and the quantity of mycopesticide available.

The risk of contamination to downwind plots is especially high with ULV drift spraying and wide separation of plots is necessary (see *Table 5.2*).

Figure 5.5 shows a regular pattern of plots with minimum spacings when using vehicle mounted sprayers.

N.B. in many terrains you will have to use less regular arrangements.

Assess the size of the area of grasshopper infestation and the amount of formulation available. Plot size is determined by the mobility of the insects. One hectare plots may be adequate for *Zonocerus* spp. nymphs but *Oedaleus senegalensis* needs 50ha. For a proper statistical analysis you need at least 3 replicates for each treatment. You can get interesting results even if the conditions are not perfect, and the experience gained will be useful for future trials.

Success comes with experience

Table 5.2
PLOT SIZES

Treatment type	Minimum plot size	preferred plot size	track spacing (typical range)	Minimum downwind distance to next plot
ground-based: electrostatic, VLV	100 m²	0.25 ha	1 - 5	10 m
ground-based: oil, 'MULV' sprays	0.25 ha*	1 ha	3 - 10	100 m
vehicle, aerial @<3 m off ground	4 ha*	9 ha	15 - 50	200 m
aerial @ª 5 m off ground	1 km²*	10 km²	50-200	500 m
aerial @ 10-20 m off ground				1 km

**: avoid smaller plots except in exceptional circumstances.*

Spray using normal method, as shown in *Figure 5.5*. Separation of the down-wind plot from the last upwind spray track by at least one swath width. The distance to the assessment zone depends on application methods and spraying conditions, but will be at least 200 m for vehicle mounted sprayers; aerial spraying may require 1km separation. This is especially important when laying out adjacent untreated control plots where only the assessment zones may be marked.

Figure 5.5
LAYING OUT SMALL AND MEDIUM SIZED (4-50) PLOTS FOR DRIFT SPRAYING

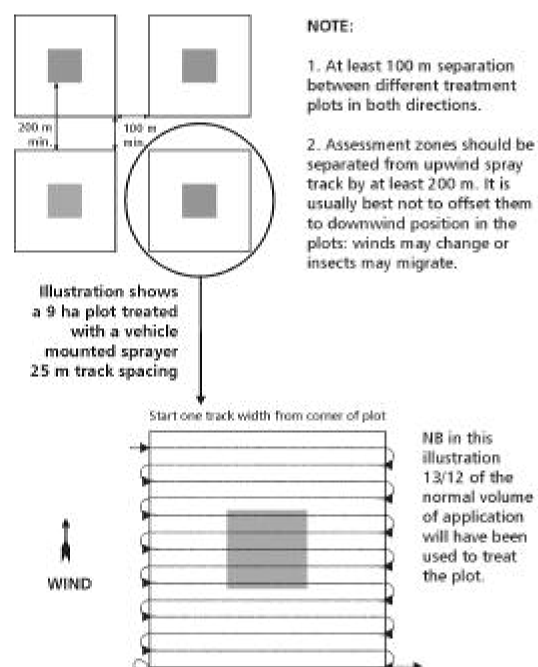


Figure 5.6 shows the procedure for marking out a 9ha plot. Markers should be aligned (in step 1) with the wind. Marking can be carried out on foot with a 100 m tape, however it is much easier to use a vehicle. The odometer may not be very accurate even if it has a kilometre counter reading to 0.1 km, and counting tyre revolutions is better. Paint mark one of the vehicles tyres with white paint and count the number of rotations per 100 m; a tally counter is useful for this purpose.

LAYING OUT LARGE SCALE TRIALS

If the trial size is scaled up above 10ha, problems of positioning and co-ordination increase dramatically. You will need to prepare a map of the site and develop a robust system of communication. Although not absolutely essential, Global Positioning Systems (GPS) and walkie-talkie radios are extremely useful for laying out and executing large trials. GPS can also be very helpful for locating and following bands of migratory pests.

GPS instruments receive signals from approximately 24 satellites that orbit the Earth twice a day at 11,000 nautical miles in six fixed planes. Micro-processors calculate the position of the unit by triangulation (provided signals can be received from at least three satellites). Hand-held devices have now become widely available for <$500. They can obtain a positional fix in ½ to 2 minutes and typically are accurate to 10-30 m. Selective availability is the deliberate incorporation of random errors into the satellite signals in order to "maintain optimum military effectiveness"; it affects all equipment and can decrease positioning accuracy by as much as 100 m. More sophisticated configurations involve the use of differential GPS, where the mobile units communicate with a fixed control unit, placed in a

known position. This measures correctable positioning errors for the area which are then removed from fixes made by the mobile units but errors of up to 30 m may still occur. These low cost mobile units are very useful for laying out plots and general positioning but are not suitable for accurate tracking of sprayers.

49ha plots

1 Use GPS or a conventional mapping technique to find the centre of each plot. Mark them with large marker posts (at least 2.5 m, with flags).

2 Calculate the sides and diagonal distances for the plot size required (e.g. for a 49 ha plot, the sides must be 700 m and half the diagonal is 495 m).

3 Decide on a suitable plot orientation (depending on the "typical" direction of the wind). It is convenient to position the four corners due North, South, East and West of the central point. In tropical areas the prevailing wind will usually be at an angle to the lines of longitude.

4 Use a compass to take bearings and measure to the four corners of the plot (e.g. 495 m from the plot centre). Place large marker posts at each corner and record them as way-points on your GPS.

5 Vehicle tracks provide useful temporary guides for positioning the four corners of the assessment zone in the centre of each plot. In the example above, the corners were positioned 100 m from the centres.

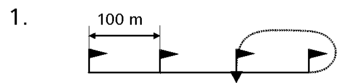
6 Make sure that the four sides of the plot are marked, either with vehicle tracks or rows of small flags. These are to orientate the flagmen when they spray.

7 When spraying in vehicle-based trials in savannah woodland, a third flagman may be needed to walk across the centre of each plot. It may only be possible to treat one >50 ha plot per day.

8 Carry out assessments as above (*Figure 5.9*).

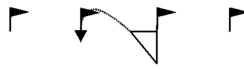
Figure 5.6
MARKING OUT A 9HA PLOT
with 16 markers posts, using a vahicle

1.



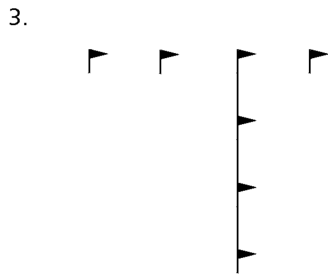
Place first 4 posts in a straight line, at 100 m intervals.

2. 1 st flagmant walks to 2nd post



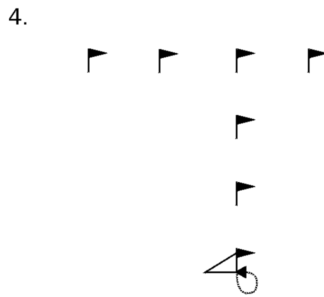
Using 2 temporary posts and a 30-40-50 mrope align vehicle at right angles to the 3rd post.

3.



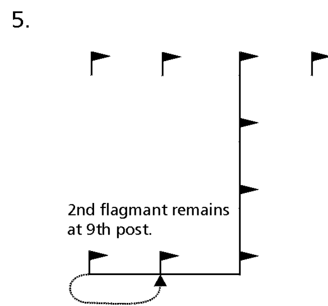
Position posts 5 to7

4.



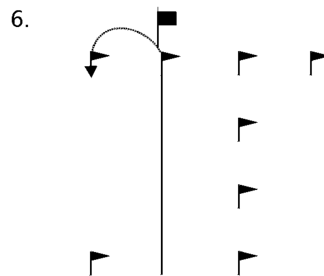
Use 30-40-50 m rope to mark out 2 nd perpendicular and re-align vehicle

5.



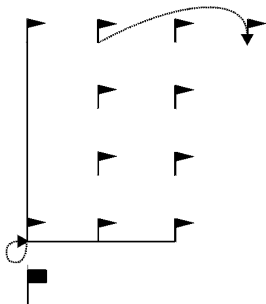
Mark out posts 8 and 9 then return to 8th post.

6.



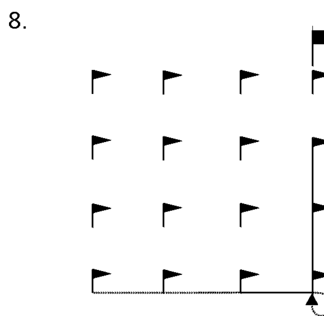
Align vehicle with flagmant at 2nd post then mark out 2 remaining corners of the assessment zone.

7. 1st flagmant walks to4th post.

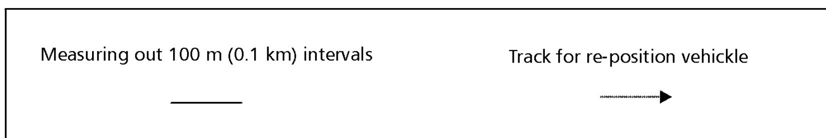


Aligh vehicle with flagmant at 9th and post insert 2 side markers.

8.

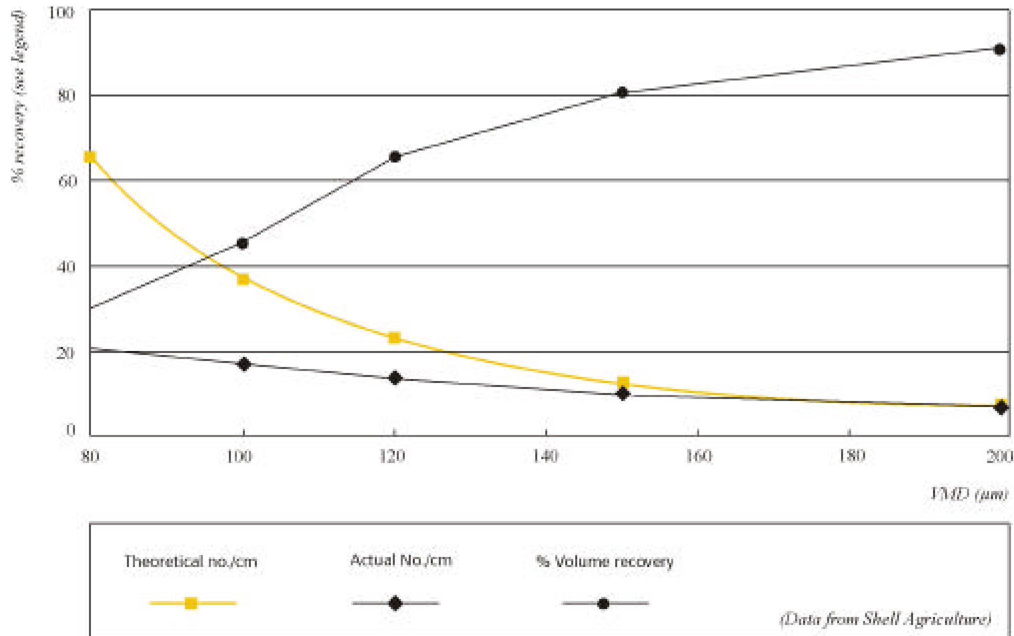


Retrace track to 7th post, mark out final corner and 2 side markers.



5. SPECIAL CONSIDERATIONS FOR AERIAL APPLICATION TRIALS

Figure 5.7.
AERIAL SPRAY RECOVERY
theoretical and actual droplet cover



Large difference between theoretical and actual droplate numbers recovered at smaller sizes

0

Aerial spraying is the most important method of control for the desert locust and certain other species. A detailed description of aerial application is outside the scope of this manual and is a matter for specialist operators. An outline of the equipment that is commonly used is given in Section 4.

The objective of this section is to give a check list of the important features to note in spray aeroplanes equipped for ULV application. This may be helpful in the selection of a spray operator (if there is a choice) and prepare you for discussions about trial requirements and calibration with the spray pilot. You should note:

1 **Aeroplane type**

a) operating speed

b) payload

2 **Atomisers**

a) type

b) number on each wing

3 **Flow control**

a) Pumping system: air turbine pumps must be calibrated in flight, independently driven pumps (powered by electricity, hydraulic mechanism or 2 stroke engine) are more suitable for trial purposes.

b) Restrictors: Micronair VRUs usually fitted with odd numbered disc, even numbered discs are sometimes fitted for higher flow rate ranges.

c) Diaphragm check valves; hydraulic valves used in older systems.

d) Well maintained pressure gauge visible to pilot is essential, flow meter desirable but not always fitted.

4 Tank and plumbing system

a) wastage for pipe priming. This may be up to 50 l and is a problem if supply of experimental samples is limited. Wastage is much less with high winged aircraft (e.g. the BN2b 'Islander') fitted with the Micronair 'pod' system (approx. 8 l) or with light and micro-light aircraft.

b) normally oil- or water-based formulations used in the tank?

c) cleanliness (check the inside of the tank carefully).

5 Experience and dedication of pilots & support staff (perhaps the most important factor of all).

One of the first questions you may be asked is "what droplet size do you want?"

There is no simple recommendation that can be made for mycoinsecticides at this stage. Secondary pick-up of formulation occurs for 1-2 hours after application, so that the extra "coverage" that may result from spraying small droplets may be less important than the total volume of formulation recovered on plants.

Spraying small (<120 µm) droplets often results in a low volume recovery of a formulation, for a relatively small increase in droplet numbers per cm^2 (see *Figure 5.7*). Much depends on the operating conditions; ALWAYS follow the advice of an experienced spray pilot.

DO NOT SPRAY

1 At temperatures of >25° (you will usually spray early in the morning); never spray under inversion conditions

2 With little or no cross-wind

3 At volume application rates of <1 l/ha for initial trials.

6. FIELD TRIALS

GENERAL

A great variety of conditions occur during field trials on locusts and grasshoppers and objectives of different trials vary.

We give general guide-lines which are no substitute for experience.

1 Assemble your team and gain experience by carrying out small field tests and arena trials.

2 Where possible include in the team people who are experienced in locust and grasshopper control.

3 Look for target pest mortality, and signs of death due to the pathogen. For example, death due to mycosis can be checked by incubating individual cadavers in a plastic box containing moistened filter paper or similar.

4 Catch and cage specimens for observation at base.

5 Work out the logistics of transporting cages of treated and control insects separately both from each other, and from any equipment that has been used for spraying live formulation.

6 You may have to continue sampling biopesticide trials for 3 weeks or more after treatment.

SOME “DO'S AND DON'TS”

1 Only ONE person can run a field trial;.

2 Discuss the trial design and details with colleagues, but in the end make sure that one person follows it through from start to finish.

3 This person should have complete authority to take operational decisions and is as the focal point for any questions or decisions.

PREPARATION

DO check over the field site carefully several times.

DO a germination test on the formulation the day before application.

DO lay out the plots the day BEFORE you spray - DON'T try to do it all in one day.

DO take cage samples immediately after spraying.

DO be clear about the objectives of the trial - if you are looking for a population reduction, make sure your sampling precision is good enough (variance small relative to the mean).

DO prepare a list of things you will need on the day of application.

DO carry out replicated trials and do randomise. It seems a lot of trouble at the time, but, if you don't do this your results may be meaningless, and will certainly be unpublishable.

ON THE SPRAY DAY

DON'T rely on anyone else to put things in the vehicle.

DO take water and food.

DO make sure that everyone involved knows what is going on.

DO make full use of all the people you have available without losing control.

DO learn about the behaviour and biology of the target insect.

DO take time to think about the trial - try to think what might go wrong, because if it can go wrong, it inevitably will.

DO make the design comprehensive and fool-proof, so that you can obtain a partial result even if some aspects go wrong.

DO talk to the local farmers and explain what you are doing. Employ a watchman if necessary.

DO test your sampling and counting procedures in advance, and check that the variance is low.

A possible sequence of events

Make full use of local information.

Consider sending an advance scouting team.

DAY 1

Assess the site

1 Identify species present. Are they known to be susceptible to any fungal pesticide? Which is the dominant species present?

2 Assess the terrain. What sort of spray application will you use?

3 How mobile are the insects? Factors: instar and vegetation.

4 Decide on plot size. Factors: man power, formulation, terrain, vegetation, sprayers, number of replicates, mobility of insects, previous field trials.

5 Do you hope to see a reduction in the population? If so, try to do population counts using different techniques.

6 Sample 25-50 grasshoppers from each plot and incubate to check for background levels of fungal infection

DAY 2

Plan the experiment (you may be able to do this on day1)

1 Think about the objectives of the trial.

2 Decide on the number of plots, replicates, treatments and sampling procedures. What are your limitations - time, money, personnel, formulation, security? If possible, start laying out the plots, or do this on day 3

3 Check the viability of the formulation; put up a germination test.

4 Check the toxicity of formulation ingredients.

5 Check that the sprayer works and that spares are available!

6 What is the flow rate?

7 Check droplet size (see Section 4).

8 Brief the team members on the experimental design and their roles.

9 Think about the weather - especially the speed and direction of the wind.

DAY 3

Lay out the trial

1 Lay out plots using corner posts, flags, labels and a compass. Mark on a plan; calculate areas and application rates.

2 Do pre-treatment population counts; check that the variance is acceptably low.

3 Take a pre-treatment sample for cage incubation.

4 Assess the germination test.

DAY 4

Treatment

N.B. If weather conditions are unsuitable, you may have to delay spraying until the evening or the next day.

1 Check wind direction and finalise experimental design.

2 Spray (see Section 4). Always measure out the correct amount of formulation for each plot and check that it has been used. Have some in reserve in case of over-dosing.

3 Periodically clean the disc and check the batteries.

4 Check deposition with oil sensitive paper or black cards.

5 Check target impaction if you have used tracer; collect samples of treated insects and vegetation.

6 Collect samples for cage incubation.

DAY 5 to 28

Monitoring

1 Collect more samples for cage incubation (usually days 1, 3, & 7 post application).

2 Keep an eye on grasshoppers and ants; observe their behaviour in the field.

3 Keep up population counts.

4 Look for cadavers.

5 Monitor cage incubations.

POSSIBLE PROBLEMS

Weather

- heavy rain may affect the application and cause high control mortality
- wind speed will affect spray application.

Insect behaviour

- insects may mature and leave the plot, or move out of the plot if food is scarce.

Agricultural practices

- livestock may be taken through the plot;
- farmers may start clearing or burning the land, or spray with insecticides.

Pathogen

- spores may not be viable.
- certain grasshopper species may not be susceptible to the fungus (although this should normally have been checked with bioassays, before going into the field).

Spray coverage may not be even.
Possible causes:

- wind variable in speed and /or direction;
- high, dense vegetation.

TRIALS ON LOCUSTS

Locust adults and nymphs are very mobile and are particularly difficult to work with in the field.

Adults

As locusts swarms are very mobile it is difficult to follow a swarm to check the effect of treatments. The best method is to cage a of locusts after application.

Nymphs

Hopper bands can be treated in 3rd to early 5th instar. Follow the bands for 1-3 weeks. Take cage samples to check on the application. Increased predation on the sick insects can pose a problem. Population counts are particularly difficult, but have a try. Use your imagination! A method developed by LUBILOSA is described in Langewald *et al.* IN PRESS. Problems may be encountered with droplet deposition, in which case consider the use of mist-blower ULV combination.

7. SAMPLING, DATA COLLECTION AND ANALYSIS

Some guide-lines on assessing mortality with slow acting pesticides are given by FAO (1991). It is most important to carefully record all relevant data during a trial: the FAO Pesticide Referee Group set minimum standards of reporting. A sample recording sheet is shown in *Figure 5.8.*

Cage samples are almost invariably taken to establish that dose-transfer and infection have taken place, and to ensure that at least some information is recovered even if other assessments fail. The most substantial measure of success will always be a demonstration of pest population reductions. *Table 5.3* is a guide to sampling methods appropriate for Sahelian grasshoppers and locusts (from P.A. Shah, 1995, PhD thesis, University of London). An arrangement for carrying out both population counts and sweep net sampling in large plots is shown in *Figure 5.9.*

Results must be recorded carefully on pre-prepared data forms. Data entry is easy if you use forms printed from the computer spread-sheet which is also used for analysis and presentation. *Figure 5.10* shows a spread-sheet template used by the LUBILOSA project (this is continued downwards for 3 day intervals up to the end point of the trial). You can also make graphs from data arranged in this way.

CAGE SAMPLES

Collecting samples of grasshoppers in the field after treatment and incubating in cages can provide some useful data even when it is not possible to carry out a full field trial with population counts. The method is full of pitfalls and data should be treated with caution and should not used as evidence for field efficacy.

- Insect may be cross-contaminated during collection or while in the cage.
- Insects may be stressed in the cages and die more quickly than in the field.

Nevertheless, some inferences can be gleaned from careful consideration of the incubation curves (Langewald *et al.*, 1996). Data from cage incubations can be treated similarly to bioassay results (Section 2). Data may be presented graphically, or you may wish to summarise the results with a few key statistics. These could include:

1 Total mortality on days 7, 14 and 21

2 The median lethal time (MLT): the number of days to achieve an accumulated 50% mortality (using linear interpolation of cumulative, daily mortalities)

3 The average survival time (AST) can be calculated as follows (where nt is the number of individuals that die on day t out of a population N treated insects, between day 1 and the end point of the assay - u):

$$AST(t) = \frac{\sum_{t-1}^{u} n_t \text{ x } (t-0.5)}{N}$$

It is useful to obtain a measure of the time span over which mortality occurs (analogous to the slope of a dose response line). For this purpose the variance of the AST can calculated as follows:

$$\text{variance} = \frac{\sum_{t=1}^{u} n_t \text{ x } (t-0.5-t)^2}{N-1}$$

MLT and AST have been used most often for summarising bioassays rather than field tests; both have advantages and weakness. MLT is calculated using little more half the insects inoculated/retrieved, however it is simple to calculate and robust. The sigmoid mortality curve may not be symmetrical so AST is used in order to incorporate all the data. The shareware LUBILOSA Meltimor programme can be used to calculate AST and MLT (Bateman *et al.*, 1996) but these statistics are most appropriate for basic bioassays. The result obtained with the AST formula shown is affected by sample size and the end point of the experiment if all the insects do not die. Kaplan and Meier's (1958) survival analysis method overcomes these problems, and takes all mortality and all survivors into account; this is available within SPSS and Kaplan-Meier analysis of a mycopesticide trial is described by Jenkins and Thomas (1996)

FIELD COUNTS

Population counts carried out as described in Figure 5.9 will be used here for illustration. It is important that during the experiment each person counts along the same lines, because of the variability between individuals. Using this method it should be possible to

sample fixed quadrates. Due to variation in the vegetation, populations of grasshoppers are heterogeneously distributed and the variability of counts over time can be considerably reduced using this method. In cultivated areas such as cassava fields count insects per plant and replace the diagonal assessment set up, with one taking account of the alignment of crops.

A field trial of the type illustrated in Figure 5.9 was analysed as a split-split-plot design. It consists of 25 single quadrat counts (split-split-plots), nested in each assessment line; four assessment lines (split-plots) being nested in each plot; the plots were replicated three times (both treatments and controls). The assessments were repeated at regular intervals, so that time after application (repeated measures) could be included as an additional factor.

STATISTICAL ANALYSIS

Counts are most likely log-normal distributed. A log transformation of data is also necessary to achieve homogeneous variances, assumptions which have to be made before applying ANOVA techniques. At low population densities many counts may be zero. In this case single counts of one assessment line can be pooled. Differences between the counts before treatment can be corrected using the pre treatment counts as covariate. The final statistical procedure is a double nested repeated measure analysis of covariance. The data can also be analysed on a day by day basis excluding the factor "repeated measures" from the analysis.

For graphical presentation the average log counts should be presented to provide correct error bars. A curve corrected for changes in the control population density should have the typical shape of a sigmoid cumulative mortality curve.

Percent efficacy can be estimated using the formula of Henderson and Tilton:

$$\%\,\text{efficacy} = 100\left(1 - \frac{T_a}{C_a} \text{ x } \frac{C_b}{T_b}\right)$$

where

infestation in treated plot:	T_b:	before application
	T_a :	after application
infestation in control plot:	C_b:	before application
	C_a:	after application

CADAVER COUNTS

Another obvious measure of the effectiveness of mycopesticide application is the occurrence of dead grasshoppers or locusts in the field following application. This is particularly convincing for outside observers, especially when the symptoms match those demonstrated in samples. Cadavers may be difficult to find unless populations have been enclosed in field cages.

Cadavers can be collected and incubated to check for sporulation; samples should be retained for subsequent biochemical analysis, to check that the strain recovered is the same as that used for treatment. The counts can be quantified by using quadrat searches, or by searching for a fixed length of time.

The number of cadavers recovered is often disappointingly low, and this may be due to:

1 lower mortality than expected,

2 loss of sick insects to predators,

3 failure to obtain an accurate absolute population estimate of live insects,

4 loss of cadavers to scavengers,

5 difficulty of finding cadavers once they have fallen to the soil, or because sick insects have hidden themselves before dying.

SPORE LOAD IN THE FIELD

As discussed above, searches for cadavers in the field may yield disappointing results. A more objective estimate can be obtained by installing cages with open bottoms in the field and stocking with healthy grasshoppers. After a fixed exposure time (normally 2 days), the grasshoppers are removed and incubated in cages; the field cage is moved and re-stocked.

The method gives an estimate of the initial applied inoculum, the decay of this inoculum and any increase in the level of inoculum as a consequence of the sporulation of cadavers.

Figure 5.8

RECORDING SHEET FOR MYCOINSECTICIDE TRIALS AGAINST ACRIDIDS

One form to be completed for each plot sprayed

GENERAL

DATE:	TRIAL CO-ORDINATOR:
COUNTRY:	TRIAL NO:
NEAREST TOWN/VILLAGE:	
LATITUDE:	LONGITUDE:

HABITAT/VEGETATION

DOMINANT SPECIES:
MEAN HEIGHT:
PERCENTAGE COVER:
No. TREES/HA.
STATE OF VEGETATION (green, dry etc.):
SOIL TYPE/STRUCTURE:

TARGET

MAJOR SPECIES:	%	{STAGE: (or ratio }:
OTHER SPECIES:	%	{of nymphs:adults)}:

APPLICATION

SPRAYER:
RESTRICTOR/SETTING:
MEASURED FLOW RATE:
SPRAYER RPM (No. of batteries):
EMISSION HEIGHT (m):
FORWARD SPEED (m/s):
TRACK SPACING (m):
VOL. APPLN. RATE:
DROPLET SAMPLING REF:

FORMULATION (for SU mycoinsecticide)

STRAIN/ISOLATE:	BATCH No.:
SPORE CONCENTRATION:	SPORE VIABILITY:
DILUENT AND RATIO (if applicable):	
G. DRY PRODUCT/L (if applicable):	
RESERVOIR SAMPLE REF.:	

CONDITIONS

	START	**FINISH**
TIME:		
DRY BULB TEMPERATURE (C):		
RELATIVE HUMIDITY (% or wet bulb C):		
WIND SPEED (m/s):		
WIND DIRECTION (bearing)		
CLOUD COVER (%)		
SUNLIGHT (y/n)		

COMMENTS AND PROBLEMS

Table 5.3

A DECISION MATRIX FOR FOUR PRINCIPAL SAMPLING METHODS OF ORTHOPTERA

METHOD	MOBILITY			DENSITY			VEGETATION		
	Fast	Intermed	Slow	Hign 1	Medium 2	Low	Low 3	Patchy 4 5	Tall
Quadrat counts	!!!	X			X		X		!!!
Flush Counts	X		!!!	X					X
Sweep netting			X		X		X		
Timed catches		X				X	←	X	→

1 > 25m2 or 50 100 m2: 2 < 1m2 or 5 100 m2: 3 < 300 mm height: 4< 30% ground cover: 5 < 300 mm height

CLASSIFICATION OF SELECTED SPECIES AND STAGES BY IMOBILITY

	ADULT STAGES	NYMPHAL STAGES
Fast fliers or strong jumpers	*Acorypha glaucopsis,* *Anacridium* spp. *Cryptocatantops haemorrhoidalis* *Cyracantharis taratica* *Diablocatantops axillaris* *Gastrinargus* spp. *Locusa* spp. *Ornithacris* spp. *Schistocera gregaria* *Truxalis* spp.	later stages of *Acorypha clara* *Acorypha glaucopis* *Krauselle amabile*
Intermediate	*Ailopus* spp. *Hieroglyphus daganensis* *Morphascris fasciata* *Oedaleus senegalensis* *Trilophidia* spp. *Zonocerus varieegatus*	*Acrotylus* spp. *Ailopus* spp. *Morphacris fasciata* *Oedaleus senegalensis* *Trilophidia* spp.
Slow fliers or week jumpers	*Acrotylus* spp. *Chrotogonus* spp. *Pyrgomorpha* spp.	*Chrotogonus* spp. Nymphs of *Catantopinae* *Krauuaria angulifera* *Schstocerca gregaria* *Trixalis* spp. *Zonocerus variegatus*

Figure 5.9

ASSESSMENTS IN MEDIUM-SIZED (6-10) PLOTS

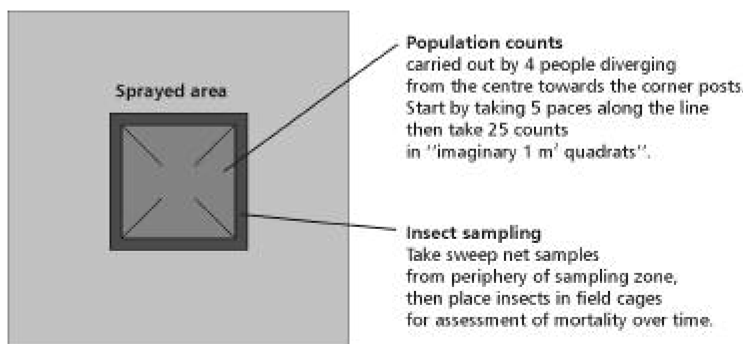


Figure 5.10

DATA RECORDING SHEET

Cage sample monitoring sheet																							
Experiment																							
Samples	Plot		Day																				
	Sample size		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Pre-spray	Plot 1	Mortality																					
	25	Sporulation																					
	Plot 2	Mortality																					
	25	Sporulation																					
	Plot 3	Mortality																					
	25	Sporulation																					
	Plot 4	Mortality																					
	25	Sporulation																					
	Plot 5	Mortality																					
	25	Sporulation																					
	Plot 6	Mortality																					
	25	Sporulation																					
	Plot 7	Mortality																					
	25	Sporulation																					
	Plot 8	Mortality																					
	25	Sporulation																					
	Total control	Mortality																					
		Sporulation																					
	Total treated	Mortality																					
		Sporulation																					
	Sample size		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Day 0	Plot 1	Mortality																					
	25	Sporulation																					
	Plot 2	Mortality																					
	25	Sporulation																					
	Plot 3	Mortality																					
	25	Sporulation																					
	Plot 4	Mortality																					
	25	Sporulation																					
	Plot 5	Mortality																					
	25	Sporulation																					
	Plot 6	Mortality																					
	25	Sporulation																					
	Plot 7	Mortality																					
	25	Sporulation																					
	Plot 8	Mortality																					
	25	Sporulation																					
	Total control	Mortality																					
		Sporulation																					
	Total treated	Mortality																					
		Sporulation																					
	Sample size		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Day 3	Plot 1	Mortality																					
	25	Sporulation																					
	Plot 2	Mortality																					
	25	Sporulation																					
	Plot 3	Mortality																					
	25	Sporulation																					
	Plot 4	Mortality																					
	25	Sporulation																					
	Plot 5	Mortality																					
	25	Sporulation																					
	Plot 6	Mortality																					
	25	Sporulation																					
	Plot 7	Mortality																					
	25	Sporulation																					
	Plot 8	Mortality																					
	25	Sporulation																					
	Total control	Mortality																					
		Sporulation																					
	Total treated	Mortality																					
		Sporulation																					
	Sample size		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Day 6	Plot 1	Mortality																					
	25	Sporulation																					
	Plot 2	Mortality																					
	25	Sporulation																					
	Plot 3	Mortality																					
	25	Sporulation																					
	Plot 4	Mortality																					
	25	Sporulation																					
	Plot 5	Mortality																					
	25	Sporulation																					
	Plot 6	Mortality																					
	25	Sporulation																					
	Plot 7	Mortality																					
	25	Sporulation																					
	Plot 8	Mortality																					
	25	Sporulation																					
	Total control	Mortality																					
		Sporulation																					
	Total treated	Mortality																					
		Sporulation																					

8. NOTES

CHECKING SPRAY DRIFT AND COVERAGE WITH DYES, PIGMENTS AND CARDS.

Droplets can be sampled by various methods e.g.:

Artificial targets

Dyes or pigments on glossy ('Kromekote'), cards Ciba Geigy cards (2 types: water sensitive and "oil" [i.e. solvent] sensitive cards, Magnesium oxide slides, Paraffin or silicone oil matrix in Petri dishes.

Natural targets

Ultra violet tracer pigments are added to the spray formulation at an appropriate rate, so that droplets can be seen clearly (e.g. approximately 1:30 in the case of formulated 'Lumogen'). Droplets counts can be estimated in various ways

- droplets per 1 cm of plant stem or grass leaf
- droplets per cm² on large leaves
- droplets per insect

Field assessments of controlled droplet application (CDA) techniques often include the estimation of spray droplet numbers deposited in different parts of a crop to determine coverage on the target, appropriate swath widths and contamination outside the treated area. Do not spend too much time counting individual droplets. Speed of is important so that you can reduce carelessness due to fatigue and enable large numbers of observations to take place under conditions of limited manpower.

A gauge has been developed for use CDA experiments (Bateman, 1993). It is based on a standardised 0-9 scoring system and has undergone several modifications to make it more widely applicable and "realistic". A computer was used to produce randomly generated populations of circles representing droplet images. *Figure 5.10* is composed of randomly generated positive images suitable for use with daylight tracer dyes or oil-sensitive cards. A similarly generated negative image for assessment of droplets marked with fluorescent tracer materials with ultra violet light is shown in Section 4.

The numbers of marked droplets on targets are compared with a sheet containing dotted areas which increase in a doubling progression with each index number. These may be converted back into estimates of droplet numbers with the aid of computer spreadsheets or a specific computer programme. Each index (I) represents a range *where*:

the equivalent median droplet number = $2^I - 1$

You can also simply check for an "adequate" coverage averaging say 30 droplets/cm (use gauge no 5).

The indices heading each column represent unit mean diameters. Multiplying the index by 100 µm gives the approximate mean mark diameter, and dividing by a spread factor of 2 will give a crude estimate of actual droplet diameter with most types of card. The increase of dose from left to right is therefore related to the cube of each index, and a mean droplet volume factor (compared with the left-hand sample in each row) is shown underneath each column. Columns 3, 5, 7 and 9 contain randomly generated normally distributed spectra where the Volume Average Diameters (VAD) have been matched to these units; they represent VMD/NMD ratios of approximately 2 (or spans of 0.9).

Figure 5.11
DROPLET SCALE: POSITIVE IMAGE

CHECK LISTS

"ARENA" TESTS

0.25 ha plot, mown or cleared

Supply of insects

Cages

Paper clips (if adult insects are used)

Cassava cuttings or maize plants

ULV sprayer (one for each formulation/isolate)

UV-tracer (Lumogen)

UV lamp

Fungus formulation

Thermometer/Hygrometer

Anemometer

Measuring cylinders, funnels, buckets

Recording sheets on clip board

Artificial targets (below) at each sampling site

Artificial targets for checking droplet deposits:

Glass slides

Oil-sensitive paper/Black glossy cards

Target posts (plus spade/ mallet)

Elastic bands/ clips/ double-sided tape

Petri dishes containing antibiotic agar (under development)

FIELD TRIALS

2-3 assistants and suitably sized field site infested with grasshoppers or locusts.

ULV sprayer (if possible: one for each treatment, 1 spare)

Batteries: as required plus spare set

Tool kit, spare discs, motor, pump etc.

Fungus formulation

Flags and marker posts (see below)

Thermometer/Hygrometer

Anemometer

Measuring cylinders, funnels, buckets

Cages

Sweep nets

Recording sheets on clip board

UV-tracer (Lumogen) / UV lamp; if you feel you need to check droplet deposits more intensively bring artificial target kit listed above.

Marking out plots (*Figure 5.9.*)

As well as the vehicle or tape measure you will need:

16 marker posts for each plot (2 m tall and made more visible with bright paint or fluorescent marker tape)

spade or mallet for inserting posts into ground

A prismatic "optical square", or a 120 m rope loop marked at 30, 40 and 50 m for forming right angles

2 temporary posts or canes

2 flags (1 x 2 m minimum)

VERY LARGE PLOTS

The larger the trial site, the more useful walkie talkie radios become (where permitted); binoculars are also very useful. Global Positioning Systems (GPS) are becoming more affordable and rapidly become "essentials" in remote areas.

APPLICATION TO OTHER INSECTS

The general concepts discussed in detail above are applicable to any highly mobile insect. For less mobile insects, trials are often much simpler, and adequate, statistically valid results may be gained on very much smaller plots. In all cases, several important points must be recognised: Familiarity with the insect and its behaviour Using accepted methods for counting the insects, but spending time analysing them statistically to be sure they will yield sound data. Spending enough time in the field to observe unexpected effects.