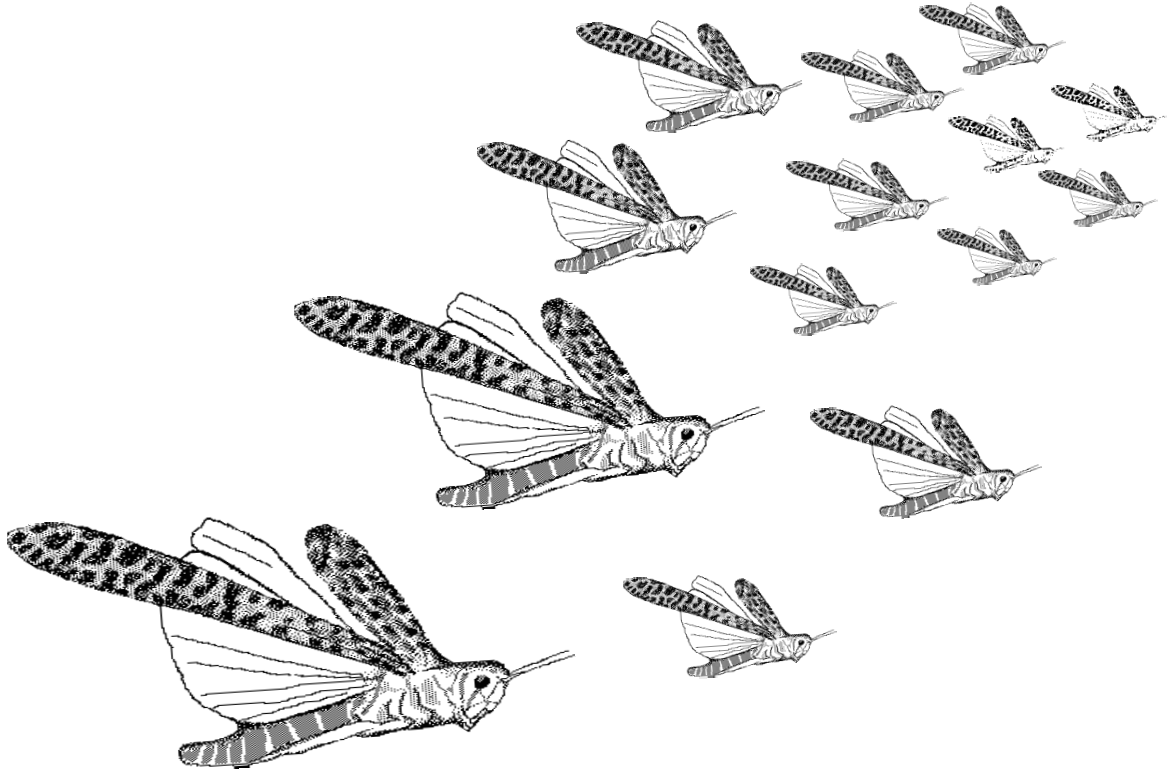
Guidelines for Pesticide Trials on Desert Locust Hoppers



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Guidelines for pesticide trials on hopper bands of Desert Locust, *(Schistocerca gregaria)* using ultra low volume (ULV) applications

1. INTRODUCTION

The objective of these guidelines is to give advice on the design of pesticide trials on Desert Locust infestations and on their reporting. The aim is brief standardised reports with no significant information omitted and enough detail to enable the trials to be repeated. The design of trials depends on the characteristics of ULV application, the characteristics of the insects and the characteristics of the pesticide effects. These will vary from trial to trial so the guidelines here are not hard and fast rules for every situation. It is important to think carefully before and during the trial, to expect difficulties which may invalidate results and to have flexibility to modify the trial accordingly.

The Locusts and Other Migratory Pests Group of FAO (Rome, Italy) would appreciate receiving reports of trials carried out on Desert Locusts to add to their information base.

2. TRIAL DESIGN

Test organisms: Nymphs of the Desert Locust (*Schistocerca gregaria*) living in gregarious groups (hopper bands).

Area: Areas with sparse and clumpy vegetation and a great deal of bare ground are suitable. The vegetation should neither be too dense (where hopper bands are difficult to trace and pesticide residues are too much diluted) nor too light (speed of hopper bands is too high, pesticide is lost on soil). Area and vegetation type should in principle be representative of Desert Locust habitat conditions, but uniform habitat (flat terrain, regular vegetation pattern) makes evaluation easier without invalidating the results.

Test product: This should be a pesticide which from field trials, laboratory data or operational experience is expected to perform well. Products with undesirable characteristics such as high mammalian toxicity or environmental side-effects are unlikely to be worth testing.

Reference product: This is useful to check that all else is well with the trial, for example a failure with the test product may indicate a defective atomiser or unfavourable meteorological conditions, which would otherwise have given a falsely bad impression of the test product. Use a registered product at an area dosage which has proved satisfactory in practice. In general, formulation type and mode of action should be close to that of the test product, but this will depend on the aim of the particular trial.

Unsprayed control plots: These are not necessary for fast acting pesticides since the effects on the hopper bands begin quickly and the band can then be watched. For slower acting pesticides, an unsprayed control gives an indication, although not a completely certain one, of what would have happened to the locust populations within the sprayed plots had they not been sprayed. They are useful to check on major changes in background populations such as mass exodus after fledging, or mass hatching of new generations.

Treatments: Each different pesticide and each different dose rate constitute a treatment which must be replicated.

Replicates: To be sufficient to allow for between plot variance in a statistically sound way, probably close to 10 replicates, including some in less than ideal conditions. The temptation to do one or two trials with many different treatments must be avoided.

Plot size : Size and location of a plot will depend on:

- application equipment
- place of the specific hopper band to be studied;
- expected direction and distance of displacement;
- expected speed of action of the pesticide.

Minimum plot size for **quick acting pesticides** is determined mainly by the requirement to produce a reasonably sized, uniformly treated sampling area using ULV equipment.

Hand held sprayer:	1 ha
Vehicle mounted sprayer:	4 ha
Aircraft:	1 km^2

Minimum size for **slow acting pesticides** (e.g. insect growth regulators) is more dependent on the behaviour of the insect and its response to the pesticide than on the spray equipment. Plots must be very large so that bands do not march out of the plot before the hoppers have had time to acquire a lethal dose. Ideally a plot must be several times larger than the net displacement a band can achieve in its lifetime. A band will move at least 5 km and can move much further. In the case of barrier treatment, ideally a very large block is treated of which only a central plot of 50 or 100 km^2 is sampled.

Trial plots should be well separated to prevent spray drift from one to another or interaction of locust populations. Separation should be at least:

Hand held sprayer:	100 m
Vehicle mounted sprayer:	200 m
Aircraft mounted sprayer:	500 m

Target stage: Trials may start as soon as sufficient hopper bands appear in the area under study. Hoppers should ideally be older than 2nd instar (too susceptible) but should not have developed beyond the early fifth instar (fledging soon). The actual spray target may be the insects themselves (fast acting contact pesticide) or the vegetation (slow-acting stomach pesticide).

Sprayer: Rotary spray devices give the narrowest drop spectra and should always be used in trial work. If the apparatus is used in a known way the emitted drop spectrum can be determined later using a laser analyser. Laser analysis data may be already available but they must have been produced using the formulation being tested or at least one with a similar viscosity. There is no reason to suppose ULV spraying from the air will be either more or less effective than ULV ground spraying, or that different ground methods will produce different results, provided the same sized droplets are used in the same circumstances.

Area dosage: The aim of the trials is to determine an optimal area dosage for effective control. The approximate amount of pesticide required may be known from previous trials or operations. This should then be the target area dosage. If there is less certainty about this, 2 or 3 area dosages can be tested, always remembering that each area dosage constitutes a separate treatment that will serve only as a very rough guide unless there are sufficient replicates.

Spray technique: Applications should be made in tracks at right angles to the wind. To obtain a reasonably even deposit, the distance between spray tracks (track spacing) should be less than the total downwind distance that the spray is carried (swath width), (see Figure 1). Flagmen or some other system of accurately marking track spacing, are essential to ensure even coverage.

3. TRIAL PROCEDURES

Calibration of equipment: Before the trials start, the spray equipment should be calibrated and the operators fully familiar with it. Manuals should be referred to and the sprayer adjusted to produce a suitable nominal drop size.

The potential swath width of the sprayer should be determined either by referring to previous work or by testing in the field. The testing is most easily done using an array of oil sensitive papers to collect the droplets at different distances downwind. Papers should be mounted vertically, facing into wind, just above vegetation height on sticks which are 1 cm or less in width. Horizontal samplers are not suitable since they do no catch the small drop produced by ULV equipment. 10 sampling distances should be sufficient, but to allow for variation in distribution and deposition of the spray, 3 parallel lines of 10 samplers, separated by at least 10 m, are recommended. Typical downwind sampling distances for sprayers are:

Hand held sprayer:	0, 2, 4, 7, 10, 15, 20, 25, 35, 50 m
Vehicle mounted sprayer:	0, 5, 10, 15, 20, 25, 35, 50, 70, 100 m
Aircraft mounted sprayer:	0, 10, 20, 40, 70, 100, 150, 200, 250, 350, 500 m

The sprayer should be filled with pesticide and run across wind past the upwind end of the sampling layout. If the samplers are not becoming overloaded with drops (which will make them difficult to count) more than one spray pass can be made to help to even out the large variations in distribution and deposition normally occurring. The droplets on the papers can be counted and a graph drawn of deposition downwind. To count droplets is not strictly an accurate measure of volume deposition since larger droplets are deposited closer to the sprayer, but the width of the droplet spectrum should be quite narrow from ULV equipment and the technique is simple and adequate. From the swath width of the sprayer, a track spacing which will overlap the swaths to give a reasonably uniform deposit should be chosen for use in the trial.

Nominal flow rate to give the required volume application rate must be calculated. Flow rate checks must be made using the actual pesticide and sprayer restrictor settings adjusted until flow rate is satisfactory. Flow rate can often vary from day to day, so it is advisable to recheck it at the beginning of each spray day. This may be difficult with an aircraft, or unnecessary if it is fitted with an accurate flow meter, but every effort must be made to apply the pesticide as near to the nominal dosage as possible otherwise replicates will not be comparable.

Speed of ground based sprayers can also be calibrated at this stage. Using a marked out track of measured length and a stop watch, operators can time their passes, whether on foot or in a vehicle. They can familiarise themselves with the required speed to give the nominal dosage.

It is also useful for flagmen to calibrate their pace so that they can measure track spacings as they go without pre-marking. The best way is for the flagman to walk comfortably and count the number of paces in a measured 100 m track. This is easier than him trying to adjust his pace to be exactly 1 m. (Total plot size should always be measured afterwards by some more accurate means such as a distance wheel or tape measure).

Laying out the plot: Spraying must be carried out approximately crosswind. The plot must be laid out immediately before spraying since wind direction is rarely constant for very long. It is difficult to lay out a large plot quickly and very difficult indeed when an aircraft is used. Walkie talkie radios are very desirable when laying out large plots and radio communication with the aircraft is essential. It is a common experience to lay out a plot and find that the wind direction has changed by the time the aircraft has arrived, so that the plot must be re-aligned in great haste. A pentagonal prism "optical square" which lays-off a precise right angle is a great help. The simplest technique is to mark a base line at right angles to the wind, lay-off right angles to each end, and mark the ends of each spray run as you go. The plot can be measured later.

Application conditions: Spraying should start early morning and finish before the onset of heat convective turbulence, characterised by the wind beginning to vary considerably in strength and direction. The time that this occurs will depend on factors such as cloud cover and temperature so no absolute time can be given. Further spraying can be carried out in the hour or so before sunset if for example, spraying barriers when it will not be necessary to make a mortality assessment soon after. Windspeed should be greater than 1 m/s for hand held sprayers and greater than 2 m/s for vehicle mounted and aircraft mounted applications, to ensure that spray is carried over a reasonable swath. The stronger the wind, the better, up to a windspeed of around 5 m/s - droplets carried horizontally will be more likely to impact on insects or vegetation than on the ground.

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Area dosage measurement: The exact volume of pesticide actually applied per unit area of plot will never be precisely what is intended so every effort must be made to accurately determine it. With a hand-held sprayer, it is easy to measure the area of the plot and the volume applied. With large plots it is more difficult to measure the area and the volumes applied are usually too small to be measured in relation to the pesticide tank. In this case emission rate, speed of the aircraft or vehicle, time actually spraying, track length and track spacing should be determined as carefully as possible.

4. ASSESSMENT OF MORTALITY

Place: Mortality assessments should not be made within roughly one swath width of the upwind plot boundary since this area will be underdosed (see Figure 1). Strips of plot at the sides and downwind edges should also not be assessed due to movement of insects into and out of the plot. The width of these strips will depend on the mobility of the insects and the speed of action of the pesticide. The sampling area is typically a much smaller area than the sprayed plot, situated towards the downwind edge rather than centrally.

Method and timing: The preliminary assessment should be made immediately before spraying. Frequency and timing of following assessments depends on the type of pesticide used.

With **quick acting pesticides**, the hoppers will not move very much so the process is relatively simple. Using quadrats, count hoppers dead and alive soon after spraying (2 - 4 hrs) then about 1 hour before sunset. If there are too many alive to count, a rough estimate is sufficient since there is little interest in quantifying a poor kill. Immigration, emigration, scavenging and natural mortality can be ignored. Parallel

unsprayed "control" plots are unnecessary. Care must be taken with some pyrethroid pesticides where the initial rapid "knockdown" gives the impression of complete kill. The moribund insects must be monitored for 2 - 3 days to check whether they eventually die or recover.

Determining the efficacy of **slow acting** pesticides is much more difficult since the band will move, leaving behind the dead and dying. Estimates of dead, say by quadrat counts, must cover the whole sampled area, as must estimates of those alive, since the dead and the living will not be in the same place. Band densities may be too high for quadrat counting and the insects too mobile. Density of the live insects may stay the same as they regroup to replace dead ones. On the other hand the pesticide may cause the band to disperse so that quadrat counts of live insects give an over estimate of efficacy - one needs to present evidence that the bands have been killed, not merely broken up. If scavenging of corpses occurs on a significant scale by other insects or birds, no estimate can be made of numbers dying. Care is needed to ensure that counts give a reliable estimate of mortality.

Hopper bands have to be followed for a few days and with insect growth regulators, until at least the next moult. Ideally two or three observations should be made each day, e.g. about 1-2 hours after sunrise, around mid-day and about 1 hour before sunset. Close observation is not only necessary to monitor the efficacy of the product, but also to avoid losing the hopper band. Counts are only comparable for particular behaviour patterns, eg when marching or roosting. In practice it is easiest to observe and count hopper bands when they are marching.

During the observations the following records should be made:

- size of the hopper band (in m^2)
- an estimation of the density of hoppers in the band (no. of nymphs/m^2)
- distance the hopper band covered since the last observation (place of observation to be marked in the field)
- type of activity of the hopper band (marching, ground grouping, roosting)
- abnormalities in behaviour or development of hoppers

Further counts over several days after this are **not a test of persistence.** If hopper numbers remain low, the reason may simply be that the insects are not very mobile and are not re-entering the plot. Conversely, if insect numbers increase, it may be that the pesticide is still killing insects by secondary contact or by stomach action, but that the insects are re-entering faster than they are being killed.

A method to determine the efficacy of slow acting pesticides in large plots with a large number of hopper bands, is to compare the "percentage band infestation" before and at intervals after spraying. This can be done by crossing the plot on parallel tracks and noting at regular intervals whether one is in a band or not. The proportion of points in a band is a valid measure of the proportion of the area covered by band. Density estimates can be improved by assigning density categories to each point and calculating the percentage of points of each density category before and at intervals after spraying. Density categories might be called "band", "sub-band", "scattered" or "present" and plausible density figures for 3rd instar hoppers for these are 200/m^2, 50/m^2, 10/m^2 and 1/m^2.

NOTE: It is emphasized that the design of trials with slow acting and relatively persistent pesticides is determined to a very large extent by the specific characteristics of the pesticide. It is therefore recommended to consult experts in pesticide testing and before staging such a trial to avoid potential mistakes in the trial procedures.

Time and frequency:

Preliminary assessment:	immediately before treatment.
1st assessment:	day of treatment
Further assessments:	daily or more often, at least until next moult with IGRs, often not more than 24 hours with fast acting pesticides.

Caging: This is a useful supplement with a slow acting pesticide. It is of little use with a quick-acting, mainly contact, pesticide since most nymphs should be dead before you can collect a sample. It must be remembered that caging mimics natural conditions only if the area treated is large enough for the band to remain with sprayed vegetation for the time nymphs caged with sprayed grass take to die. Caging sprayed locusts with unsprayed grass as well as with sprayed grass, will show whether the pesticide is just slow acting or whether it is accumulated over a period. It is always necessary to keep a few cages of untreated locusts with unsprayed grass as controls to check whether the pesticide or the stress of caging is killing them. It is very difficult to keep larvae alive in gauze cages for any prolonged period. Even with every effort to provide the right amounts of sunlight, shade, humidity, vegetation, resting sites, moulting sites etc and to protect the insects from predation by ants, lizards, spiders and birds, high mortality is common before 2 weeks. Catching the insects may be a cause, since the process of sweep netting often damages them. Their cuticles may be abraded by the net or by grass and seeds stuck to it. The least damaging way to catch insects for caging is to drive along in a vehicle or motorbike and hold a sweep net just above the grass. Disturbed locusts will jump or fly up and be caught. If the speed of the vehicle is kept just fast enough to hold insects to the back of the net they will suffer far less physical damage than if they had been caught by netting from side to side on foot.

Another possible cause of cage mortality is catching them with a net contaminated with pesticide. Sweeping over sprayed grass can quickly contaminate a net, so the net must either not be allowed to touch the vegetation, or it must be regularly exchanged for a freshly washed one. It is a bad mistake to use a different net or set of nets for catching sprayed insects and unsprayed control insects. The **same net or set of nets** must be used to catch the control insects to check whether net contamination is causing some or all of the mortality in the test insects.

Alternatively, it may be that what is being observed is simply the large scale natural mortality of larvae which must be occurring in the wild.

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Large plastic flasks with holes in the lid make quite satisfactory cages and have the advantage that the vegetation does not dry out quickly if they are kept in the shade. Wood framed cages (50x50x50 cm) draped with mosquito netting can also be used. Problems with these are that insects often rest on the netting rather than on the dosed vegetation, often get trapped between netting and frame, sometime bite holes in the netting and can be attacked by ants unless the cage legs are put into oil baths.

If the cages are reused they must be decontaminated. This is very difficult to achieve with synthetic pyrethroids.

Mortality expectations: One cannot usually expect to kill all the locusts in a sprayed area. There will usually be a few individuals which escape the spray - they may be sheltered by vegetation or other insects or in the case of a stomach acting pesticide, happen not to feed on dosed vegetation. A mortality of 95% is considered very good but mortality less than 90% is inadequate.

Testing persistence: Indications on the length of time that the pesticide residues remain effective is important for developing efficient control strategies. Cage tests may provide these indications. Field sprayed vegetation is offered to hoppers in cages at

different intervals after spraying, until mortality is less than 50%. The mortality in these cages should be compared to the mortality in cages where hoppers are offered unsprayed vegetation. This requires a caging system that hoppers can survive - itself quite a challenge in the field, and a steady supply of hoppers over a period of several weeks. However, the alternative is even more unlikely - to monitor the sprayed area and hope that hoppers appear at intervals to assay the insecticidal activity of the vegetation.

Residue analysis: Laboratory testing of vegetation samples collected at intervals after spraying is useful to check on the physical breakdown/dispersal of the pesticide, although it may not tally with the observed field persistence. Active pesticide may persist on parts of the plant which are not eaten or touched by the insects, for example the dried out bottom leaves of grasses.

5. DETAILS TO BE DETERMINED AND NOTED

All the details below should be carefully recorded:

Pesticide: Common name, trade name, manufacturer, formulation concentration (g a.i./l), diluents/dilution ratio (if applicable).

Equipment: Make and type of aircraft/vehicle/atomiser

Application details:

- rotational speed of atomiser (RPM) - blade angle (if applicable)
- nominal droplet size (VMD)
- variable restrictor unit setting or color of flow restrictor
- emission rate (and how measured)
- total time spent spraying
- vehicle/aircraft/walking speed (speedometers and airspeed indicators are not accurate measures of spray vehicle speed)
- emission height
- track spacing
- number of runs and area sprayed
- nominal area dosage (l/ha and g a.i./ha)
- measured area dosage (l/ha and g a.i./ha) and how measured
- problems encountered
- droplet deposition data from oil sensitive paper
- estimated swath width
- droplet size if the means are available for sizing.

Location: The approximate location of the trial should be described in such a way that it can be found again on a map by someone not familiar with the country. It should thus include:

- name of the nearest town or village (appearing on a standard country map).
- distance/direction of the trial area in relation to this town of village
- map coordinates

Habitat: A detailed description of the terrain and vegetation is required since this influences both pesticide deposition and insect behaviour. The following characteristics of the trial plots have to be listed:

- dominant species of plants and trees
- height of grass/herb/shrub/tree layer
- patchiness of vegetation

- density of cover (overall/within patches/between patches)
- state of vegetation (green, dry, in seed etc)
- soil type
- soil structure (e.g. if many cracks in the surface where locusts could hide)

Locust population:

- species: (*Schistocerca gregaria*)
- stage/composition of population (trials are preferably carried out on fourth or early fifth hoppers, since these are generally the least susceptible stages to pesticides)
- size of the band(s)
- approximate density
- details on band behaviour: e.g. speed of hopper band marching

Meteorological conditions (record before, during and after spray):

- temperature
- daily max and min temperatures over observation period (if slow acting pesticide)
- relative humidity
- mean wind speed and range at standard height (2 metres)
- wind direction relative to spray tracks
- cloud cover (estimated %)
- rainfall in mm per day and details e.g. if all rain fell in short rain storm

Pre and post spray population assessments: To be reported are:

- method of assessment
- count data
- mortalities at different post-spray times

Effects on other organisms: Any effects on other insects, on wildlife, birds, fish etc should also be recorded. This cannot replace full environmental side-effect trials but may provide useful background information for them.

Any clinical and/or biological effects on the applicators drivers, loaders, or pilots should also be recorded, as appropriate.

Appendix 1 shows a record sheet which can be used to record the details . It is only an example and will have to be altered for some types of trial.

6. REPORTING

All information listed above in Section 5, as well as other methods, details and results, should be reported in a brief, systematic form. The report should include original (raw) data and an analysis and evaluation. Statistical analysis should be used, where appropriate, by clearly stated methods.

7. PLANNING TRIALS

To mount a trial one must have hoppers. Bands will occur for a few weeks only and the timing can be forecast confidently only a few weeks ahead of that. Even then, suitable infestations may prove difficult to find.

A trial must be planned on the basis of minimum support and maximum mobility. Every extra person increases the logistic problems, especially the number of vehicles needed.

A work programme (sometimes called a protocol) should be drawn up for discussion with involved parties for comments and advice. Such an initial plan is very important to help people think about the problems, but it must be emphasised that it should not be rigid and unchangeable.

There is a danger in planning over-ambitious trials with too many products and different dosages. This can lead to poorly executed trials and inconclusive reports. It is better to concentrate on one or two products to produce trial results and reports which become a reliable reference for others. Such reports can be submitted before the FAO Pesticide Trial Referee Group for possible inclusion in the FAO list of publications.

The trials should be planned carefully with action taken only when needed, so that the exercise can be cancelled with the least loss. A suggested procedure might be:

1. Contact FAO Desert Locust Information Service to find out where breeding is likely, say 6 months ahead.
2. Make outline plans - decide size of team, method of application, amount of pesticide, etc.
3. Three months later find out from DL IS where breeding is likely 3 months ahead.
4. Decide on area and provisional date for operations - contact PPD in that country to obtain permission and promise of cooperation.
5. Draw up detailed plans with dates (decide on a provisional "D" day and count back), e.g.:

liaison with PPD in the country		D - 90
dispatch of pesticide		D - 70
acquisition of equipment	by	D - 60
dispatch of equipment		D - 30
provision of vehicles	by	D - 20
arrival on site of liaison/preparation officer		D - 20
departure of main party		D - 5

6. Regular contact with country and FAO to advance or retard time table, or cancel exercise.

It must be emphasized that to make rigid plans involving many people, which cannot be altered or cancelled, can result in great waste.

8. EQUIPMENT NEEDED

This is not an exhaustive list but covers most things:

Walkie talkie radios (UHF or VHF) for larger trials
Surveying tape (100m),
Anemometer (0 - 10 m/s, preferably with averaging function),
Thermometer
whirling hygrometer
Pentagonal prism (optical square)
Measuring cylinders
Funnels
Buckets
Basic tool kit
Protective clothing, (soap, water, overalls, gloves, masks, boots etc.)
Binoculars
Poles
"Day-glo" cloth
Tally counters
Stop watch

Wire "quadrats" (can be made easily with rings of wire or plastic tubing)
Cages or plastic flasks with perforated lids (5 litre)
Secateurs or shears (for cutting vegetation samples)
Scissors
Adhesive tape (if oil sensitive papers are used, double sided tape is needed)
Oil-sensitive paper
Oil soluble fluorescent tracer
Ultra-violet lamp
Pocket magnifiers
Clip boards
Camera and film
Mallets
Tachometer for measuring RPM
Sprayers and spares
Compass
Torch
Sweep nets
Calculator
Cages (5 litre plastic containers with perforated lids)

Hans Dobson, 8 May 1991

NB. The electronic version of this document does not contain figures and appendices. These may be requested from the author (Hans@Dobsons.demon.co.uk).