SOP Title 6: Semi Field Trials- Open Field Experiments for Aerial Applications

Objective

The objective of small-scale studies is to determine the efficacy and optimum application dosage of the formulated space spray product or space spray machinery under relatively controlled settings. The aim is to determine the dosage or operational setting that achieves a minimum of 90% mortality. Dosages are selected to produce a range of efficacy that includes >95% mortality and at least one between 80% and 95%, in order to demonstrate that the minimum volume is being used to create the maximum effect. Semi field tests should be located in an open field, with minimal obstruction of the spray plume by vegetation or other obstacles. The testing of a positive control, of a standard insecticide, is encouraged.

Trial Design

For aerial applications, there is a clear distinction between chemical and equipment testing. For chemical testing, a matrix of the sampling stations is prepared centrally within an application zone. The sampling stations are spaced in a square each 20 m apart with a minimum of five lines in each direction (Fig 1). The target zone must be large enough to contain both the flux and deposition of the spray. When testing new formulations and active ingredients, application equipment with known and uniform distribution pattern should be used and a positive control of a known compound is encouraged.

For equipment testing, the aim is to calculate the effective distance of the spray plume and deposition profile. This is done by flying a single flight line: one pass can be used but flying two or three passes over the same line at the same altitude increases the amount of chemical released and aids analysis. The sample stations should be arranged in a downwind transect with the first station under the flight line (Point Zero). The transect should be a minimum of 5 km with sample stations separated by 200m. Where resources allow, run duplicate sample stations at each downwind distance (Fig 2). An active ingredient with a known toxicity should be used at full application rate to ensure that it is the efficiency of the equipment that is being tested and not efficacy of the compound.

Sample stations

These small field trials repeat experiments in the same location maintaining control over habitat to increase the likelihood that change is due to treatment parameters and not to an environmental input. Therefore, using traps for assessing the impact on the wild population is not appropriate. Instead, efficacy is assessed by observing the mortality of susceptible laboratory-reared mosquitoes in field bioassay cages. Alongside the bioassay cage, information on the volume, the droplet size distribution of the spray plume and deposition to the ground is collected.

For the field bioassay, cylindrical screen cages with open mesh, are used to confine the test mosquitoes. A minimum of two duplicate cages with 25 insecticide susceptible nonblood fed female mosquitoes are positioned at each sample station. Rotating impactors are placed alongside the mosquito bioassay cages to provide information on the airborne volume, droplet density, and droplet size distribution of the spray.

Deposition on the ground is measured using filter paper. A fluorescent tracer (Tinopal OB for oil-based formulations; Tinopal CBS-X for water-based formulations) is added to the spray mix to enable volumetric analysis via fluorimetry, which allows inexpensive and rapid analytical techniques. Gas Chromatography (GC) analysis can also be used but this technique can be cost prohibitive. Even when using GC analysis, the tracer should still be included as, during the microscopic analysis, the fluorescent tracer allows the operator to discern the fluorescing pesticide drops from naturally occurring oil aerosols which will be non-

fluorescing. Where water-based compounds are to be tested, magnesium oxide (MgO) coated slides can be used but these surfaces are not suitable for measuring droplet sizes of <10 µm. MgO slides can only be assessed microscopically, but a volume can be derived by droplet number and size per unit area. The fluorescent tracer helps identify craters on the MgO slides[1].

Atmospheric conditions

The droplet size distribution used in space spraying is very small, which makes the spray cloud highly susceptible to atmospheric conditions. The meteorology at time of application is one of the most important factors effecting control and, therefore, accurate measurement of wind speed, direction, temperature and humidity is essential. Atmospheric stability is important, which can be calculated by measuring wind speed and temperature at two heights (1.5 m and 10 m are preferred with 5 m being a suitable alternative) to facilitate the calculation of a stability parameter such as the Richardson number. Optimal conditions for spray trials occur under neutral or stable atmospheric conditions. This is typically at or near sundown and up to an hour after sunrise. At this time, there is an increase in temperature with height above ground level compared to unstable (daytime) conditions. Unstable conditions are radiative and convective and, considering the small droplet size distribution, the spray can be easily taken up and out of the target area; therefore, unstable conditions are to be avoided. Neutral and stable conditions are optimal in helping to keep the small droplets from rising above the target zone. Testing should occur at windspeeds between 3-15 km/hour, measured within 10 m of ground level, and should not occur during precipitation events. If replicate experiments are carried out on the same day, there should be a minimum of a 30-minute interval between each replicate.

The actual sampling intervals (time before pickup) should be determined based on size of test application area, prevailing meteorology and estimated time taken for spray cloud to fully clear the test area. When the most distant sampler is located more than 500 m from the spray line, the waiting time should be 4 times the length of time that it would take the spray cloud to reach the most distant sampling location if it was moving at the speed of the prevailing wind. For example, if the spray was made under 5 km/hr wind conditions and the furthest sampling location was 1000 m from the spray line, a minimum wait time would be 48 mins [4 *1000 m * 60(min/hr)/ (5000 m/hr)].

Procedure Semi Field Trials- Open Field Experiments for Aerial Applications

Materials list

- Insecticide susceptible, laboratory reared, non-blood-fed, 2–5 day old female mosquitoes
- Sprayer
- Formulated chemical and diluent (where applicable)
- Weather station(s); wind speed, wind direction, temperature and humidity
- Sample station stands (minimum of 24)
- Tape measure
- Boards for sedimentation samplers covered in aluminum foil
- Rotating impactor
- Slides (Teflon for oil-based formulation sprays or magnesium oxide for water based)
- Slide holder
- Mosquito bioassay cages

[1] Volume data can be returned by placing a non-coated slide in the other position. Although the droplet will not maintain integrity, the volume should be retained for volumetric analysis.

- Holding cups
- Gloves
- Filter paper or acetate sheeting
- Forceps
- Clock, notebook and pens
- Amber vials for slides and amber vials for filter paper
- Wash solution
- Fluorimeter
- Microscope (preferably fluorescent)

Preparation

1. Calibrate the equipment to deliver droplets with a $Dv_{0.5}$ of <25-45 µm and a $Dv_{0.9}$ of <100 µm. The droplet size distribution required will depend on the aircraft altitude and the specific gravity of the compound being sprayed with guidance being provided by the chemical manufacturer. The nozzle type and application settings will dictate droplet size distribution and should be carefully considered.
2. Build a frame to hold the mosquito bioassay cages and rotating impactor 1.5 m above the ground. If possible, the rotating impactor and bioassay cages should be positioned at least 1 m apart.
3. Prepare the meteorological station with sensors at two heights (if possible) 5-10 m and 1.5 m.
4. Prepare the boards to hold the sedimentation sampler.
5. Label amber glass vials for each experimental compound, dose, species, replicate and sample number. The glass vials should be of an appropriate size to hold the folded sedimentation samples and slides.
6. Build disposable mosquito bioassay cages.
7. Prepare a label for each cage with an appropriate annotation for each experimental compound, dose, species, replicate and sample number.
8. Aspirate 25 susceptible, non-blood-fed, 2-5 day old female mosquitoes into each cylindrical field cage (a minimum of two cages per sample station).

Station set up

1. Use historical weather data to find an area that runs in line with a predominant wind direction, typically a road or opening that allows access to the sampling sites, for the transect design. DO NOT conduct the transect spray if the wind direction is greater than 30 degrees off the direction of the transect.
2. Mark out the location of the sample station locations as per Fig 1 and Fig 2.
3. Set up sample stations, placing the board holding the filter paper at the base of the station, and mount the rotating impactors loaded with appropriate slides.

Spraying Procedure

1. Before the spray run, place the mosquito bioassay cages on the sample stations, and switch on the rotating impactors.
2. Record the delivery characteristics of the treatment, including parameters such as discharge rate, application altitude, vehicle speed, nozzle angle and pressure.
3. Record the time of the spray application, so that the relevant temperature, humidity, wind speed and direction information can be extracted from the logged data. *Suggestion: Record in a notes*

section any significant events that occur throughout the experimental run and manually note the atmospheric conditions (temperature, humidity wind speed and direction) as a backup to the data logger information collection.

4. Apply treatment.
5. Do not conduct tests when wind direction is more than 30° from the sample line (Fig 3), in transect studies. Correct distance accordingly for wind direction in the data recording and report. The sprayer is turned on a minimum of 1000 m before reaching the test area and turned off at a minimum of 1000 m beyond the test area.
6. Wait for >30 mins and then start the post spray procedure of collecting samplers. The spray plume travels a long distance and needs to settle out from altitude, so the waiting period should reflect that.

Post Spray

1. Ready enough field personnel to rapidly collect and transfer mosquitoes.
2. Collect the bioassay cages, lightly anesthetize the mosquitoes in the field and transfer into clean labelled holding cups. Place holding cups in a protective container (a cooler box is ideal) with 10% sugar solution on cottons. As soon as possible, transfer the mosquitoes in clean holding cups to a room at 27±2 ˚C temperature and 80±10% RH for 60 min knockdown and 24-hour mortality observations.
3. Using forceps, fold the ground deposition samples into their prelabeled amber glass vials, rinse the forceps with acetone between each sample. Store the vials in a cool box and return them to the laboratory store them in a refrigerator (cool area) until analysis.
4. Transfer the slides to the laboratory in a protective cool box for droplet size and volumetric assessment. Place the volumetric slides in amber vials and store in a refrigerator (4 - 10 °C).
5. If there are concerns over volatility of the formulation, the team must conduct the microscopic analysis immediately, if not they can be stored for deferred analysis.
6. Measure the droplet size distribution: During microscopic analysis a minimum of 200 droplets should be measured for each slide, reading across the width of the collector as many times as necessary[2]. If you are using image analysis software the area covered will be recorded and the relevant statistics calculated. Manual droplet sizing requires that the number of complete traverses is recorded until> 200 droplets sizes are recorded. Only count droplets within the eyepiece graticule are counted so area can be calculated.
7. Fluorometric analysis: For the slides and the ground deposition samplers, add a predetermined type and volume of wash solution to the amber vials. Shake the vials for a fixed time (e.g. 20 seconds per jar) to ensure all the tracer goes into solution. Compare the fluorescent reading of the sample to the calibration standard to determine the volume deposited on the sample.

Recording knock down and mortality

1. Record knockdown 60 minutes after exposure. A mosquito is classified as knocked down if it cannot stand (e.g. has one or two legs), lies on its back-moving legs and wings but unable to take off, cannot fly in a coordinated manner or takes off briefly but falls immediately.

[2] As the air flows around an object (the slide) suspended particles will continue in their original direction due to their inertia. Smaller particles have less inertia, streamlining with the air, so the impact probability will increase closer to outer the edge.

2. Record mortality 24 hours after exposure. A mosquito is classified as dead if it is immobile, cannot stand or shows no signs of life. If control mortality is >10%, the test must be repeated.

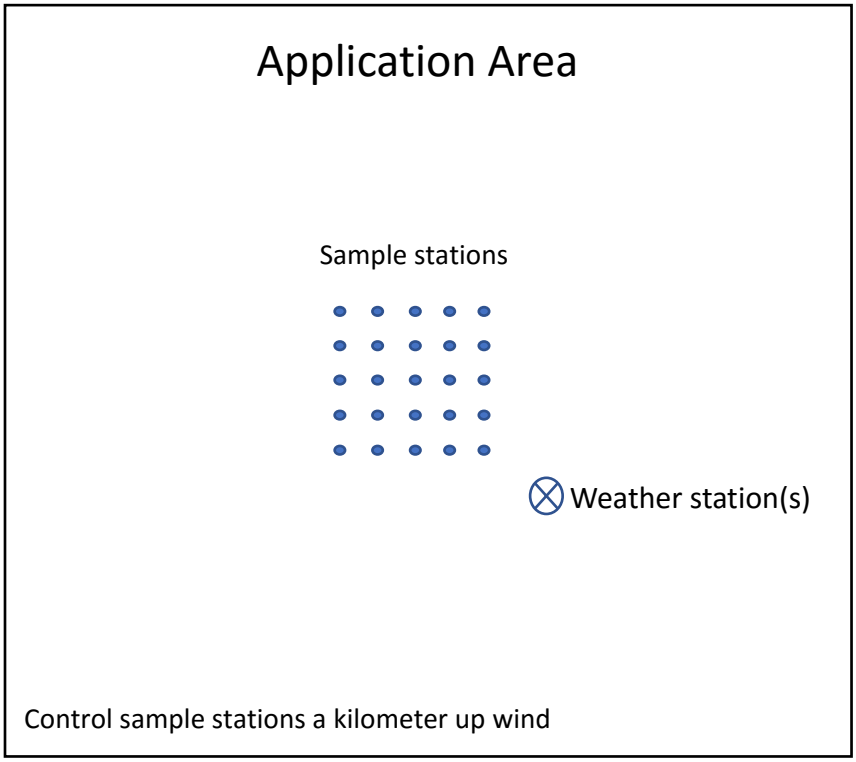

Figure 1 The design of an aerial open field trial set up for assessment of chemical efficacy

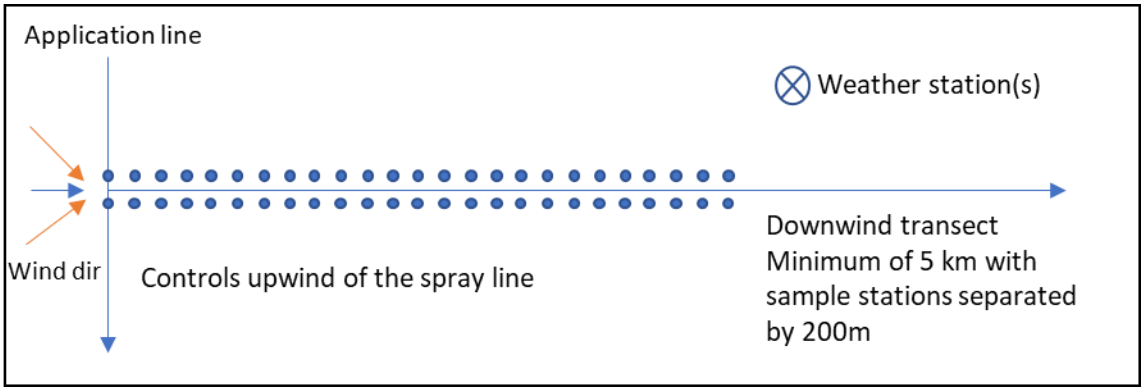

Figure 2 The design of an aerial open field trial set up for equipment assessment via a downwind transect.

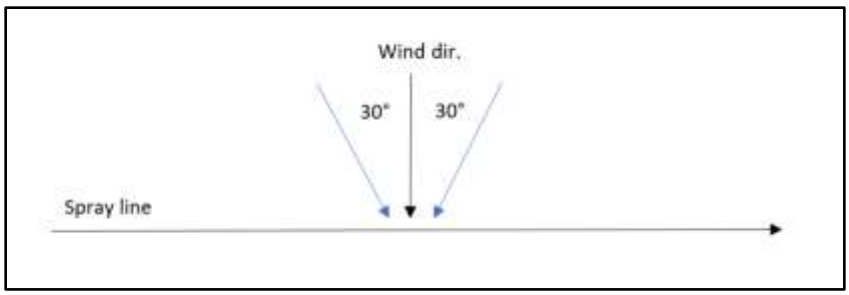

Figure 3 Diagram to show the experimental set up in regard to wind direction and sample location and spray line