

SOP Title 5: Semi Field Trials - Open Field Experiments for Ground-based Applications

Objective

The objective of small-scale field trials is to determine the efficacy and optimum application dosage of the formulated space spray product or to assess new space spray machinery under relatively controlled settings. The aim is to determine the dosage or operational settings that achieves a minimum of 90% mortality in laboratory-reared, insecticide susceptible mosquitoes. 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.

Design

At this stage of testing, although the objectives are different, there is little difference between chemical testing and equipment testing due to the scale of the trials. Semi field tests should be conducted in an open field, with minimal obstruction of the spray plume by vegetation or other obstacles (and an unobstructed fetch upwind if possible). The testing of a positive control, of a standard insecticide or application technique, is encouraged. This study utilizes a simple design of downwind samplers, where the spray application line is considered line zero and a minimum of three lines of sample stations are placed at 30, 60, and 90m downwind for truck mounted. For hand or back portable spray equipment, the distances should be 10, 20 and 30m. Each sample line should be separated by 10 m. Control sample stations should be located a minimum of 50 m up wind from the spray line.

Sample stations

Small field trials repeat experiments in the same location maintaining control over habitat to increase the likelihood that change is due to treatment parameters not an environmental input. At this point trap data of 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 and the droplet size distribution is collected using rotating impactors and deposition to the ground is collected with sedimentation samplers.

Atmospheric conditions

The droplet size distribution used in space spraying is very small which makes the spray cloud highly susceptible to atmospheric change. The meteorology at time of application is one of the most important factors effecting control, and therefore measurement of wind speed, direction, temperature and humidity is required. Information should be provided at the highest resolution possible. Atmospheric stability is important, which can be calculated by measuring wind speed and temperature at two heights (1.5 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 or up to an hour after sunrise. At this time there is an increase in temperature with height above ground level as opposed to the decrease in temperature seen during unstable (daytime) conditions. Unstable conditions are radiative and convective, and considering the small droplet size distribution, the spray plume is not distributed uniformly through the test area and can be taken up and out of the target area; unstable conditions are to be avoided. In contrast to this, neutral and stable conditions help 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 30-minute interval between each replicate. The actual intervals should be determined based on size of test application area, prevailing meteorology and 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 [$4 * 1000 \text{ m} * 60(\text{min/hr}) / (5000 \text{ m/hr})$].

Procedure Semi Field Trials- Open Field Experiments for Ground Applications

Equipment list

- Insecticide susceptible, laboratory reared, non-blood-fed, 2–5 day old female mosquitoes
- Sprayer
- Formulated chemical and diluent (where applicable)
- Data logger weather station(s); wind speed, wind direction, temperature and humidity
- Sample station stands (minimum of 12 including controls)
- 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 storage container (airtight)
- Mosquito bioassay cages,
- Holding cups
- Gloves
- Clock, notebook and pens

If conducting fluorimetric analysis:

- Filter paper or acetate sheeting
- Forceps
- Amber vials for slides and amber vials for filter paper
- Wash solution
- Fluorimeter
- Microscope (preferably fluorescent) with calibrated eyepiece graticule

Preparation

1. Calibrate the equipment to deliver droplets with a $Dv_{0.5} < 30 \mu\text{m}$ and a $Dv_{0.9} < 50 \mu\text{m}$, unless otherwise indicated by the manufacturer.
2. Build the sample station frames to hold the mosquito bioassay cages and rotating impactor approximately 1.5 m above the ground.
3. Prepare the metrological station with data logging sensors at two heights (if possible) 5- 10m and 1.5 m or one at 1.5 m.
4. Aspirate 25 susceptible, non-blood fed, 2-5-day old female mosquitoes into each cylindrical field cage.

5. Prepare the holding cups and slide holders (appropriately sized slide boxes to hold the slides vertically and separately). Alternatively, larger plastic boxes with frames to accommodate complete rotating impactor arms with slides still attached.
6. Prepare a label for each cage with an appropriate annotation for each experimental compound, dose, species, replicate and sample number.
7. Prepare the boards for the sedimentation samples.
8. Label amber glass vials for each experimental compound, dose, species, replicate and sample number for both the sedimentation samplers and the slides.

Station set up

1. Using weather forecasts, estimate the wind direction and arrange the spray line perpendicular to the anticipated wind direction. Change the set up if the wind direction is consistently more than 30 degrees from perpendicular.
2. Mark out the grid for the sample station locations from the spray line (30, 60, 90m for vehicle mounted sprayers and 10, 20, 30m for handheld sprayers) with a minimum of three spray lines separated by approximately 10 m.
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. Shortly 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, 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. Recorded in a notes section any significant events that occur throughout the experimental run and manually note the atmospheric conditions because the dataloggers can fail.
4. Apply treatment.
5. Tests should not be conducted when wind direction is more than 30° off the sample line angle (perpendicular to the spray line), because this creates an excessive increase in the distance between the spray line and the collection stations (Fig 1). Wind speeds need to be below 15km/hr. Correct distance accordingly for wind direction in the write up. The spray machine is turned on at a minimum of 100 m before reaching the test area and turned off at a minimum of 100 m beyond the test area.
6. Wait for a minimum of 30 mins (based on wind speed and time taken for spray cloud to completely clear the test area) and then start the post spray procedure of collecting samples.

Post Spray

1. Have enough field personnel ready to rapidly collect and transfer mosquitoes.
2. Collect the bioassay cages, lightly anesthetize the mosquitoes in the field and transfer into clean holding cups. Provide the holding cups with 10% sugar solution on cotton wool and place in a protective container. As soon as possible, transfer the mosquitoes to clean bioassay cages or holding cups in a room at 27 +/- 2° C temperature and 80 +/- 10% RH for 60 min knockdown (including the 15 min exposure time) and 24-hour mortality observations.

3. Transfer the slides to the laboratory for droplet size and droplet density analysis. If volumetric and/or fluorimetric analysis is being conducted place the volumetric slides in amber vials and store in a refrigerator.
4. Conduct the microscopic analysis immediately if there are concerns about the volatility of the test item. If no concerns samples can be stored for deferred analysis.
5. Calculate droplet size distribution, accounting for the formulations spread factor ($Dv_{0.1}$, $Dv_{0.5}$ and $Dv_{0.9}$).
6. Fluorometric analysis (if required in protocol):
 - Using forceps fold the ground deposition samples into their pre-labeled 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 (4-5 °C) until analysis. This step can be conducted in the field, or if a suitably designed deposit board holding box is available, taken back to the laboratory and performed there.
 - For both 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 predetermined 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. 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 >20%, the test must be repeated.

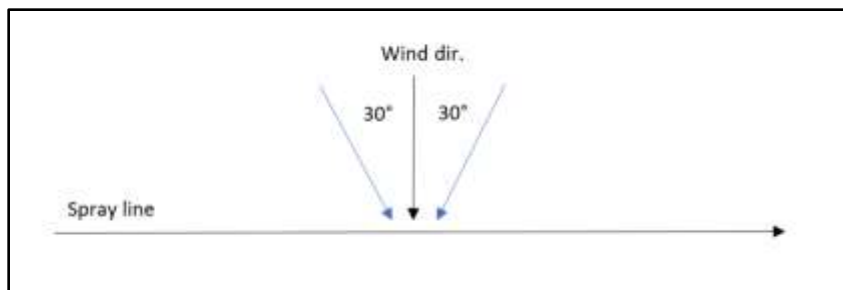


Figure 1 Diagram to show the experimental set up in regard to wind direction and sample location and spray line