SOP Title 7: Operational Outdoor Field Trials Ground and aerial

Introduction

Operational field trials assess the effectiveness of the formulated space spray product or equipment against field populations of the target species. The methods of collecting adult mosquitoes for measuring relative changes in density must be appropriate to the target species. Alongside the assessment of the natural population there should be controlled measures of effect using cage mosquito bioassays, volume and droplet size distribution of the spray plume and ground deposition.

Operational trials with truck mounted ground equipment will be conducted most typically within an urban environment, as a road system is required. Backpack aerosol generators are available for smaller areas with less of a road network. Aerial applications are wide area applications whether they be urban or suburban, there are no limitations to aerial applications that are typically made from release height of 30-100 m based on terrain and man-made towers in the area. Single operations with truck mounted equipment typically cover 0.5-5 square kilometers, whereas aerial applications may cover up to 100 square kilometers at a time.

The dose recommended on the label or the equipment settings that were found to provide >90% mortality from open field small scale studies using caged mosquitoes should be used. However, when moving from a small-scale, open-field trial to an operational trial, higher application rates may be needed due to the increased number of obstructions, harborages and other factors. In addition, if resistance is present in the natural population the dose may need to be adjusted along with other application factors. The susceptibility of the local target species to the insecticide must be verified beforehand, as described in SOP 7-1.

Design

The area and location of test sites should be representative of the target species' habitat. The street plans, layouts or vegetation characteristics should be carefully surveyed, mapped and recorded. Extensive surveillance is necessary to provide detail of the population and its natural fluctuations over time. The optimal design is a random allocation of treated and untreated areas to habitats that would be considered comparable. There should be a minimum of three replicate sites and control sites for each intervention using Country-approved control products. As a general guideline, each site should be a minimum of 1 and 5 km^2 for ground and aerial trials, respectively.

Operational protocols often require that once the mosquito population exceeds a threshold the intervention is applied. A threshold may be a predetermined population number that triggers the need for mosquitoes to be controlled, or it may be a combination of factors including mosquito population numbers, increasing prevalence of disease agents and heightened risk to the public. After a single intervention recruitment from larval sites and invasion from unsprayed areas over the successive days may lead to a rebound in mosquito population numbers. If the scope of the experiment was to simply look at an acute response to the intervention post application population decline alongside comparative analysis of the sample stations would be used as the measure of effect. Where the scope of the experiment is to ascertain whether longer term population suppression can be achieved additional applications will be required with a suggested minimum of 4 consecutive sprays spaced 5-7 days apart. These types of multiple applications are often referred to as "sequential" and may be timed based on observations of the mosquito population rebounding past a predetermined threshold, or alternatively a set number of days later based on historical knowledge of mosquito population dynamics and the

mosquito species biology. The sequential applications are continued until the scope of the investigation is reached. Acute experiments should be replicated over time in the same location whilst suppression experiments should be replicated over space.

The controlled measure of effect is required to determine that the chemical has entered the target zone. This is achieved with a caged mosquito bioassay as a biological measure of effect and a rotating impactor and ground deposition sampler to physically define the chemical distribution. A variety of open and sheltered habitats should be established to provide replicated observations of resulting mortality for statistical analysis throughout the trial. The locations should be mapped and repeated. A detailed description of each sample station location should be given along with a measure, along the lines of leaf area index, to gauge the obstruction level of the location. The sample stations are a very useful comparison to open field studies so that the effect of obstructions on the spray plume can be assessed. It is important that rotating impactors be side by side with bioassay cages at each location to determine if the sprayed material reached each location. This is critical in foliage-dense areas.

Timing and Atmospheric Conditions

The droplet size distribution used in space spraying is very small (volume median diameters of truck sprays are typically <20μm, while aerial sprays are typically 30-45μm), which makes the spray cloud highly susceptible to atmospheric change and wind speed and direction. The meteorology at time of application is one of the most important factors effecting control; therefore, measurement of wind speed, direction, temperature and humidity is required. Information should be provided at the highest resolution possible. Understanding the atmospheric stability is important so weather conditions should be measured at two heights (1.5 and 10 m, or minimum of 5 m) to calculate the Richardson number. Optimal conditions for spray trials occur under neutral or stable atmospheric conditions (Richardson number <0.5), which typically occur 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 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, meaning unstable conditions are to be avoided. 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. Space sprays are best applied when mosquitoes are active and flying, which is usually at dusk and dawn. If daytime applications are required, then it may be necessary to at least avoid the hottest times in the middle of the day and possibly use a slightly larger droplet size distribution.

Procedure Semi Field Trials- Open Field Experiments

Equipment list

- Trapping and surveillance systems relevant for the target species
- Non-blood-fed, 2–5 day old female mosquitoes
- Gloves and goggles
- Sprayer
- Formulated chemical
- Fluorescent tracers (if needed)
- Weather station(s); wind speed, wind direction, temperature and humidity
- Sample station stands
- Boards for sedimentation samplers covered in Aluminum foil
- Rotating impactors

- Slides
- Slide holder
- Mosquito bioassay cages and clean transfer containers
- 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. Begin surveillance of the target species within an appropriate time prior to the application- minimum of 1 month is the suggested timeframe.
2. Determine the threshold population level prior to commencement of the experiment. Applications are triggered in operational field trials when the threshold is reached.
3. Map and locate the position of each sample station and provide a detailed description of the location with GPS coordinates
4. Calibrate the ground or aerial application system- see the relevant calibration guideline.
    - Machines must be calibrated to deliver a spray droplet distribution with a $Dv_{0.5}$ of <20 µm and a $Dv_{0.9}$ < 50 µm for ground equipment and $Dv_{0.5}$ of <45 µm and a $Dv_{0.9}$ < 100 µm for aerial equipment (as determined by the waved or spinning slide method), unless the manufacturers or label instructions indicate otherwise.
5. Build frames to hold the mosquito bioassay cages and rotating impactor 1.5 m above the ground.
6. Prepare the meteorological station with sensors at two heights (if possible) 5-10 and 1.5m or one at 1.5m.
7. Aspirate 25 susceptible, non-blood-fed, 2-5 day old female mosquitoes into each cylindrical field cage. Prepare a label for each cage with an appropriate annotation for each experimental compound, dose, species, replicate and sample number.
8. Prepare the ground deposition samplers ready for deployment in the field.
9. 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 filter paper or acetate sheeting.
10. Field vehicle(s) need to be prepared for sample transportation.
11. Two people should position, collect and transfer the mosquitoes. One person should be in charge of the rotating impactors and one in charge of the filter papers. A separate person should take overall charge of the experiment, communicate with the spray operator, oversee the meteorological station, and the running, timing and notation of the experiment. A separate person (pilot or truck driver) should oversee the spray machinery and stay isolated from the rest of the experiment.

Station set up

1. Survey the target area defining representative habitats, map the locations and set out a minimum of 20 sample stations throughout the target zone.

2. Map the sample station locations and provide a numerical indication of obstruction. This could be a qualitative index of 1-100 where 1 is open and 100 is completely obstructed. Or a quantitative measure using a fisheye camera for a measure of leaf area index or vegetative density.
3. Set up sample stations, placing the board holding the filter paper at least 1 m from the base of the station, holding the rotating impactors loaded with appropriate slides and the mosquito cages.

Spraying Procedure

1. Place the mosquito bioassay cages on the sample stations shortly before the run, 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. Record 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. Ensure the area sprayed sufficiently covers the test site, that the test side is a smaller area positioned centrally in the larger application zone
5. Collect sample stations for post treatment procedures 15 min after a ground application and >40 mins after an aerial application.

Post Spray

1. Prepare for the post spray surveillance: surveillance should be conducted the night before, the night of the experiment (at least one hour after the application) and the following evening.
2. Collect the bioassay cages and 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 holding cups containing the mosquitoes at to a room at 27 +/- 2˚ C temperature and 80 +/- 10% RH for 60 min knockdown and 24-hour mortality observations.
3. Collect the slides for volumetric and droplet size distribution measures (i.e. droplet density such as drops/mm^2). If there are concerns over volatility of the formulation, the team must conduct the microscopic analysis immediately. Otherwise the slides can be stored for deferred analysis.
4. Transfer the slides to the laboratory in a protective cool box for droplet size and volumetric assessment. Place the volumetric slides in slide holder boxes and store in a refrigerator.
5. Accounting for the formulations spread factor the droplet sized distribution (Dv0.1, Dv0.5 and Dv0.9) is calculated. A spread factor of 0.62 is common for most oil-based sprays
6. Collect the ground deposition samples and place into their prelabeled amber glass vials, rinsing 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 -10 °C) until analysis.

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.