The biological effect of cage design corrected for reductions in spray penetration

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Abstract: In-field measures of physical spray concentration do not tend to correlate well with caged insect mortality data. This is partly due to the reduced penetration of the spray into the cage. Spray penetration is hindered by the structure of the cage. Wind tunnel studies were conducted to investigate the accuracy of those calculations developed to correct for filtration levels in caged mosquito bioassays. Zenivex E20 (Etofenprox) was applied at rates ranging from an LD_{10} to an LD_{90}. Three cage types were used, each with different penetration levels. The dose approaching the cage was converted to the dose entering the cage using cage penetration data from previous research. The penetration conversion factor returned a data set that directly correlated dose with mosquito mortality (R^2 = = 0.918). The mortality percent was a function of the dose within the cage. The mesh type acted as a regulator. Although the conversion factor was effective, the differences between cages was not always significant due to within-group variation.

Key words: bioassay cage, cage insects, field mortality, spray filtration

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Introduction

Evaluating the efficacy of vector control treatments is dependent on accurate measurements of the amount of spray material applied across a given location as well as the insect mortality within the treated area. Both the physical characterisation of the applied spray and the biological measures of efficacy have sampling inefficacies. A number of experiments have been conducted by the authors to investigate the effects of these inefficiencies. This research project is the initial step in synthesising this data to provide more accurate field measures.

Bioassay cages are typically used in field evaluations to confine mosquitoes for a controlled, reproducible, and comparable field count of mortality. The expectation is that returned data will provide comparable information on the treatments applied. Where conditions are equal, and suitable replication can be achieved, bioassay cages are effective tools. The use of these bioassays to determine the mortality of the natural population is typically not advised as the cage is an unrealistic model. Better understanding, and correcting for the effects of the cage could, however, provide a better estimate of natural field mortality. Moreover, where conditions (primarily wind speed) are highly variable, a correction factor would normalise data. Advances in measures of volume and drop-size distribution in the field make such corrections possible.

Penetration inefficiencies of applied sprays into bioassay cages are due to screen porosity (Breeland 1970; Boobar *et al*. 1988; Barber *et al*. 2006) and cage type and geometry (Breeland 1970; Boobar *et al*. 1988; Bunner *et al*. 1989; Hoffmann *et al*. 2008). All of the above affect spray and air flow penetration into the cage. The screening material itself will tend to collect the spray material and there is a relatively high collection efficiency (Fox *et al*. 2004; Fritz and Hoffmann 2008a). As a result, mortality rates of confined mosquitoes do not always correlate well with other observed parameters used to monitor wild mosquitoes populations (Boobar *et al*. 1988).

There is also concern that the variations in cage construction used by researchers, mean that mortality data between field studies using different cages cannot be compared without accounting for the differing penetration amounts (Boobar *et al*. 1988). Hoffmann *et al*. (2008)

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looked at two bioassay cage designs, a flat disk and a cylinder. Airspeed, spray drop-size, and spray fluxes (where flux is defined as volume of spray material moving through a vertical area over the complete duration of a spray treatment) inside and outside the cages, was measured. It was found that spray concentration reductions ranged from 50 to 70%. However, the measured spray flux data was not corrected for the collection efficiency (CE) of the sampler used, which would have differed for the external and internal samplers as a result of the reduced airspeed inside the cage (May and Clifford 1967). Here, CE refers to the percentage of spray material that is presented to the sampler that actually deposits on the sampling surface and is recovered as part of the analysis process. The study by May and Clifford (1967) looked at different sampling surface shapes (flat plates, cylinder, rods, spheres) as well as different droplet sizes and approach velocities. They found that for the same sampler and droplet size, droplets moving at a higher velocity were deposited on the sampling surface with greater efficiency. As a further engineering analysis of different bioassay cages, Fritz *et al*. (2010) examined 12 different mosquito bioassay cages used in the field or previously reported. They measured airspeed, spray drop-size, and spray flux inside and outside of the cages. Spray fluxes were corrected for the collection efficiencies of the samplers used and for the conditions under which the measurements were taken (i.e. the different air velocities inside and outside of the cage). Fritz *et al*. (2010) found that spray fluxes inside the cage were reduced 30 to over 75%, as compared to that outside the cage. There were higher reductions at lower wind speeds. This data set was used to create the correction factors applied in this study.

The first objective of this work was to demonstrate how data from previous research should be applied to correct spray flux data for a sampler's CE. The second objective was to estimate the actual spray dose presented to caged mosquitoes, and the third objective was to illustrate the consequences of not correcting these data.

Materials and Methods

As a brief overview, a unique set of cage mosquito mortality data was collected by applying an insecticide at varying dosage levels in a controlled wind-tunnel environment. A constant airspeed of 2 m/s was maintained. Three different cages types were evaluated, with each being tested at each of the selected spray dosages. For each treatment replicate, a cage with mosquitoes inside was positioned in the tunnel, downwind of the spray nozzle. The spray flux immediately in front of the cage was measured using a fine wire deposition sampler. Using previously developed relationships for both sampler CE and cage filtration correction terms, insect mortality data was compared to both corrected and uncorrected spray exposure levels. This comparison was done to illustrate the critical need for these corrections when examining field data.

The mosquito species used was *Aedes taeniorhynchus* (Central Life Sciences®). The chemical used was Zenivex (Etofenprox, EPA Reg No. 2724-79), and it was also provided by Central Life Sciences®. Four doses (10, 20, 30, and 40 μg/ml) were used to provide mortalities ranging between an LD_{10} and an LD_{90}. Preliminary studies were conducted over a wider range of dosages to more narrowly define those used as part of this work. These dosages represent the rates at which Zenivex was diluted into acetone. Prior to dilution in acetone, Uvitex OB dye (M. F. Cachat Company, Columbus OH) was added to the Zenivex at a rate of 0.2% (w/v). Each treatment replication was applied by feeding 10 ml of the treatment solution into an air-assisted, dual-venture-style, stainless steel nozzle (Advanced Special Technologies, Winnebago, MN) operated at an air pressure of 552 kPa (80 psi) using an extremely fine spray class – according to ANSI/ASAE S572.1 (2009).

The three cages used in this study included a fabric disc cage, a metal disc cage, and a metal cylinder (Fig. 1). These three cages were previously examined for spray penetration by Fritz *et al*. (2010). The cages were shown to have internal spray concentration reductions of 34, 52, and 64% in a 2 m/s airstream for the fabric cage, metal disc cage, and metal cylinder cage, respectively. It should be noted, that these reductions factors varied not only by cage type, but by wind speed. Also, the metal disk cage was not originally evaluated for spray flux reduction as part of Fritz *et al*. (2010), but was evaluated following the same methods for this study. While only basic construction and performance details are described here, more extensive details on these cages can be found in Fritz *et al*. (2010). The fabric disc cage was constructed from concentric, friction-fit cardboard rings that secured the screen material to each face. The overall dimensions were ∅16 cm diameter and 4 cm depth. Screen material was T-310 tulle (Walmart®). The tulle fiber diameters were roughly ∅0.05 to ∅0.09 mm with a distance between fibers of 1.1 mm and a porosity of 83.6%. The metal disc cage was a metal

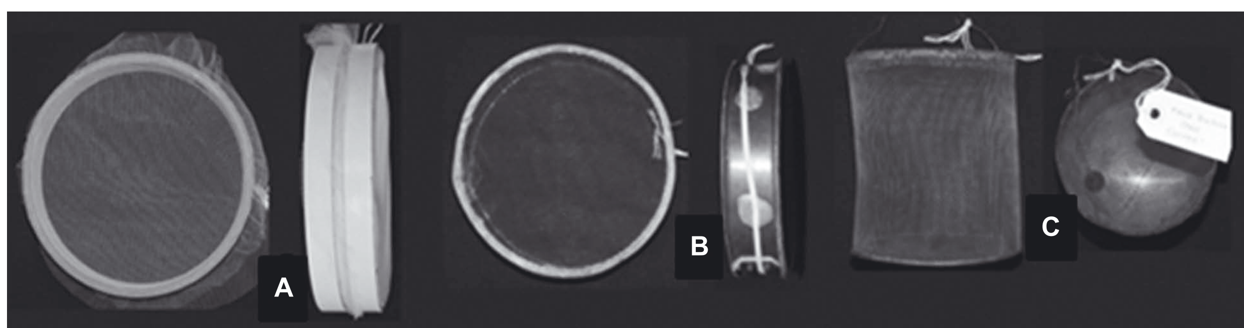

Fig. 1. The three cage designs under investigation: A – the fabric cage, B – the metal disc cage, C – the metal cylinder cage

Fig. 2. Mosquito bioassay cage in position in the wind tunnel: the metal disk cage with volumetric line samplers up-wind of the cage

disc constructed from a copper ring with copper screen soldered to each face. The overall dimensions were ∅15 cm diameter and 3.5 cm depth. Screen material was copper mesh with ∅0.28 mm diameter wire and mesh openings 1.22 ´1.60 mm for a porosity of 57.9%. The metal cylinder cage is a metal cylinder with the dimensions of ∅12 cm diameter and 14 cm height. The copper mesh is the same as that of the metal disc cage.

Each cage was loaded with 25 non-blood fed female mosquitos (*Ae. taeniorhynchus*). Prior to each spray replication, cages were positioned 5 m downstream of the nozzle (Fig. 2). Stainless steel wires (∅0.56 mm diameter × 150 mm length) were positioned 30 cm upstream of the cages. The wires were held in place with hemostats, and the wires were used to assess the volume of spray presented to the cage. After each spray replication, the wire samplers were collected into individually labeled plastic bags. Exposed cages were immediately removed and the exposed mosquitoes aspirated into holding cups (0.5 pint cups with screened tops). This transfer process was performed less the 5 min after treatment insuring little if any effects from contact toxicity (Barber *et al.* 2006). Before re-deployment of the wire samplers for the next replication, the hemostats were cleaned in acetone. Clean gloves were used for each new deployment to eliminate contamination. A total of ten replications were made for every dosage/cage pair. Additionally, control cages were also collected prior to, and between replications five and six as well as after each set of dose/cage treatments. The control samples consisted of placing caged mosquitoes, identical to those tested, into the tunnel and spraying an acetone-only solution at the same 10 ml rate. The control mosquitoes were then aspirated to the holding cups.

Spray drop-size was measured 60 cm upstream of the cage using a Sympatec HELOS laser diffraction drop-sizing system (Sympatec Inc., Clausthal, Germany). The system was fitted with a lens which resulted in a dynamic size range from ∅0.5 mm to ∅875 mm across 32 sizing bins. Tests were performed within the guidelines provided by ASTM Standard E1260: Standard Test Method for Determining Liquid Drop Size Characteristics in a Spray Using Optical Non-imaging, Light-Scattering Instruments (ASTM 2003).

Wire samplers were processed for spray deposition in a laboratory by pipetting 15 ml of hexane into each bag, agitating the bags, and decanting 6 ml of the effluent into a cuvette. The cuvettes were then placed into a spectrofluorophotometer (Model RF5000U; Shimadzu, Kyoto, Japan) with an excitation wavelength of 372 nm and an emission at 427 nm, and a minimum detection level of 0.00007 mg/cm^2. Fluorometric readings were converted to volume of spray material per area sampled, using comparative analysis with fluorometric standards of known tracer dye concentration.

Spray flux data from the wire samplers were corrected. The determined sampler's CE for each spray replication was used for the correction. The full drop-size spectrum, measured as described earlier, was used to determine the CE of the wire samplers following the methods outlined by Fritz and Hoffmann (2008b). In summary, drop-size and velocity data were used to calculate droplet Reynolds numbers. Droplet Reynolds numbers were the used to determine Stokes numbers for each drop-size bin measured. Drop-size specific Stokes numbers were then fitted to data presented by May and Clifford (1967) to determine CE of a cylinder. For the spray droplet size and airspeeds used in this study, the sampler CEs ranged from 62–65%. There was some slight variation between replications due to minute changes of the airspeed in the tunnel. The sampler CE corrected values were defined as Applied Flux. The uncorrected spray flux data were not used in the analysis. The amount of material that actually penetrated into the cage was determined using the spray flux reduction values mentioned previously (34, 52, and 64% reduction in spray flux for the fabric cage, metal disc cage, and metal cylinder cage in a 2 m/s airstream, respectively). These cage reduction corrected values are defined as Spray Penetration.

Mortality counts were made at 24 h after treatment for all the treated and control cages. Insects were considered dead if ataxic. Overall insect mortality (M) for each cage was calculated from the observed mortality in the cages (MO) and any mortality observed in the control (MC) via Abbott's corrected mortality equation (Abbott 1925):

$$M = [(MO - MC)/(100 - MC)] \times 100.$$

All statistical analysis was done using JMP, Version 10 (SAS Institute Inc. 2012) with a least squares regression Fit Model ($\alpha = 0.05$ level).

Results

Drop size for all spray treatments resulted in $D_{V0.1}$, $D_{V0.5}$, and $D_{V0.9}$ values of ∅3.6, ∅12.5, and ∅23.6 μm, respectively. With respect to mortality, and taking cage, dose and cage × dose as the main effects, only dose ($p < 0.0001$) and cage × dose ($p = 0.0033$) were significant. This indicates that the targeted dosage levels did in fact result in a range of applied spray fluxes and hence, spray penetrations and mortality levels within each cage-type test. Initial examination of the data showed that the applied spray flux levels for the metal cylinder cage at the 40 μg/ml spray dosage, fell between the actual applied spray fluxes seen at the 10 and 20 μg/ml dose levels. Additionally, the corresponding mortality numbers also fell between those seen for the same 10 and 20 μg/ml dose levels. These measured and observed mortalities indicated that there were potential problems with the 40 μg/ml dosage treatments, therefore these data were dropped from the overall analysis. The mean and standard deviation of applied spray fluxes; spray penetrations and mortalities for each cage and dosage level, are given in table 1. However, when examining mortality with cage, spray penetration, and cage × spray as the main effects, only spray penetration was significant ($p < 0.0001$). This justified pooling all of the cage data and fitting mortality to spray penetration. The examining of the standard deviations of all the data, showed an indication of a high level of variability. The primary reason for this, was the configuration of the spray system and cages in the wind tunnel used in this work. The spray itself was generated from the nozzle positioned in the tunnel's vertical centered area. While the cages were positioned as far downwind in the tunnel as possible, the length was still not sufficient enough that the spray evenly dispersed across the entire vertical area of the tunnel. The result was the observed variability. While this likely contributed to some of the non-significant effects seen, the individual data for spray applied flux, spray penetration, and mortality for each replication was maintained in the pooled data.

Mortality data was fit to Morgan-Mercer-Flodin (MMF) sigmoidal models (Morgan *et al*. 1975) for both the applied spray flux and spray penetration data using CurveExpert (Version 2.0.2; Daniel G. Hyams©). The plot for the mortality data *vs*. spray penetration and applied spray flux are shown in figures 3 and 4, respectively. Derived from these two relationships, the LC_{50} based on applied spray flux is 0.0094 μl/cm² *vs*. 0.0043 μl/cm² based on spray penetration. This was an overestimate of approximately 2.2 times.

$$\text{24 hour mortality} = \frac{ab + cD^d}{b + D^d} \ (\%),$$

where:

D – spray flux as either applied spray flux or spray penetration (μl Zenivex/cm²);

a, b, c, d – spray penetration, respectively: 8.86, 0.0000016, 97.7, 2.53;

a, b, c, d – applied flux, respectively: 12.1, 0.0000029, 93.2, 2.7.

Discussion

Assessing the success or failure of a mosquito adulticiding application treatment and providing guidance for future improvements hinges on understanding where the spray went, how much of it was present, and what fraction of that spray present actually entered the cage and interacted with the caged insect. The structure of both the sampler and the cages used, the characteristics of the applied spray, and the environmental conditions present, all play a role. Typical field assessments either use spray flux measured directly from the sampler, or in many cases do not measure flux at

Table 1. Dosage, penetration, and mortality levels for the tested cages

Cage	Dosage [μg/ml]	Applied flux [μl/cm²]	Spray penetrations [μl/cm²]	Mortality [%]
Metal cylinder	10	0.0040±0.0035	0.0027±0.0023	21.8±13.5
	20	0.0110±0.0040	0.0073±0.0026	77.1±28.5
	30	0.0178±0.0066	0.0118±0.0044	94.1±7.0
Metal disk	10	0.0099±0.0033	0.0036±0.0012	39.9±14.8
	20	0.0163±0.0060	0.0059±0.0021	66.3±13.0
	30	0.0238±0.0073	0.0086±0.0026	78.8±23.6
	40	0.0163±0.0121	0.0060±0.0042	60.7±36.1
Fabric	10	0.0065±0.0026	0.0031±0.0013	15.9±20.9
	20	0.0091±0.0032	0.0044±0.0015	52.4±23.6
	30	0.0146±0.0071	0.0070±0.0034	76.3±21.3
	40	0.0227±0.0075	0.0109±0.0036	91.5±4.0

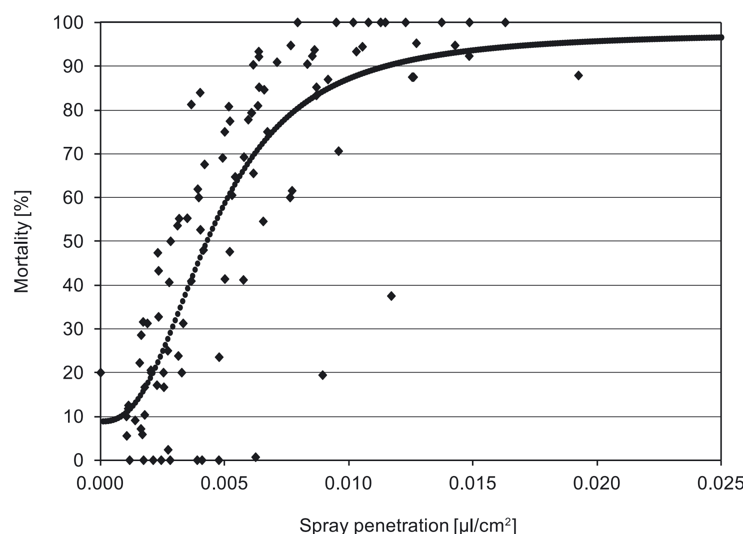

Fig. 3. Relationship between percent mortality and spray penetration

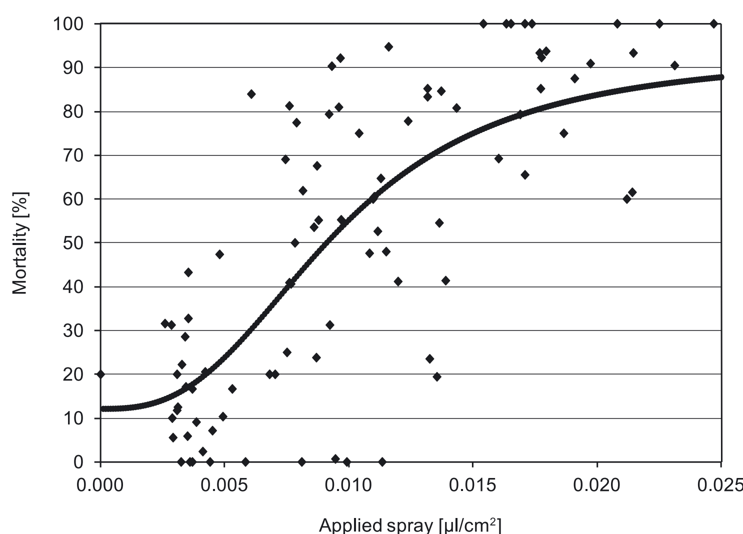

Fig. 4. Relationship between percent mortality and applied spray

all. Similarly, there is typically no accountancy for the filtration due to the cage. This work examined the effects on perceived spray exposure levels to caged mosquitos from both the spray measurement method and physical structure of insect bioassay cage. The sampling device used to measure spray concentration at the location where caged mosquitos are help has its own efficiency at which it captures droplets from the spray cloud that is dependent on both the droplet sizes in the spray and the airspeed carrying the spray through the sampling location. Using relationships developed, this efficiency was determined that the actual spray concentration presented to the bioassay cage was calculated. Further, the bioassay cage structure serves to impede spray material in the form of both a physical barrier preventing air and spray penetration and as a filtering surface which removes a portion of the spray from the total volume presented. Using developed relationships the actual spray penetrating the cage and presented to the bioassay mosquitos was calculated. The combination of the collection efficiency of the measurement system and the impedance of the bioassay cage can result in significant errors in estimating actual exposure levels to the cage insects and in turn misrepresent actual dosage levels required for effective control. As the results of this work show, not taking into account the role of both the sampler and bioassay cage can overestimate the required spray dosages needed for effective mosquito control by more than 220%.

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