

## EFFECTS OF WIND SPEED ON AEROSOL SPRAY PENETRATION IN ADULT MOSQUITO BIOASSAY CAGES<sup>1</sup>

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**ABSTRACT.** Bioassay cages are commonly used to assess efficacy of insecticides against adult mosquitoes in the field. To correlate adult mortality readings to insecticidal efficacy and/or spray application parameters properly, it is important to know how the cage used in the bioassay interacts with the spray cloud containing the applied insecticide. This study compared the size of droplets, wind speed, and amount of spray material penetrating cages and outside of cages in a wind tunnel at different wind speeds. Two bioassay cages, Center for Medical, Agricultural and Veterinary Entomology (CMAVE) and Circle, were evaluated. The screen materials used on these cages reduced the size of droplets, wind speed, and amount of spray material inside the cages as compared to the spray cloud and wind velocity outside of the cages. When the wind speed in the dispersion tunnel was set at 0.6 m/sec (1.3 mph), the mean wind speed inside of the CMAVE Bioassay Cage and Circle Cage was 0.045 m/sec (0.10 mph) and 0.075 m/sec (0.17 mph), respectively. At air velocities of 2.2 m/sec (4.9 mph) in the dispersion tunnel, the mean wind speed inside of the CMAVE Bioassay Cage and Circle Cage was 0.83 m/sec (1.86 mph) and 0.71 m/sec (1.59 mph), respectively. Consequently, there was a consistent 50–70% reduction of spray material penetrating the cages compared to the spray cloud that approached the cages. These results provide a better understanding of the impact of wind speed, cage design, and construction on ultra-low-volume spray droplets.

**KEY WORDS** Sentinel mosquito cages, ULV, droplet deposition, wind tunnel, laser diffraction

### INTRODUCTION

The use of ultra-low-volume (ULV) insecticide droplets for adult mosquito control was developed by the United States Department of Agriculture in the 1960s (Knapp and Roberts 1965), and is broadly used for controlling adult mosquito populations today. Assessment of insecticide efficacy provides the foundation for pest management programs. In addition to insecticide concentration, dose, and release rate, important considerations in ULV efficacy are droplet size and meteorological influences such as wind (Mount 1998). A considerable number of studies have investigated the effects of the aforementioned factors on droplet entry into sentinel cages, but few studies have investigated how varying wind speed may affect ULV droplet entry into cages.

Several studies have found that for ULV to be efficacious the optimal droplet size is less than 20  $\mu\text{m}$  in diameter. Using scanning electron microscopy, Lofgren et al. (1973) found that droplets ranging from 2 to 16  $\mu\text{m}$  in diameter were most likely to impinge on the wings and

antennae of mosquitoes flying through ULV aerosol clouds, and although droplets up to 32  $\mu\text{m}$  in diameter were found on slides, no droplets larger than 16  $\mu\text{m}$  were found on mosquitoes exposed to the same aerosol. Haile et al. (1982) determined that mortality is optimized when droplets are between 10 and 15  $\mu\text{m}$ , and that with increased distance downwind mortality of caged mosquitoes was reduced. Confining adult mosquitoes in sentinel cages for bioassay tests continues to be used as a standard method for evaluating the impact of insecticides on adult mosquitoes in the field (Boobar et al. 1988). However, the mortality rates of confined mosquitoes are not always well correlated with other indices for monitoring insecticidal impact on wild mosquito populations (Boobar et al. 1988). Barber et al. (2006) found that significant mortality could be caused by spray material deposited on the screen materials used in cage construction, and that mosquitoes should be transferred to clean containers as soon as possible after tests. Boobar et al. (1988) suggested that filtering of droplets by cage screening may also contribute to inconsistencies between mortality observed in caged and free-flying mosquitoes. Breeland (1970), using ultra-low-volume sprays and cards placed in and around cages of varying materials, found that droplet count was reduced by varying amounts depending on the material used in the construction of the cages. Rathburn et al. (1989) did not find any differences in the mortality of either *Aedes taeniorhynchus* Wiedemann or *Culex quinquefasciatus* Say when the mosquitoes were 1) confined in 2 cage types, 2) sprayed with 2 different

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volumes of the insecticides, or 3) sprayed with 2 different insecticides. Bunner et al. (1989) studied aerosol penetration through many configurations of solid and screened cages and found that a cylindrical screened cage, with the longitudinal axis perpendicular to the ground, provided a consistent profile to the wind, regardless of wind direction. Boobar et al. (1988) studied the effect of screen materials on droplet size distribution of aerosols entering sentinel mosquito exposure tubes. The cages used were solid, with different meshes or screens at the ends. Although the results showed significant reduction in the number of droplets penetrating the cages, the level of reduction varied with the different screening materials tested.

To correlate adult mortality readings to spray application parameters better, it is important to know how the cage used in the bioassay interacts with the spray cloud containing the applied insecticide. Due to the effects of the boundary layer of the cage mesh, it is expected that differences in wind speed would result in differences in droplet penetration into cages. This study explored this interaction with the following objectives: to evaluate the size of droplets that entered the cages as compared to droplet size presented to the cage, to measure the wind speed inside of the cages as compared to wind speed outside of the cages, and to measure the amount of spray material that penetrates the cages.

## MATERIALS AND METHODS

### Dispersion tunnel

A dispersion tunnel was constructed from standard lumber and plywood. The tunnel cross-sectional area was a  $0.9 \times 0.9$  m ( $3 \times 3$  ft) square area. The overall tunnel consists of a 2.4-m- (8 ft) long section where the spray nozzle is mounted entering into a  $1.8 \times 1.8 \times 6.7$  m ( $6 \times 6 \times 12$  ft) mixing chamber and a 7.3 m (24 ft) section ( $3 \times 2.4$  m [8 ft] sections) leaving the mixing chamber with a fan pulling air throughout (Fig. 1).

The test portion of the tunnel is the center of the last 2.4 m (8 ft) section before the fan. An access panel was created to allow for placement and recovery of cages and soda straws used in this study. Additionally, 2 holes were cut on opposing, vertical walls 1.8 m (6 ft) upwind of the sampling location to allow for placement of a Sympatec Helos laser diffraction droplet sizing system (Sympatec Inc., Clausthal, Germany).

### Atomizer

An air-assisted nozzle (Advanced Special Technologies, Winnebago, MN) was used in this study. The nozzle was designed to be used on

the Terminator ULV Sprayer (Advanced Special Technologies, Winnebago, MN). The Terminator's 4.7 hp (219 cc) Yanamar diesel engine powers a direct-drive air compressor that produces the air blast that creates the pesticide droplet spectrum at the dual venture style, stainless steel nozzles. This nozzle was selected for this work as previous work by Hoffmann et al. (2007) demonstrated that it produced a volume median diameter of  $20.4 \mu\text{m}$  and  $21.7 \mu\text{m}$  for water-based and oil sprays, respectively, which is typical to vector control applications. The nozzle was removed from the sprayer and plumbed to a shop compressor with a pressure regulator set at 690 kPa (100 psi). The self-feed tube from the nozzle was attached to a plumbed graduated cylinder with an inline shut-off valve. This allowed for a metered release of 10 ml of the spray solution over approximately 10 sec, thereby constituting a spray run or replication.

### Spray solution

The spray solution was Orchem 796 mineral oil (Calumet Lubricants Co., L.P., Indianapolis, IN) with Uvitex fluorescent dye at the rate of 1 g/liter of oil. The oil was selected because it is commonly used as a diluent in vector control applications. During each spray replication, 10 ml of the spray solution was sprayed through the nozzle and released into the dispersion tunnel. Solution samples were analyzed in the laboratory to determine the exact amount of dye in solution and used to standardize deposition measurements across the various tests.

### Cages

Two different cages used in adult bioassay tests were evaluated in these studies. The Center for Medical, Agricultural and Veterinary Entomology (CMAVE) Bioassay Cage was a cylindrical cage that can be collapsed during storage and was fully described by Cooperband et al. (2007). The cage was framed by embroidery hoops that are approximately 26 cm in diameter and form the top and bottom of the cage. Wooden dowels are used as internal structural supports making the cage approximately 31 cm tall. The cage was covered in nylon tulle. The 2nd cage is referred to as the Circle Cage and was based on a design provided to the authors by Tom Janasek and David Sykes. The cages were constructed by forming a  $60 \times 6$ -cm paperboard strip into a circle approximately 18 cm in diameter and covering it with bridal veil material. The Circle Cage was designed to be oriented vertically with screen opening positioned into the wind.

Each of the screen materials used had fiber widths of approximately 0.075 mm and openings

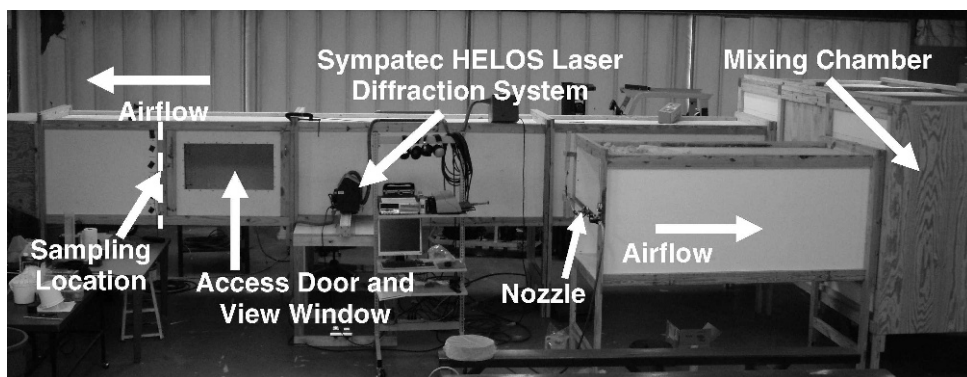


Fig. 1. Spray dispersion tunnel with spray nozzle and Sympatec Helos laser diffraction system in place.

of approximately 1 mm. Teitel and Shklyar (1998) defined screen porosity,  $\alpha$ , as

$$\alpha = \frac{(m - d)(n - d)}{mn}, \quad (1)$$

where  $m$  and  $n$  are distances between 2 adjacent weft (horizontal) and warp (vertical) fibers, respectively, and  $d$  is the diameter of the fiber. Therefore, the 2 screen materials had a screen porosity of 85.5%. However, the bridal veil screens tended to be of a looser weave than the nylon tulle screen.

#### Droplet size equipment

A Sympatec Helos laser diffraction droplet sizing system (Sympatec Inc., Clausthal, Germany) was used to measure the droplet size of the spray material in the dispersion tunnel and presented to the cage and screen samples. The Helos system utilizes a 623-nm He-Ne laser and was fitted with a lens (denoted by manufacturer as R5) with a dynamic size range of 0.5–875  $\mu\text{m}$ , which is divided across 32 sizing bins. The laser system has 2 components, the emitter and the receiver, which were positioned across from each other and outside of the wind tunnel. Holes that were slightly larger than the laser optics were cut into the tunnel at a height of 0.36 m (1.2 ft) as a precautionary measure to minimize air-flow disruption in the wind tunnel and to allow measurement of the spray cloud across the entire tunnel.

The most common term used to describe spray droplet size spectra is volume median diameter ( $D_{V0.5}$ ).  $D_{V0.5}$  is the droplet diameter ( $\mu\text{m}$ ) where 50% of the spray volume or mass is contained in droplets smaller than this value.  $D_{V0.1}$  and  $D_{V0.9}$  values, which describe the proportion of the spray volume (10% and 90%, respectively) contained in droplets of the specified size or less, were also calculated. The percent of spray volume contained in droplets less than 20  $\mu\text{m}$  (%Vol <

20  $\mu\text{m}$ ) was calculated for all tests. The term (%Vol < 20  $\mu\text{m}$ ) is an indicator of the portion of the applied material that will most likely stay aloft after an application and potentially impinge on a flying insect.

#### Droplet size tests

The purpose of the droplet size tests was to compare the size of droplets that penetrated the 2 screen materials to the size of droplets presented to them. A wooden test stand was constructed to span the width of the tunnel to facilitate testing and changing of screen materials. Holes were cut in the vertical sides of the frame to allow the laser beam to pass through the frame unobstructed. The screen material was stretched taut over both upstream and downstream faces of the frame (0.9 m wide  $\times$  0.3 m high). The screen material was replaced with new screen material after 3 replications at a given air velocity. Evaluations were made with and without the screening material across the tunnel (referred to in Tables 1 and 2 as Screen in Place and Open Tunnel, respectively). The air velocities tested were 0.6, 0.9, 1.3, 2.2, and 4.5 m/sec.

#### Air velocity and spray deposition measurement

Each cage was suspended in the center of the wind tunnel and away from the ceiling and floor (Fig. 2). Two hot-wire anemometers (Extech Instruments, Model 407119A, Waltham, MA) were used to measure air velocities. One anemometer was positioned outside of the cage, whereas the other was positioned inside of the cage. Simultaneous readings of the 2 anemometers were made during each of the 3 replicates for each of the air velocities tested. Although the 3 replicates were completed during 1 air velocity setting, the air velocities were randomized throughout the testing.

Concurrent with air velocity tests, soda straws (19.1 cm long  $\times$  0.6 cm diameter) were used to

Table 1. Effect of the screen material used in the CMAVE Bioassay Cage on the droplet size parameters in the open tunnel versus passing through the screen material.

Wind speed (m/sec)	Laser optical path	D <sub>V0.1</sub> (μm ± SD) <sup>1</sup>	D <sub>V0.5</sub> (μm ± SD)	D <sub>V0.9</sub> (μm ± SD)	%Vol < 20 μm
0.6	Open tunnel screen in place	11.0 ± 0.0	18.5 ± 0.2	34.2 ± 1.0	57.2 ± 1.1
		10.2 ± 0.4 <i>P</i> = 0.02*, <sup>2</sup>	17.7 ± 0.2 <i>P</i> = 0.007**	33.1 ± 0.3 <i>P</i> = 0.16 (ns)	60.9 ± 0.5 <i>P</i> = 0.007**
0.9	Open tunnel screen in place	11.6 ± 0.0	20.2 ± 0.2	40.8 ± 0.5	49.2 ± 0.7
		11.1 ± 0.9 <i>P</i> = 0.001**	19.7 ± 0.1 <i>P</i> = 0.01**	40.8 ± 0.1 <i>P</i> = 0.77 (ns)	51.4 ± 0.5 <i>P</i> = 0.01**
1.3	Open tunnel screen in place	11.7 ± 0.1	20.8 ± 0.2	43.7 ± 0.7	46.4 ± 0.9
		11.2 ± 0.1 <i>P</i> = 0.002**	20.1 ± 0.0 <i>P</i> = 0.003**	42.7 ± 0.4 <i>P</i> = 0.09**	49.7 ± 0.1 <i>P</i> = 0.003**
2.2	Open tunnel screen in place	11.6 ± 0.1	20.7 ± 0.2	44.5 ± 0.5	47.3 ± 0.7
		11.3 ± 0.1 <i>P</i> = 0.02*	20.4 ± 0.6 <i>P</i> = 0.43 (ns)	44.7 ± 1.9 <i>P</i> = 0.88 (ns)	48.4 ± 2.3 <i>P</i> = 0.42 (ns)
4.5	Open tunnel screen in place	11.1 ± 0.0	19.7 ± 0.1	44.3 ± 0.3	51.4 ± 0.3
		10.7 ± 0.1 <i>P</i> = 0.002**	18.9 ± 0.0 <i>P</i> = 0.001**	43.1 ± 0.1 <i>P</i> = 0.003**	54.9 ± 0.1 <i>P</i> = 0.001**

<sup>1</sup> D<sub>V0.1</sub>, D<sub>V0.5</sub>, and D<sub>V0.9</sub> values describe the median diameter for the specified volumetric proportion of the spray (10%, 50%, and 90%, respectively) that falls below the size in micrometers given in the table.  
<sup>2</sup> Means within each data pair for the different droplet size parameters were analyzed for statistical differences with the use of the paired Student's *t*-test (ns = not significant).  
\* Significant at α = 0.05.  
\*\* Highly significant at α = 0.01.

collect spray deposits in order to measure the spray flux or amount of material that passed by. Two straws were used, 1 straw positioned inside each cage to measure the amount of material that penetrated the cage, and a 2nd straw positioned outside each cage to measure amount of material presented to each cage (Fig. 2). After each replication, the straws were carefully placed in individually labeled plastic bags and stored out of the light to prevent any photodegradation of the dye. The bags were brought back to the

laboratory for processing. After pipetting 20 ml of hexane into each bag, the bags were agitated, and 6 ml of the effluent was poured into a cuvette. The cuvettes were then placed into a spectrofluorophotometer (Shimadzu, Model RF5000U, Kyoto, Japan) with an excitation wavelength of 372 nm and an emission at 427 nm. These wavelengths optimized the fluorescence of the Uvitex dye. The fluorometric readings were converted to μg/cm<sup>2</sup> with the use of a projected area of the straw of 11.6 cm<sup>2</sup>. The

Table 2. Effect of the screen material used in the Circle Cage on the droplet size parameters in the open tunnel versus passing through the screen material.

Wind speed (m/sec)	Laser optical path	D <sub>V0.1</sub> (μm ± SD) <sup>1</sup>	D <sub>V0.5</sub> (μm ± SD)	D <sub>V0.9</sub> (μm ± SD)	%Vol < 20 μm
0.6	Open tunnel screen in place	10.7 ± 0.2	18.9 ± 0.2	36.4 ± 0.6	54.7 ± 1.0
		10.5 ± 0.1 <i>P</i> = 0.11 (ns) <sup>2</sup>	18.5 ± 0.4 <i>P</i> = 0.14 (ns)	36.4 ± 1.0 <i>P</i> = 0.96 (ns)	56.9 ± 2.1 <i>P</i> = 0.18 (ns)
0.9	Open tunnel screen in place	11.4 ± 0.1	20.2 ± 0.1	41.1 ± 0.3	49.0 ± 0.6
		11.1 ± 0.1 <i>P</i> = 0.006**	19.7 ± 0.0 <i>P</i> = 0.004**	41.4 ± 0.2 <i>P</i> = 0.21 (ns)	51.1 ± 0.2 <i>P</i> = 0.004**
1.3	Open tunnel screen in place	11.7 ± 0.1	20.9 ± 0.3	43.4 ± 0.9	46.5 ± 1.2
		11.2 ± 0.1 <i>P</i> = 0.001**	20.2 ± 0.3 <i>P</i> = 0.04*	42.9 ± 0.9 <i>P</i> = 0.55 (ns)	49.4 ± 1.2 <i>P</i> = 0.04*
2.2	Open tunnel screen in place	11.7 ± 0.1	20.9 ± 0.2	44.9 ± 0.7	46.0 ± 0.9
		11.2 ± 0.1 <i>P</i> = 0.002**	19.9 ± 0.2 <i>P</i> = 0.004**	43.0 ± 0.7 <i>P</i> = 0.03*	50.3 ± 0.8 <i>P</i> = 0.003**
4.5	Open tunnel screen in place	11.1 ± 0.0	19.7 ± 0.1	44.3 ± 0.3	51.4 ± 0.3
		10.7 ± 0.0 <i>P</i> = 0.001**	19.1 ± 0.1 <i>P</i> = 0.001**	43.4 ± 0.7 <i>P</i> = 0.12 (ns)	54.0 ± 0.4 <i>P</i> = 0.001**

<sup>1</sup> D<sub>V0.1</sub>, D<sub>V0.5</sub>, and D<sub>V0.9</sub> values describe the median diameter for the specified volumetric proportion of the spray (10%, 50%, and 90%, respectively) that falls below the size in micrometers given in the table.  
<sup>2</sup> Means within each data pair for the different droplet size parameters were analyzed for statistical differences with the use of the paired Student's *t*-test (ns = not significant).  
\* Significant at α = 0.05.  
\*\* Highly significant at α = 0.01.



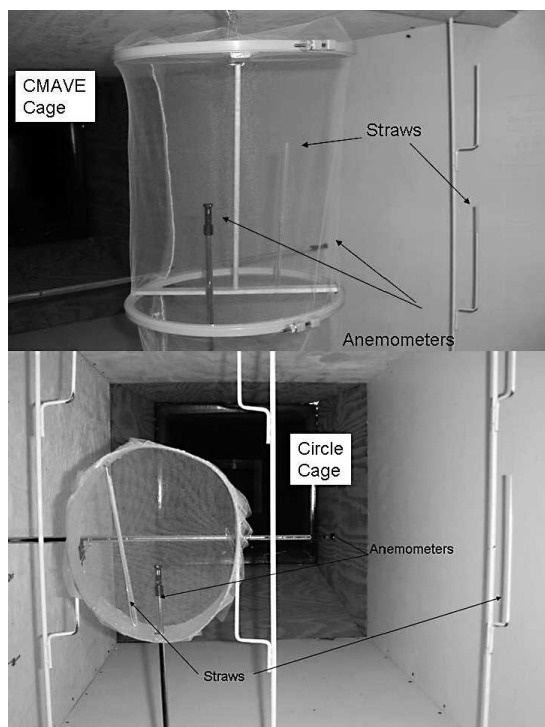


Fig. 2. Center for Medical, Agricultural and Veterinary Entomology and Circle Bioassay Cages in the wind tunnel during the air velocity measurements and straw deposition studies.

minimum detection level for the dye and sampling technique was  $7.0 \times 10^{-5} \mu\text{g}/\text{cm}^2$ .

### Statistical analysis

All tests were replicated at least 3 times and statistically analyzed. Droplet size data were analyzed with the use of the PROC GLM procedure in SAS (SAS Institute 2001). Linear regression equations were developed for the air velocity studies in Microsoft Excel (Microsoft 2003) with the airspeed in the tunnel considered the dependent variable and the airspeed in the bioassay cages the independent variable. The deposition inside and outside of the cages was analyzed with the Student's *t*-test (SAS Institute 2001). The level of significance for all tests was set at  $\alpha = 0.05$ .

## RESULTS

### Droplet sizes inside and outside of the cages

The spray dispersion tunnel used in these studies generated repeatable droplet sizing data. There was generally only 0.5–2% variance within the  $D_{V0.5}$  values for a given test. In preliminary experiments, the 2 cages were modified so that each cage and screen material would extend

across the 0.9-m width of the dispersion tunnel. The modified cages were stretched across the length of the wind tunnel so that the laser beam would pass through the center of the cages along the longitudinal axis (i.e., center line). Each end of the screen material was secured to the wall of the tunnel to prevent any droplets from passing through the laser beam without first passing through the screen. The resulting data were inconsistent, probably due to buildup of the spray material on the screens during the testing. Therefore, the testing stand presented was developed to allow the screen material to be easily changed between tests.

Differences in droplet sizes (i.e.,  $\sim 0.3$ – $3 \mu\text{m}$ ) measured with and without screening material from each cage were significant (Tables 1 and 2). Nearly all trials resulted in a significant decrease in the size of the droplets passing through the screens as compared to the open tunnel, indicating that larger droplets were being filtered by the screen material. All of the droplet size parameters ( $D_{V0.5}$ ,  $D_{V0.1}$ ,  $D_{V0.9}$ , and  $\% \text{Vol} < 20$ ) increased as the air velocity was increased from 0.6 m/sec to 2.2 m/sec. This was expected, as there was more energy at the higher wind speeds to pull larger droplets down the dispersion tunnel to the testing section before they settled out. However, the droplet size parameters slightly decreased when the air velocity was increased to 4.5 m/sec in the tunnel. This may be due to higher air speeds resulting in increased collection efficiencies of the screen material, thus removing more droplets from the air stream and decreasing the droplet size of the penetrating spray.

The percent reduction of the  $D_{V0.5}$  inside and outside of the cages was calculated for the screen materials used in the 2 cages. The percent reductions of  $D_{V0.5}$  for the CMAVE materials were 4.3, 2.5, 3.4, 1.5, and 4.1% for the 0.6, 0.9, 1.3, 2.2, and 4.5 m/sec airspeeds, respectively. The percent reductions of  $D_{V0.5}$  for the Circle Cage were 2.1, 2.5, 3.4, 4.8, and 3.1% for the 0.6, 0.9, 1.3, 2.2, and 4.5 m/sec airspeeds, respectively. The overall averages were 3.14 and 3.15% for the CMAVE and the Circle Cages, respectively. Both screen materials essentially have the same collection effect on the spray, which would be expected given that they basically have the same structure.

### Air velocities inside and outside of cage

There were significant decreases in air velocities inside the cages as compared to the air velocities in the wind tunnel. When the wind speed in the dispersion tunnel was set at 0.6 m/sec (1.3 mph), the mean wind speed inside of the CMAVE Cage and Circle Cage was 0.045 m/sec (0.1 mph) and 0.075 m/sec (0.17 mph), respectively (Fig. 3). At air velocities of 2.2 m/sec (4.9 mph) in the dispersion tunnel, the mean wind speed inside of

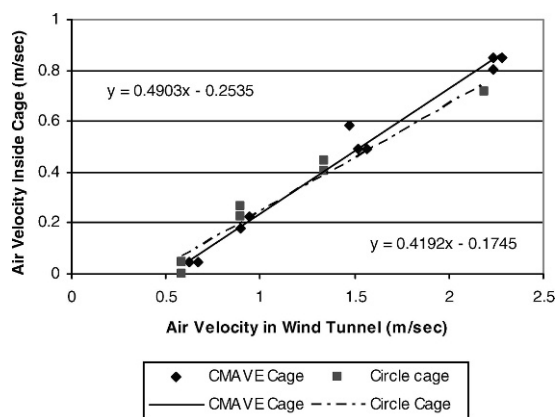


Fig. 3. Air velocities inside versus outside of the Center for Medical, Agricultural and Veterinary Entomology and Circle Bioassay Cages.

the CMAVE Cage and Circle Cage was 0.83 m/sec (1.86 mph) and 0.71 m/sec (1.59 mph), respectively. The implication of these measurements is that if these cages are used in low wind speeds (i.e., <0.67 m/sec [1.5 mph]) very little of the spray being carried by the wind is likely to penetrate the cage. Based on the regression equations from the data collected, no spray-laden air would be expected to penetrate the cages at ambient wind speeds of 0.25 m/sec (0.56 mph) ( $R^2 = 0.986$ ) and 0.17 m/sec (0.38 mph) ( $R^2 = 0.968$ ) for the CMAVE Cage and Circle Cage, respectively. The reduction in air velocity inside the cages correlated to lower levels of spray penetrating the cages.

#### Deposition on soda straws

Significantly lower deposition values were measured inside the CMAVE Cage and Circle Cage than measured in the open tunnel at all 4 wind speeds tested (Fig. 4). There was a consistent 50–70% reduction of spray material penetrating the cage compared to the spray cloud that approached the CMAVE Cage and Circle Cage. The amount of spray penetrating the cage increased as the wind speed increased for both the CMAVE Cage and Circle Cage. These results suggest that lethal dosages of insecticides as determined by bioassay cages may be overestimating the amount of insecticide needed to cause mortality in the wild. Although this study did not include bioassays, the methods and techniques described here could be adapted for bioassay studies.

#### DISCUSSION

Two cages (CMAVE Cage and Circle Cage) used in adult mosquito bioassays were tested to measure their effects on the size of droplets that

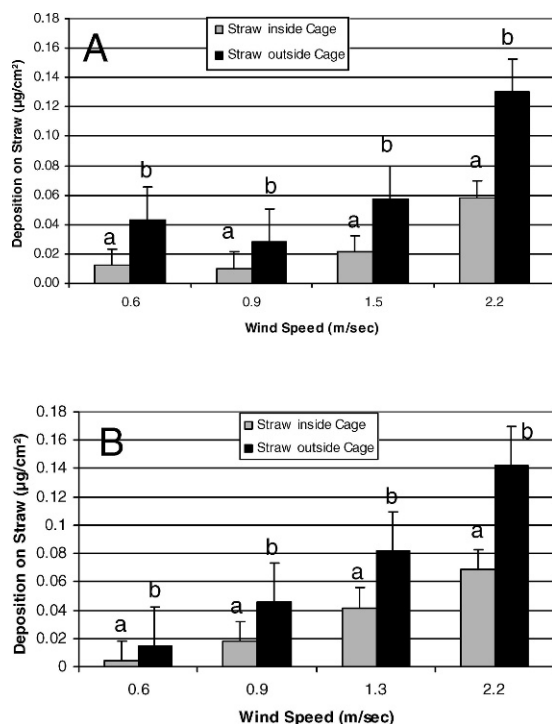


Fig. 4. Deposition on soda straws placed inside and outside of the Center for Medical, Agricultural and Veterinary Entomology (A) and Circle (B) Bioassay Cages. Different letters within each column for a given wind speed indicate significant differences.

penetrate the cages, and the amount of spray filtered by the cage screening material. The influence of the screening material on the airspeed inside and outside of the cages was also measured. The 2 types of screening evaluated in this study were found to reduce the size of droplets, air velocity, and amount of spray material inside as compared to outside of the cages similarly.

The movement of a fluid (in this case air) flowing past an object is described by an object's Reynolds number, which takes into account the viscosity and velocity of the fluid, and the length of the object (in this case the diameter of the mesh) (Patel et al. 1985). The creation of a boundary layer likewise reduces the flow of a viscous fluid past a surface. Consequently, the fiber diameter and the porosity of the mesh are the 2 main features of a screening material that affect airflow through that material (Wakeland and Keolian 2003). As predicted, because of effects of the boundary layer and Reynolds number produced by a wire mesh (Livesey and Laws 1973), the wind speed measured inside cages was significantly slower than wind speed outside of cages, which would also result in a reduction in ULV droplets entering cages, especially at lower wind speeds.

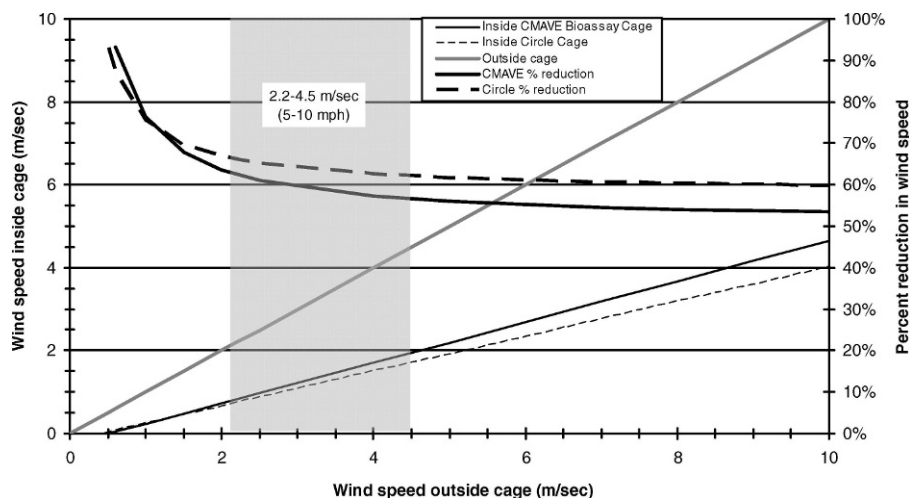


Fig. 5. Regression lines of wind speeds inside Center for Medical, Agricultural and Veterinary Entomology Bioassay Cage, Circle Cage, and outside of cages, and percent reduction in wind speed in cages compared to outside cages. Light gray region indicates the wind speed at which ultra-low-volume spray is usually applied.

As wind speed outside cages increased, the percent reduction of wind speed inside cages decreased. With tunnel wind speeds of 2.2 and 0.6 m/sec, a respective 63–93% reduction of wind speed was observed inside cages. Figure 5 shows linear regressions of wind speeds inside the tunnel, and both cages extrapolated to higher wind speeds, as well as the percent reduction in wind speed that would be expected. At a low wind speed, there would be less opportunity for droplet-laden air to enter cages, because the wind speed inside cages would reach 0 when the wind speed outside of cages was roughly between 0.4 and 0.5 m/sec. This has important implications when caged sentinel mosquitoes are used to monitor spray success in the field. With all other factors being equal, at low wind speeds much more spray would be needed to realize the same rate of droplet entry into cages, and perhaps mortality rates of caged mosquitoes, than at higher wind speeds. Additionally, when Circle Cages, which have solid sides, were used, small shifts in wind direction could further reduce droplet entry into the cages during ULV spray application. This would result in field estimations of mortality being highly variable and inaccurate when they are based on mosquito mortality in sentinel cages, as suggested by Boobar et al. (1988). It is likely that the amount of spray required to attain the desired mortality in the field has been overestimated based on mortality figures for caged sentinel mosquitoes, because of the amount of spray that is filtered out by the cage before the spray enters the cage.

Corresponding to the reduction of wind speed observed inside cages, there was a consistent 50–70% reduction of spray material penetrating the

cages compared to the spray cloud that approached the cages. The highest deposition of droplets on straws occurred outside of cages at the highest wind speed tested. The accumulation of droplets on straws outside a cage at 1.5 m/sec was more similar to the droplet deposition on straws inside the cage at 2.2 m/sec. The largest droplets were most likely to be filtered by the screening material on the cages. A statistically significant reduction occurred in the volume of droplets less than 20  $\mu\text{m}$  in diameter (the droplets most likely to persist in the air and impinge on flying mosquitoes) entering cages. Again, this indicates that lethal dosages of insecticides as determined by bioassay cages may be overestimating the amount of insecticide needed to cause mortality in the wild. Although there were significant differences in the size of droplets entering cages at different wind speeds, the biological relevance of these differences in field applications must be tested. Future studies to address how these differences inside and outside of cages affect mortality could be conducted by adapting these techniques and methods for bioassay studies.

## CONCLUSIONS

For the 2 cages (CMAVE and Circle) evaluated in these studies, the average reduction in droplet sizes inside the cages as compared to outside of the cages were 3.14 and 3.15% for the CMAVE and the Circle Cages, respectively. At 0.6 m/sec (1.3 mph), the mean wind speed inside of the CMAVE Bioassay Cage and Circle Cage was 0.045 m/sec (0.1 mph) and 0.075 m/sec (0.17 mph), respectively. At 2.2 m/sec (4.9 mph), the mean wind speed inside of the CMAVE Bioassay Cage and Circle Cage was 0.83 m/sec (1.86 mph)



and 0.71 m/sec (1.59 mph), respectively. There was a consistent 50–70% reduction of spray material penetrating the cages compared to the spray cloud that approached the CMAVE Bioassay Cage and Circle Cage.

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