METHODS OF ULV DROPLET SAMPLING AND ANALYSIS:
EFFECTS ON THE SIZE AND SPECTRUM OF THE
DROPLETS COLLECTED

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ABSTRACT. Several aspects concerning collection and analysis of ULV droplets were clarified and defined. Experimental comparison of 5 ULV droplet collection methods indicated that small settling chambers and mechanical slide rotators with Teflon® coated slides are best suited for field collection of droplets from ULV generators.

Decrease in diameter due to evaporation for 1 to 10 μm malathion droplets was insignificant (3.26%) when the droplet diameters were determined within 24 hr of treatment, and the volume decrease was more significant (7.68%) at 24 hr.

Due to lower droplet velocities and environmental effects, collections of malathion droplets 7.6 m from the nozzle, as indicated by label procedures, may not be as representative of the actual droplet spectrum as the same method used at 1.2 m.

The importance of accurate standardized insecticide ultralow volume (ULV) droplet sampling methods should be realized in the light of some recent and past research concerning the ULV droplet spectrum. Since the late 1940's workers have indicated that insecticidal droplet size directly influences the chemical's kill efficacy (LaMer et al. 1947, Yeomans 1949, Mount and Pierce 1972a, Lofgren et al. 1973). Based on a critical review of literature pertaining to droplet size of aerosol ground and aerial spray for adult mosquito control, Mount (1970) considered a droplet size range of 5 to 10 μm "Mass Median Diameter" to be optimal for ground application and 10 to 25 μm "Mass Median Diameter" optimum for aerial application. More recently, Haile et al. (1978a, unpublished) using malathion in laboratory and field equipment, indicated that the optimum size of droplets for control of at least 2 mosquito species was between 10 and 15 μm Volume Median Diameter. They stated that the efficiency of the insecticide decreased rapidly for <5 μm and >25 μm Volume Median Diameter droplets.

In using ULV sprays to control adult mosquitoes (Knapp and Roberts 1965, Glancy et al. 1966, Knapp and Pass 1966) mosquito control districts have been confronted with the problem of deciding which droplet sampling method to use for evaluating sprays from ULV aerosol generators.

Currently, the more popular methods generally use glass microscope slides with some type of coating. Such methods include: 1) hand wave, 2) slide pendulum, 3) chambered settling, 4) open air settling, and 5) mechanical impaction. Because various nonstandardized methods and terms are being used, confusion and misinformation is common among professionals involved in mosquito control. The problem is compounded when communications extend across disciplinary lines.

The purpose of this study was to compare the various ULV droplet sampling methods and evaluation procedures used by mosquito control districts. Although there are other methods for sampling droplets, such as those involving photomicrography (Rathburn and Misericocchi 1967), Coulter Counters (Haile et al. 1978b), and rupp cells, those that incorporate the use of coated glass slides are the principal ones used by mosquito control districts. Such slides were used in this study. The coating should be oleophobic to lessen the chance of droplet coalescence. Two coatings commonly employed for ULV droplet collection are Dupont Tef-
Ion® plastic film (Anderson and Schulte 1971) and General Electric Dri-Film® silicone (Yeomans 1949).

Other oleophobic and nonoleophobic coatings reviewed were found to be unsuitable (Hurtig and Perry, 1950, Higgins 1967). These coatings were either difficult to prepare or work with, or they were limited by the minimum detectable droplet size. Dye cards (Thornton and Davis 1956, Skoog and Cowan 1968) were unsuitable because ULV sprays contain substantial numbers of droplets 5 to 10 μm or less, which are difficult, if not impossible, to distinguish from spots or imperfections on the dyed paper (Rathburn 1970). Silicone coated slides are commonly used in ULV droplet evaluation. However, Anderson and Schulte (1971) stated that malathion droplets below 2 μm are only faintly visible on this coating.

Because the following study was largely based on examining a wide spectrum of droplet sizes, slides coated with Teflon FEP film, Type A, of 2 mil thickness, were chosen. Droplet collection techniques using glass slides involve either impinging the droplet or allowing the droplet to settle onto the slide. Both techniques are suitable for collecting droplets, but there are inherent differences in the characteristics of the collections. Impingement involves forcing airborne droplets onto the surface of the slide by moving the slide in some way through the spray. This has the advantages of allowing a quick sampling of sprays that may be carried by wind, and the droplets can be collected soon enough after treatment to give immediate information. A major disadvantage is the bias toward collecting large droplets over small droplets (Yeomans 1949, Mount and Pierce 1972b). There is also greater variability in the density of the droplets collected using impingement as opposed to the settling methods.

Settling methods allow the droplets to settle onto the surface of a stationary glass slide. These have the advantage of being unbiased in droplet size (Mount and Pierce 1972b), and they result in little variability in droplet density when the spray is confined in a chamber. The major disadvantage is the need for a waiting period before measuring the collected droplets, in order to let the slowly moving smaller sizes completely settle out.

The differential rates of this settling are important to consider when determining the time lapse between treatment and slide removal. Mount and Pierce (1972b) showed that malathion droplets settle in a large chamber within 24 hr after spray, with no additional droplets collected on glass slides on the floor of the chamber 24-48 hr after treatment. For example, a 1.0 μm sphere having a specific gravity of 1.0 at an ambient temperature of 21°C has a settling velocity of 2.1 mm/min (Wolf et al. 1959). Therefore, such a sphere would settle 3 m in 23.8 hr, which is consistent with Mount and Pierce’s observations for their 2.4 m (8 ft) high chamber. The specific gravity of malathion is slightly greater than one, which would result in a faster settling rate. A 10 μm sphere under the same conditions settles at a rate of 180 mm/min, and would settle 3 m in 16.7 min, or 86 times faster than a 1.0 μm sphere.

DEFINITIONS

Five different methods of sampling ULV droplets were used in this study. We considered them to be among the more common sampling methods currently used in operational evaluation. The following working definitions are given to help clarify certain terms used here that relate to collection methods. All droplets are collected with the nozzle parallel to the ground and the ULV generator stationary. BATTERY-POWERED SLIDE ROTATOR (GULVA® MODEL SPINNER). A mechanical device rotates the slide through the aerosol cloud with the coated surface facing in the direction of the motion (Rathburn 1970) at a distance of 7.6 m from the point of discharge. The slide is attached vertically to a bar 8.9 cm from the center of rotation and spins at 250
rpm. A collapsible music stand or other support is used to elevate the slide rotator to a height of 115 cm. Droplets are collected by the spinning slide for 15 min after treatment.

**Cascade Impactor.** A device that uses a series of progressively smaller air jets to increase air velocity from jet to jet, which impinges successively smaller droplets on slides (Rathburn 1970).

**Parallel Hand Wave.** The glass slide is waved as rapidly as possible toward the source through the aerosol cloud, with the coated surface of the slide facing the source, at a distance of 7.6 m from the point of discharge.

**Perpendicular Hand Wave.** The glass slide is waved as rapidly as possible perpendicularly through the aerosol cloud, with the coated surface constantly facing the source, at a distance of 7.6 m from the point of discharge (Anon. 1977).

**Settling Chamber.** A small plywood chamber (46 x 46 x 46 cm) provides a confined atmosphere that permits the aerosol droplets to settle on the coated surface of glass slides placed on the floor of the chamber before the droplets enter (modified from Mount and Pierce 1972b). The chamber is positioned 7.6 m from the aerosol source at a base height of 46 cm. A 46 x 46 cm sliding door is open and facing the source for 1 min after spray. The door is then closed and the chamber is left undisturbed for 24 hr. Once closed, the chamber may be moved a short distance into a protected area.

**Slide Pendulum.** A mechanical device swings the slide perpendicularly through the aerosol cloud with the coated surface of the slide constantly facing the source, at a distance of 1.2 m from the point of discharge. The slide is attached by a clip to the end of a 1 m stick pivoted on a vertical stick at the height of the nozzle. The free-swinging pendulum-like motion of the 1 m stick is similar to that of the perpendicular hand wave method. The slide falls from the vertical position through the aerosol cloud and is caught at the apex of its swing (Beidler 1975).

The hand wave method, obviously the quickest and most convenient to use, is perhaps the least desirable compared to the settling and impaction methods, due to bias toward larger droplet sizes (Mount and Pierce 1972b). Lower slide velocities bias the sample toward large droplets, whereas high velocities and centrifugal force create oblong droplets (Beidler 1975). Beidler (1975) explained the design of the slide pendulum and compared droplet samples taken 1.2 m (slide pendulum) and 7.6 m (parallel hand wave) from the nozzle. The "Mass Median Diameter" and size ranges were essentially the same for both distances. It was noted that the 1.2 m sample distance helped nullify error from wind drift, allowed the person collecting the droplets to stand clear of the spray, and eliminated oblong droplets.

Another method of collecting ULV droplets uses a battery-operated slide rotator or simply a spinner. As described earlier, the droplets are collected by a slide rotating at a constant speed. Rathburn (1970) stated that the low impaction velocity of the slide is not sufficient to efficiently collect droplets below 10 μm. However, the relatively high velocity of the GULVA model, which is used at New Orleans Mosquito Control, has proven effective for all droplet sizes from 1 μm to at least 50 μm.

Yeomans (1949) mentioned the use of a settling chamber as one of 2 methods of collecting droplets smaller than 20 μm in diameter. Mount and Pierce (1972b) described the use of a 2.4 x 2.4 x 2.4 m plywood chamber in which all droplets settled to the slides on the floor within 24 hr after treatment. Compared to hand wave and impaction methods, this collection method best represented all droplet sizes generated by a LECO® HD cold aerosol generator. Evans (1977, unpublished) stated that there was no significant difference between hand wave and settling methods when Volume Median Diameters were compared.
However, the Frequency (Number) Median Diameter for the settling chamber was \(<2 \mu m\) whereas the hand wave method yielded a Frequency Median Diameter of 12.5 \(\mu m\). Also, the settling method proved more efficient than the hand wave method for droplets \(<10 \mu m\).

The size of the chamber did not reduce the overall efficiency for collecting small droplets.

**DROPLET SIZE DISTRIBUTION ANALYSIS**

ULV spray droplets are collected for analysis of droplet size distribution. There are many techniques used to express this, and many factors which determine the techniques used. Such factors are the type of nozzle being tested, the dimension of the spray, the method used to collect the droplets, and others. In general, the common methods of analysis use either median droplet diameter or mean droplet diameter (Anon. 1973).

The median droplet diameter is the diameter which divides the aerosol into 2 equal portions. This can be based on droplet number, length, surface area, volume, or mass.

The Number Median Diameter is the corrected droplet diameter that corresponds to the accumulated \% \(\frac{N}{\sum N}\) at the 50\% level. The corrected diameter is the diameter (D) of the droplet collected on the glass, corrected to the diameter of a sphere with the same volume (Anderson and Schulte 1971). N is the number of droplets of that diameter.

The Length Median Diameter is the corrected droplet diameter that corresponds to the accumulated \% \(\frac{DN}{\sum(DN)}\) at the 50\% level. This is generally computed in table form as shown in figure 1.

The Surface Median Diameter is the corrected droplet diameter that corresponds to the accumulated \% \(\frac{ND^2}{\sum(ND^2)}\) at the 50\% level.

The Volume Median Diameter is the corrected droplet diameter that corresponds to the accumulated \% \(\frac{ND^3}{\sum(ND^3)}\) at the 50\% level.

The Mass Median Diameter is equal to the Volume Median Diameter.

The mean droplet diameter is the sum of all droplet measurements divided by the total number of measurements. Various kinds of mean diameters can be computed depending on the information needed. The Arithmetic Mean, Sauter Mean, Volume Mean, and Surface Mean are all accepted methods of droplet analysis.

The Arithmetic Mean Diameter is the weighted average of the corrected droplet diameters in the spray sample: \(\frac{\sum(DN)}{\sum N}\).

The Sauter Mean Diameter is the corrected diameter of a droplet whose ratio of volume to surface area is equal to that of the entire spray: \(\frac{\sum(ND^3)}{\sum(ND^2)}\).

The Surface Mean Diameter is the corrected diameter of a droplet whose surface is, if multiplied by the total number of droplets, equal to the total surface of all droplets in the spray: \(\left(\frac{\sum(ND^2)}{\sum N}\right)^{1/2}\).

The Volume Mean Diameter is the corrected diameter of a droplet whose volume, if multiplied by the number of droplets, will equal the total volume of the spray: \(\left(\frac{\sum(ND^3)}{\sum N}\right)^{1/3}\).

<table>
<thead>
<tr>
<th>Dc</th>
<th>N</th>
<th>%</th>
<th>Accumulated %</th>
</tr>
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<td></td>
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</tr>
</tbody>
</table>

Fig. 1. Form used to calculate Length Median Diameter.
Specifying the exact median or mean droplet diameter calculation used is very important, since the results can differ greatly. Common methods of analysis are the Length Median Diameter and the Volume Median Diameter. Often these are used synonymously, which is incorrect. To exemplify this, droplet diameter data randomly taken from the files of New Orleans Mosquito Control show significant differences between the Volume and Length Median Diameters of an aerosol sample. Droplets collected on Teflon coated slides in a settling chamber had a Volume Median Diameter of 19.27 μm and a Length Median Diameter of 10.58 μm. The Length Median Diameter is 55% of the Volume Median Diameter, and only 8% of the total volume of the droplets collected were below the 10.58 μm droplet.

FACTORS WHICH AFFECT DIAMETER CALCULATIONS

The velocity of the slide will affect the efficiency of impingement of various droplet diameters. In general, except for settling methods, the velocity of the slide must be increased to sample smaller droplets efficiently. Also, impingement efficiency is in proportion to droplet size. Compensation must be made in analysis for these factors. Yeomans (1949) showed that with impingement methods of collection, the rate of deposition is proportional to the square of the diameter of the droplet. Since the droplets impinge in ratio to D^2, but the volume is a function of D^3, the number of impinged droplets can be multiplied by D^3/D^2, or D, instead of D^3 when droplets are collected with equal efficiency, as in a settling chamber.

The change in droplet shape from a sphere in the air to a plano-convex lens on the slide coating is another factor to consider when analyzing droplet diameters. The increase in diameter associated with the spreading of the droplet on the oleophobic coating must be compensated for by a “spread factor.” There are 2 methods that can be used to compute the spread for a given liquid collected on a specific slide coating. One method uses the focal length and the diameter of the base of the droplet lens to calculate the thickness of the lens and then the volume (May 1945).

From this information the collection factor is derived. Anderson and Schulte (1971) stated that for certain materials on Teflon coatings, this method yielded ambiguous data, and they developed a method for physically measuring the thickness and the base of the lens so that volume and spread factor could be calculated. Spread factor is expressed as the ratio of the diameter of the droplet in air to the base of the lens on the slide: µ_droplet = spread factor. Because D_droplet

D_lens

is less than D_lens: the ratio is less than one.

Evaporation of material from the droplet is another source of diameter error and must be compensated for. Measuring the decrease in droplet diameter for a given time at a desired temperature is the easiest method.

Finally, a sample size of at least 100, preferably 200 droplets for each spray analysis is considered adequate.

MATERIALS AND METHODS

Malathion droplets were collected from a LECO HD ULV generator operating with a flow rate of 118 ml/min with 3.5 psi pressure. The ambient air temperature was 27°C and the chemical temperature was 24°C. The nozzle pointed from the rear of the truck parallel to the ground.

All droplets were collected at a distance of 7.6 m from the nozzle using 5 methods of collection: parallel hand wave, slide pendulum, mechanical slide rotator, large settling chamber, and small settling chamber. There were 3 replicates for each method except the large chamber, which used 3 slides and 1 replicate. All methods were as defined, except the slide pendulum method, which was conducted at 7.6 m from the nozzle. The large chamber was steel and measured 24 x 24 x 6 m high. All
tests were performed at the doorway of the large chamber.

The ULV unit was operated with the nozzle pointing downwind, toward the opening of the large chamber. The door to the large chamber and small chambers were left open for 30 sec after spraying. The spinners collected droplets for 15 min and were then covered and removed. The coated slides from the hand wave and slide pendulum methods were covered and were immediately removed from the large chamber. The slides from the large chamber were removed 24 hr after spray. Slides from the small chambers were removed by opening the door 1 cm and pulling them out with an attached string after 1 hr, 6 hr, 12 hr, 18 hr, and 24 hr. The droplets on each slide were measured and counted using a compound microscope at 480x, with an ocular micrometer calibrated so that each division measured 1.25 μm. The spread factor for malathion on Teflon was determined to be .70.

The evaporation rate of malathion droplets was determined by photographing a field of 46 droplets at predetermined time intervals; the change in diameter of the droplets was then measured by projecting transparencies on a large viewing screen. For absolute measurements a calibrated micrometer was available in each projected slide.

RESULTS AND DISCUSSION

Figure 2 shows the evaporation rate of technical grade malathion (Cythion®) droplets in the 1 to 10 μm range, recorded over a 4 day period. The average rate of diameter decrease was 0.14%/hr, or 3.36%/day, which represents an average volume decrease of 0.32%/hr, or 7.68%/day. The droplet Length Median Diameter, Volume Median Diameter, and the

![Graph showing evaporation rate of Cythion droplets](image)

Fig. 2. Evaporation rate of Cythion droplets (1–10 μm) collected on Teflon coated slides.
density of droplets per unit area determined for each collection method appear in Table 1. Table 2 gives the percentage distribution of droplets by diameter ranges, along with the largest and smallest droplet diameter collected for each method.

Figure 3 shows the droplet diameter

Table 1. Length median diameter, volume median diameter, and droplet density using five sampling methods.

<table>
<thead>
<tr>
<th></th>
<th>Length Med. Diam (µm)</th>
<th>Volume Med. Diam (µm)</th>
<th>Density (droplets/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAR. HAND WAVE</td>
<td>17.92±2.19</td>
<td>24.97±1.48*</td>
<td>9</td>
</tr>
<tr>
<td>SLIDE PENDULUM</td>
<td>11.07±2.05</td>
<td>21.19±4.98*</td>
<td>30</td>
</tr>
<tr>
<td>SLIDE ROTATOR</td>
<td>20.41±1.34</td>
<td>29.92±2.07*</td>
<td>315</td>
</tr>
<tr>
<td>LARGE CHAMBER (24 hr)</td>
<td>8.76±.98*</td>
<td>19.83±.70</td>
<td>317</td>
</tr>
<tr>
<td>SMALL CHAMBER (1 hr)</td>
<td>12.59±.38*</td>
<td>26.03±3.69</td>
<td>75</td>
</tr>
<tr>
<td>Small Chamber (6 hr)</td>
<td>13.27±1.08*</td>
<td>24.22±.40</td>
<td>54</td>
</tr>
<tr>
<td>Small Chamber (12 hr)</td>
<td>11.56±2.99*</td>
<td>24.72±.91</td>
<td>132</td>
</tr>
<tr>
<td>Small Chamber (18 hr)</td>
<td>12.45±.93*</td>
<td>22.18±.62</td>
<td>209</td>
</tr>
<tr>
<td>Small Chamber (24 hr)</td>
<td>10.08±1.24*</td>
<td>19.40±.24</td>
<td>190</td>
</tr>
</tbody>
</table>

* For purposes of comparison. The Length method should be used for impinged droplets and the Volume method should be used for settled droplets.

Fig. 3. Droplets collected on Teflon coated slides inside a 46 cm² settling chamber (volume method) and from a mechanical slide rotator (length method).
cumulative percentage curves for droplets collected on Teflon coated slides. The volume curve represents droplets collected in a 46 x 46 x 46 cm settling chamber analyzed by the Volume Median method. The length curve represents droplets collected with a GULVA mechanical slide rotator analyzed by the Length Median method. Although the curves differ, the fact that the median diameters for both are approximately the same supports the findings of Yeomans (1949).

One shortcoming of the Teflon coated slides was an apparent build-up of irregular electrostatic charges on the Teflon. These charges caused clearly observable anomalies, agglomeration of droplets and streaks of droplets. It is probable that the slides were electrostatically charged (Anderson and Schulte 1971) during the cleaning process. However, destaining the slides by rubbing a smooth metallic bar along the edges after the cleaning process greatly reduced the effect.

The decrease in diameter droplets due to evaporation for 1 to 10 \( \mu \text{m} \) droplet was insignificant (3.26%) when the droplet diameters were determined within 24 hr of the treatment, and the volume decrease was more significant (7.68%) at 24 hr. For droplets greater than 10 \( \mu \text{m} \) the decrease in volume is greater because of the greater surface area; but the diameter decrease is negligible due to the smaller ratio of diameter to surface area and of surface area to volume.

The settling chamber appears to be the method which most accurately samples the droplets in a unit volume of air. After 24 hr, all droplets confined in the small chamber had probably settled out, based on the stabilization of the density (Table 1). This is probably true for the large chamber also, since the droplet distributions were almost identical. The Volume Median Diameter and the standard deviation were very close for both chambers, indicating that chamber size may be insignificant.

The slide rotator appeared to be the best of the three impingement methods. It
gave a greater droplet density and a Length Median Diameter with the smallest standard deviation. In contrast, Table 1 shows the Length Median Diameter for the slide pendulum method (at 7.6 m) to be 38% lower than the Length Median Diameter in the parallel hand wave method, and it was 46% lower than the Length Median Diameter of droplets collected by the rotator method.

It is important to note that by intended design, the slide pendulum method is used 1.2 m from the point of spray discharge (Beidler 1975). At this distance, where the impingement velocity is much greater than at 7.6 m, a more representative droplet sample can be collected. Nevertheless, the malathion label specifications (Anonymous 1977) state, “A sample of the Cythion or Malathion ULV aerosol is deposited on a slide by waving the slide as rapidly as possible perpendicular through the aerosol cloud at a distance of 25 ft from the point of discharge. The slide velocity may be increased by attaching it to a 3 or 4 ft stick by means of a spring paper clip.” However, it is possible that this method does not give an adequately representative droplet sample. The following may help explain this observation.

First, the force exerted by a moving body is a direct function of its time rate of change of momentum. The momentum
\[ \dot{P} = m \dot{v} \]
is the product of the mass and velocity, so the average force \( \dot{F}_{av} = \Delta \dot{P}/\Delta t \).

The slide pendulum and perpendicular hand held methods are the only collection methods that involve a tangential force resulting in a 90° change in droplet momentum during the collection procedure.

The change in momentum \((\Delta \dot{P})\) of the droplet is always 90° downward from the initial direction of travel: (Fig. 4). In the above figure \( \dot{P}_1 := \text{initial momentum of the droplet} \); \( \dot{P}_2 := \text{final momentum of the droplet} \); \( m_s := \text{mass of the droplet} \); \( \dot{P}_{1 \text{final}} := \text{final momentum of the droplet} \); \( \dot{v}_d := \text{velocity of the slide} \); \( \dot{v}_s := \text{velocity of the droplet} \). Therefore, the change in momentum of the droplet
\[ (\Delta \dot{P}) = \dot{P}_2 - \dot{P}_1 \]

In order to produce this change in droplet momentum, there must be an impulsive force acting for some very short time \((\Delta t)\). The impulse and momentum theorem states: \( \dot{F}_{av} \Delta t = \Delta \dot{P} \), where \( \dot{F}_{av} \) is in the same direction as \( \Delta \dot{P} \), where \( \dot{F}_{av} \) is the average force as shown below (Fig. 5).

\[ \dot{F}_{av} = \text{average force perpendicular to the slide} \]
\[ \dot{F}_{av\parallel} = \text{average force parallel (tangential) to the slide} \]
\[ \dot{F}_{av} = \text{average force effect between } \dot{F}_{av\perp} \text{ and } \dot{F}_{av\parallel}, \text{ i.e. } \dot{F}_{av} \text{ is the vector sum of } \dot{F}_{av\perp} \text{ and } \dot{F}_{av\parallel}. \]

If \( \dot{F}_{av\parallel} \) does not have enough time duration, the droplet may never attain the velocity \( \dot{v}_s \) of the slide. The droplet may slip off the slide and not be collected. The \( \dot{F}_{av\parallel} \) probably depends on the cohesive force between the droplet and the slide. For a large droplet, this cohesive force may not be large enough to cause the
droplet to reach the speed $\dot{v}$ during the time $\Delta t$, and the droplet may slip off.

Surface tension or cohesive effects are proportionally much larger for very small droplets and smaller for larger droplets, since cohesive force is a function of surface area. The ratio of surface area to mass decreases as the droplets become larger, e.g. a 20 $\mu$m droplet is $1 \times 10^3$ more massive than a 2 $\mu$m droplet.

Settling rates of the droplets may also affect the size and distribution of the sample, particularly as the distance (travel time) between the nozzle and the slide increases. Whereas a 10 $\mu$m droplet settles 0.18 m/min, a 100 $\mu$m droplet settles 18 m/min (Wolf et al. 1959).

**Conclusion.** Equipment dispersing droplets that are considered too small or too large could result in environmental damage, paint spotting, lower kill effectiveness, and chemical waste. Therefore, it is not only important periodically to calibrate ULV equipment, but also important to use convenient, interpretable sampling procedures. In light of the many variables discussed, everyone concerned with droplet sampling and evaluation should attempt to standardize his methods and terms, and should specify the exact calculations used.

**REFERENCES CITED**


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INHERITANCE PATTERN OF TWO NEW MUTANTS, RED-EYE AND GREENISH BROWN-LARVA IN ANOPHELES STEPHENSI

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ABSTRACT. Two new mutants, red-eye and greenish brown-larva have been isolated and genetically analyzed in An. stephensi type form. The inheritance pattern revealed that red-eye is a recessive sex-linked mutant and greenish brown-larva is an autosomal recessive mutant.

INTRODUCTION

Malaria resurgence on a nationwide scale and its importance as the preeminent vector-borne disease have led to intensified research on Anopheles stephensi, an important transmitter of malaria. As a first step, phenotypic markers of this vector are being isolated, characterized and maintained in our laboratory. These mutants would be of great help in assigning insecticide resistant genes to their respective chromosomes.

Two eye-color mutants, colorless-eye an autosomal mutant in An. stephensi type form (Sharma et al. 1977) and white-eye, a sex-linked mutant in An. stephensi mysorensis (Aslamkhan 1973) have already been studied. In addition to these, 3 autosomal larval mutants, black-larva (Mason and Davidson 1966), Stripe (Sakai et al. 1974), green-larva (Subbarao and Adak 1978), and several biochemical variants (Bianchi 1968, Bullini et al. 1971, Iqbal et al. 1973a, b) have been reported in An. stephensi. This paper describes the inheritance pattern of 2 more mutants, red-eye and greenish brown-larva in An. stephensi type form.

MATERIAL AND METHODS

Red-eye mutant was isolated in 1977 from a laboratory colony which was established in 1973 from Sonepat, Haryana, India. The red-eye (r) mutant expresses its phenotype, red colored eyes, from the 1st instar through adult stage. The greenish brown-larva (gh) was isolated from the wild populations of Pondicherry, India in 1975. This mutant expresses its phenotype-greenish brown color at the larval and pupal stages. In instar II, this mutant is darker in color compared to the wild type. As the larvae reach late III and IV instars the greenish brown color becomes more distinct with a slightly more greenish tinge in the thorax. True-breeding colonies of these mutants were established. Mosquito rearing was carried out following a procedure described by Ansari et al. (1978). Crossing experiments were carried out in 30×30×30 cm size cages in a laboratory maintained at 27–28°C and 70–80% RH.

RESULTS AND DISCUSSION

Red-Eye. The results of a cross between red-eye (r) and wild type (+) are given in