RESIDUAL EFFICACY OF FIELD-APPLIED PERMETHRIN, d-PHENOTHRIN, AND RESMETHRIN ON PLANT FOLIAGE AGAINST ADULT MOSQUITOES

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ABSTRACT. Backpack sprayer applications of permethrin, d-phenothrin, and resmethrin to vegetation and plants at Anastasia Island, St. Augustine, FL, were evaluated for duration of residual efficacy against adult mosquitoes. All treatments produced 100% mortality (24 h) of mosquitoes in test cages placed within the vegetation. At 48 h and 1 wk posttreatment, insecticide treatments resulted in 70–100% reduction of adult mosquitoes caught by Centers for Disease Control and Prevention traps baited with 1-octen-3-ol. Insecticide residues in excised leaves from both treated and control areas of the study sites were evaluated against adult female *Culex quinquefasciatus* by laboratory bioassay. Permethrin produced 90% mortality up to 1 wk postapplication. Both d-phenothrin and resmethrin produced nearly this level of mortality for a much shorter duration of <48 h postapplication, with residual effects dropping significantly thereafter. Average insecticide concentrations in leaves were quantified by gas chromatography/mass spectroscopy, and some correlation was observed between chemical and biological results.

KEY WORDS *Culex quinquefasciatus*, mortality, mosquito traps, plant foliage, gas chromatography/mass spectroscopy

INTRODUCTION

Plant foliage and vegetation routinely provide mosquitoes with safe, sheltered resting sites and sources of food. Adult male and female mosquitoes must regularly consume carbohydrates for a variety of activities (Foster 1995), and they ingest sugars of plant origin (Schlein and Muller 1995, Burkett et al. 1999). Thus, the use of insecticides to treat plant foliage provides a means of adult mosquito control based on their need to ingest sugar meals and to rest, which can result in increased exposure duration based on their natural behavior. Recent studies have focused on evaluating the toxicity of different insecticides on foliage against various species of mosquitoes (Xue et al. 2006, Xue 2008). Some of these studies indicated that permethrin provided residual activity for 1 wk (Helson and Surgeoner 1983), deltamethrin provided residual effects for 4 wk (Cilek and Hallmon 2006), and bifenthrin and lambdacyhalothrin was efficacious for 4–6 wk (Trout et al. 2007).

Pyrethroids are the most common insecticides being used for adult mosquito control. This is due to their short persistence in the environment (Antonious et al. 1997, 2001; Angioni et al. 2005), high level of potency against a wide range of arthropods (Burgess et al. 1988), low application rate, and low toxicity to most vertebrates with the

exception of fish (Edwards et al. 1987, Croft et al. 2001). Therefore, these insecticides are frequently the compounds of choice for use in agricultural and residential environments, and it is essential to evaluate the capabilities of the various forms of pyrethroids commercially available.

Vital statistics that need to be established are the baseline values of insecticide residues in and on leaves and to determine how this correlates to the duration of insecticide residual efficacy against adult mosquitoes. There is little information on the measured level of insecticide residues in leaves to determine the amount that will suppress mosquito populations below an action threshold. Lothrop et al. (2007) focused on a high-performance liquid chromatography to separate chromatographically the postspray residues of insecticides. Our method explores the usefulness of a gas-phase chromatographic separation technique, gas chromatography, to effect separation of insecticide residues for subsequent detection and quantification by a mass spectral detector.

The focus of this study was to investigate application of pyrethroids (permethrin, d-phenothrin, and resmethrin) to vegetation by 1) evaluation of residual efficacy on treated leaves by laboratory bioassay, and 2) correlation of the bioassay results with residual content of insecticides in leaves as determined by gas chromatography/mass spectrometry (GC/MS).

MATERIALS AND METHODS

Study sites

Two field sites were selected for their similarity in habitat but with sufficient distance between areas to avoid cross-contamination. The 1st site

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Form Approved OMB No. 0704-0188 and test involved plants and vegetation along the east border of the Anastasia Mosquito Control District on East Pope Road, St. Augustine Beach City, FL. The test was conducted June 27 to July 21, 2007. The 2nd test was conducted at Anastasia State Park, St. Augustine Beach City, FL, August 8-31, 2007. The distance between sites was 1.0 km. For each insecticide, a separate test plot was chosen at each site. The plot was approximately 30 m of border plants and vegetation. Within each plot, a Centers for Disease Control and Prevention (CDC) trap was placed in the center of the 30-m-long plot, approximately 1 m deep into the vegetation. A control plot for this test site was selected 61 m (nearest distance) to the test area. Within this control plot, a CDC trap was also placed. The distance between the 2 test areas that involved insecticide treatment was 152 m at their nearest points. A major criterion for choosing this location for the study was the requirement of adequate length and presence of leafy plants and vegetation. Predominant plants and vegetation along these studied borders comprised black willow (Salix nigra Marsh), muscadine grape (Vitis rotundifolia Michx.), cherry laurel (Prunus caroliniana Ait), Virginia creeper (Parthenocissus quinquefolia Planch), poke weed (Phytolacca americana L.), cherokee bean (Erythrina herbacea L.), persimmon (Diospyros virginiana L.), live oak (Quercus virginiana P. Mill), southern red cedar (Juniperus silicicola J. Silba), beauty berry (Callicarpa americana L.), and bay trees (*Persea* spp.).

Insecticides

Permethrin application was performed using a 1:9 dilution of Aquareslin® (20% permethrin + 20% piperonyl butoxide; Bayer, Montvale, NJ) with treatment at the lowest application rate suggested by the label. SumithrinTM (d-phenothrin) in Anvil® 10 + 10 ULV (10% d-phenothrin + 10% piperonyl butoxide; Clark, Roselle, IL) was a ready-to-use formulation and thus was used undiluted. Resmethrin, an over-the-counter product (Black Flag®, Bellingham, WA; 0.2% resmethrin + 98.8% other ingredients) was also used undiluted, as suggested by the label.

Field tests

Permethrin, d-phenothrin, and resmethrin were each applied to border plants and vegetation by a certified operator and were performed as similarly as possible. The operator used a consistent walking pace and sweeping pattern from the beginning of the test site to the end and back (point A to B, and back to A), and from ground level to a height of the operator's outstretched hand using an up-and-down (about 3 m) and side-to-side sweeping rotation. Insecticide appli-

cation was performed at 6:30–7:30 a.m. when the weather was expected to be clear and dry. Application of insecticide did not commence unless the wind speed was <1.6 km/h. A portable weather station (MX 2000; Fischer Scientific, Pittsburgh, PA) was used to monitor meteorological data of wind speed and direction, temperature, humidity, and barometric pressure. Plants and vegetation were sprayed only once at a level sufficiently low to prevent runoff from leaves and vegetation.

Insecticide applications

The insecticide application equipment was a TwisterTM XL (Curtis Dyna-Fog[®], Dayton, OH) backpack sprayer with a nozzle pressure of 5 psi, orifice no. 28, calibrated to deliver a flow rate of 6.6 oz/min and a spray droplet volume median diam of 15.5 μm. Spraying time for each insecticide was limited to 15–16 min. Since the same equipment was used to apply the test insecticides, it was flushed and rinsed with water under pressure for approximately 5 min between applications.

Field application bioassay

Six mesh-screened (0.7-mm mesh size) cylindrical cages were used (10 cm diam, 5 cm deep; 3 cages for treated sections and 3 for control sections at 1 cage for each section). Each cage contained 20–25 adult female *Culex quinquefasciatus* Say, each 5–7 days old. The cages were tied to stalks or plant stems within the spray path of each insecticide to establish their primary potency and toxicity against adult mosquitoes. Caged mosquitoes were brought to the laboratory and maintained on a 10% sucrose solution and water. Mortality of mosquitoes in each cage was assessed at 24 h postapplication. Mosquitoes remained in cages for the duration of the test.

Mosquito monitoring

Natural mosquito populations were monitored once a week from 1 wk pretreatment to 3–4 wk posttreatment. Twenty-four hours after treatment, a CDC light trap baited with 1-octen-3-ol (BioSensory Inc., Willimantic, CT) was set in the treatment site and the other CDC trap set in the control site. Traps were separated by 152 m and operated once weekly for 24 h for 4 wk. Total rainfall and temperatures in both sites were also monitored.

Laboratory leaf bioassay

Excised-leaf bioassays were performed on the same day as light-trap collections to determine residual activity of treated plant foliage to adult Cx. quinquefasciatus. A single leaf was exposed to 10–15 laboratory-reared, insecticide-susceptible female mosquitoes, each 5–7 days old. The mosquitoes were mouth aspirated into separate transparent 300-ml conical cups secured with mesh screens and rubber bands. Six randomly picked leaves from each of the test areas were used for the bioassays. Mortality was recorded for both control and treatment leaves after 24 h posttreatment. Mosquitoes had access to 10% sucrose solution. Effort was made to sample leaves of similar sizes, and weekly samples were not smaller in collection volume than the 1st week's average size.

Chemical analysis

Leaf samples were taken from areas throughout the vegetation at various heights and depths to achieve a representative sample from the treatment area. At each site, for each insecticide, and at each posttreatment collection time up to 2 wk, a treated and control sample of leaves were collected. The only 3-wk samples collected were for permethrin (treatment and control) at the Pope Road site because the 2-wk results produced >50% mortality in bioassays. The leaves were packed tightly into widemouthed quart mason jars (Ball, Muncie, IN) and labeled with their collection location and posttreatment collection time. The jars were transported to the laboratory and stored in a freezer until sample processing. The processing consisted of inserting collected leaves from each jar into a commercial food chopper (Hobart model 8185D; Hobart Corporation, Troy, OH) with approximately 250 g dry ice and chopping for roughly 1 min. Chopped samples were transferred to appropriately labeled jars and frozen until extraction. The sample weight for extraction was measured using a Sartorius model LC621P analytical balance (Goettingen, Germany) to 10.00 ± 0.01 g and recorded. Each vegetation sample was extracted using approximately 150 ml acetonitrile and placed in a Fisher model FS140 sonic bath (Thermo Fisher Scientific, Waltham, MA) for 5 min. Samples were filtered using a funnel stuffed with silanized glass wool and concentrated to approximately 10 ml using a Turbovap II (Caliper Life Sciences, Hopkinton, MA). The samples were then quantitatively transferred to 15-ml QuEChERs tubes (Restek Corporation, Belefonte, PA) and shaken for approximately 1 min before 5 min of centrifugation using a Centrific model 228 centrifuge (Thermo Fisher Scientific). The supernatants were transferred to 15-ml Class A graduated centrifuge tubes and further concentrated under nitrogen gas using an N-Evap 11 sample concentrator (Organomation Associates, Inc., Berlin, MA) to 1 ml. Ten µl of formic acid was added prior to analysis.

An aliquot of 1.0 µl was injected onto a Trace Ultra GC connected to a ThermoQuest DSQ MS equipped and a TriPlus autosampler (Thermo Fisher Scientific). The GC column used was a DB-5MS (Agilent, Wilmington, DE) (30 m × 0.25 mm inner diam, film thickness of 0.25 µm). The injection port of the GC was a programmable temperature vaporization injector set at 35°C for injection with a split flow of 12 ml/min helium carrier gas, operated in splitless mode for 0.75 min postinjection. The inject time was set at 6 sec, with evaporation and transfer rates set at 14.5°C/sec, up to 260°C, and held for 2.0 min. The GC oven temperature was set initially at 40°C and held at that temperature for 0.1 min after sample injection. The oven was ramped at 12°C/min to 310°C and held at that final temperature for 2.5 min. The GC/MS was operated with the source in electron ionization mode with 70 eV electron energy at 200° C and scanned from m/z 35–350 at 0.5 sec/ scan, with the transfer line set at 250°C. The total run time was 65 min.

Quantitation was accomplished by calculating peak areas of selected characteristic ions at m/z 123 for permethrin, m/z 183 for d-phenothrin, and m/z 123 for resmethrin. Calibration curves were constructed using 5 standards from 1.6–80 ppm ($\mu g/g$) for permethrin, 1.36–68 ppm for d-phenothrin, and 1–50 ppm for resmethrin. Samples that had responses above the standard curve were diluted $20\times$ to produce peak area counts that were within the range of the standards. The permethrin peak areas were summed by using both cis and trans isomers of this compound.

Data analysis

Weekly mosquito collection data from CDC traps in the treatment and control areas were noted for species abundance and number. Percent reduction of mosquitoes caught by CDC traps at 48 h and 1 wk posttreatment was calculated by the following formula:

% reduction =
$$\left[(P+C) - T_{/(P+C)} \right] \times 100,$$

where P = number of mosquitoes pretreatment, T = number of mosquitoes posttreatment, C = number of mosquitoes in control.

Percent reduction at the 2- and the 3-wk posttreatment was calculated by

% reduction =
$$\left(C - T/C\right) \times 100$$
,

where C is the number of mosquitoes captured in the control and T is the number of mosquitoes in the trap located in vegetation where insecticide was sprayed.

Mortality values in the laboratory bioassay were converted to percentages and corrected by

Table 1. Species abundance of adult female mosquitoes collected from Anastasia Island, St. Augustine Beach City, FL, by Centers for Disease Control and Prevention light traps baited with 1-octen-3-ol.

Mosquito species	Species composition (%)		
Ae. taeniorhynchus	59.3		
An. quadrimaculatus	2.3		
An. crucians	11.8		
Cx. quinquefasciatus	10.8		
Cx. erraticus	6.5		
Cx. nigripalpus	2.8		
Ae. albopictus	6.6		

Abbott's formula (Abbott 1925). Data were transformed by log10(n + 1), and means were compared using Student–Newman–Keuls test, with level of significance at P = 0.05 (CoStat Statistic Software 2004).

RESULTS

During treatment applications, environmental conditions at the test sites were not significantly different from each other. For Site 1, mean temperature and humidity were 26.3°C and 85.3%, respectively. Site 2 had mean temperature of 27.9°C and relative humidity of 84.3%. Wind speed was nearly 0 (<1.6 km/h) at both sites. For the duration of the study, temperatures ranged from 24.7 to 35.6°C, with total rainfall of 2.8 cm at the 1st site. Temperatures ranged from 23.3 to 35°C, with a mean of 29.4°C at Site 2, and the total precipitation was 4.0 cm of rainfall during the duration of the test. Rainfall did not occur within 24 h of a sampling period and thus is not expected to influence results.

A 100% mortality of caged *Cx. quinquefasciatus* females was observed within the spray paths of the 3 insecticides, while in contrast, no mortality was detected in the control cages 24 h posttreatment. This clearly established the primary potency and toxicity of each of the insecticides and indicates that treatments were performed with adequate precision to avoid unintended deposition of insecticide residue onto the vegetation at designated control sites.

Throughout this study, the numbers of adult mosquitoes collected in CDC traps baited with 1-octen-3-ol were very low. A total of 376 female mosquitoes was collected from the 2 sites, and species composition was comprised primarily of *Aedes taeniorhynchus* Walker. In addition, *Culex* spp., *Anopheles* spp., and *Aedes* spp. were collected (Table 1). It was noted that there was no precipitation for 8 wk prior to the field evaluations.

Percent reduction of mosquitoes, translated from numbers of mosquitoes counted in CDC traps posttreatment, differed for all of the tested pesticides. The compound d-phenothrin was the

Table 2. Percent eduction of mosquitoes caught by Centers for Disease Control and Prevention traps baited with 1-octen-3-ol at Anastasia Island, St. Augustine Beach City, FL, at posttreatments.

	Reduction (%) in mosquito catch posttreatment			
Treatment	48 h	1 wk	2 wk	3 wk
Permethrin	86	82	64	50
Resmethrin	56	70	20	0
d-Phenothrin	75	38	29	0
Mean	72	63	38	17

least effective of those tested for residual activity, producing a 75% reduction at 48 h, which dropped to 38% at 1 wk (Table 2). Resmethrin produced a 56% reduction at 48 h (believed to be anomalously low resulting from sampling error) and 70% reduction at 1 wk posttreatment. Permethrin produced 86% reduction in catches at 48 h, remained nearly as potent at 1 wk (82%), and was the only treatment with remarkable reduction in trap catches (50%) reduced at 3 wk posttreatment compared to the control.

The laboratory bioassay results of leaves collected from the sites at specific posttreatment intervals are shown in Table 3. Residues of the 3 insecticides on leaves, excised at 48 h, killed 100% of female *Cx. quinquefasciatus* in both trials. However, by 1 wk posttreatment, permethrin exhibited 97–100% knockdown of mosquitoes, while d-phenothrin produced 39% and 85% knockdown in samples from the East Pope Road (Site 1) and Anastasia State Park (Site 2), respectively. Resmethrin, on the other hand, accounted for 57% and <1% mortality in the 1st and 2nd trials, respectively, at 1 wk posttreatment. In the 2 trials, permethrin was the only insecticide that gave consistent results in both trials at 48 h and 1 wk after application.

Generally, there was not a significantly different level of knockdown for the 3 tested insecticides at 48 h posttreatment. Significant differences were evident starting at 1 wk, when the performance of permethrin treated leaves was significantly greater than d-phenothrinand resmethrin-treated leaves (P < 0.05), and the effectiveness of the 3 pesticides decreased significantly thereafter.

Chemical residue data, presented as the mass of pesticide per gram of leaves sampled, at the sites are presented in Table 4. Controls are excluded from the table because all 19 controls did not contain a detectable level of any of the insecticides. The residual levels of d-phenothrin and permethrin were detectable out to 2 wk, while resmethrin levels were detectable only out to 1 wk, and from only 1 of the 2 test sites.

Collections were made only once per time interval and per site; however, several sample

Table 3. Corrected mean (± SE) percent knockdown/mortality of female *Culex quinquefasciatus* from excised leaves treated with permethrin, d-phenothrin, and resmethrin from 2 test sites, Anastasia Island, St. Augustine Beach City, FL.

	Correct mean ± SE knockdown (%) posttreatment ^{1,2}				
Treatment	48 h	1 wk	2 wk	3 wk	
Trial 1: East Pope Road					
Permethrin d-Phenothrin Resmethrin	$100 \pm 0.0a$ $100 \pm 0.0a$ $100 \pm 0.0a$	$100 \pm 0.0a$ $39.3 \pm 2.1b$ $57.0 \pm 2.0b$	$65.0 \pm 22b$ $3.0 \pm 3.7c$ $3.0 \pm 3.7c$	13.3 ± 2.7c N/S N/S	
Trial 2: Anastasia State Park					
Permethrin d-Phenothrin Resmethrin	$100 \pm 3.3a$ $100 \pm 3.5a$ $100 \pm 3.1a$	97.0 ± 3.6a 85.4 ± 2.9b 0.3 ± 3.6c	$10.3 \pm 3.3b$ $4.4 \pm 3.7c$ $1.4 \pm 3.6c$	N/S N/S N/S	

¹ Means within a row or within a column not followed by the same letter are significant (P < 0.05).

extractions from collections can be made, if necessary, to verify the chemical results. For example, the concentration of permethrin residue was much lower than expected during the initial analysis of samples from one of the sites. After samples were reinjected and nearly identical results obtained, a 2nd subsample from each site was taken and extracted. The results were nearly identical, confirming that this was an issue that occurred with the sample collection procedure at 48 h. The 2nd case in which subsamples of original samples were extracted and analyzed a 2nd time was due to both the relatively high concentrations of d-phenonthrin from Site 2 at 48 h (302 ppm), and the unexpected low value at 1 wk (73.3 ppm compared to 92.5 ppm at 2 wk). The results from the 2nd subsample (309, 73.6, and 126 ppm, at 48 h, 1 wk, and 2 wk, respectively) indicated that these values were satisfactory estimates and that the aberrations were likely to be due to the sample collection process and naturally occurring variability.

DISCUSSION

In both trials, *Ae. taeniorhynchus* was the predominant mosquito species caught by the CDC traps, with values of 62% and 56% in sites 1 and 2, respectively. *Anopheles crucians* (Wiedemann) and *Cx. quinquefasciatus* were captured at both sites in decreasing order of magnitude. Overall mosquito abundance during the period of experiment was low. The low numbers caught might be a reflection of the drought experienced by Northeast Florida at the test time, other meteorological factors, population biology of mosquitoes, or a combination of these factors (Cilek and Hallmon 2008). The use of blends of 2

Table 4. Average permethrin, d-phenothrin, and resmethrin residue quantified as mass of insecticide per mass of leaves (μg/g) collected at 48 h, 1 wk, 2 wk, and 3 wk posttreatment from 2 test sites, Anastasia Island, St. Augustine Beach City, FL.

		* *			
	Insecticide concentration in collected leaves (μg/g) ¹				
Treatment ²	48 h	1 wk	2 wk	3 wk³	
Trial 1: East Pope Road					
Permethrin	17.7^{4}	37.0	29.0	<loq< td=""></loq<>	
d-Phenothrin	64.6	19.3	3.3	N/S	
Resmethrin	0.13	0.09	<loq< td=""><td>N/S</td></loq<>	N/S	
Trial 2: Anastasia State Park					
Permethrin	2.45^{4}	5.7	4.0	N/S	
d-Phenothrin ⁵	302	73.3	92.5	N/S	
Resmethrin	0.09	<loq< td=""><td><loq< td=""><td>N/S</td></loq<></td></loq<>	<loq< td=""><td>N/S</td></loq<>	N/S	

 $^{^{1}}$ <LOQ (limit of quantitation), less than the detectable limit of the instrument and method, which were 0.68 μ g/g, 0.8 μ g/g, and 0.5 μ g/g for d-phenothrin, permethrin, and resmethrin, respectively. The LOQ for each corresponds to one-half of the concentration of the lowest standard.

² N/S, not sampled based on low knockdown results of week 2.

² All controls contained no detectable insecticide residues.

³ N/S, not sampled based on low knockdown results of week 2.

⁴ Extractions were repeated and nearly identical residual concentrations were obtained. This indicates that it is an artifact of the sample collection.

 $^{^{5}}$ A 2nd extraction was performed with the d-phenothrin samples, resulting in residual concentrations of 309, 73.6, and 126 μ g/g for the 48-h-, 1-wk-, and 2-wk-collected samples, respectively.

or more attractants often increase trap collections, more than the use of a single attractant (Kline et al. 1990, Bernier et al. 2003). During the period of study, the use of only 1-octen-3-ol (octenol) as the attractant may also be a contributing factor to the low number of mosquitoes trapped.

Different residual effects of insecticide treatments were noticeable in the difference in percent reduction of mosquito populations caught by CDC traps. Permethrin treatment provided >50% reduction for up to 3 wk, resmethrin lasted up to 1 wk, and d-phenothrin lasted only 48 h. The bioassay results for permethrin, effective up to 1 wk after treatment, are consistent with previous reports (Helson and Surgeoner 1983, Cilek and Hallmon 2006). The results for dphenothrin and resmethrin barrier treatments are the first report that we are aware of for these insecticides. They were effective up to 1 wk for dphenothrin and 48 h or 1 wk for resmethin, depending on the site, but had negligible effect in both trials at 2 wk posttreatment.

The reason for this discrepancy is not apparent to us at present. It appears that both products may provide maximum effectiveness against mosquitoes lasting somewhere around 1 wk posttreatment. Generally, pyrethroids rapidly degrade in the environment through photolysis, hydrolysis, and biodegradation. No residues on plant leaves have been found for resmethrin at 5 days postapplication (WHO 1989). In our study, the residual levels of insecticides were detectable by our GC/MS methods up to 2 wk posttreatment for permethrin and d-phenothrin, and up to 1 wk posttreatment for resmethrin. Comparison of biological results (Table 3) to the chemical results (Table 4) indicates some correlation between the 2 sets with respect to site. What creates difficulty in the comparison may be partly due to the sampling. However, it should be kept in mind that the relative toxicity of these compounds may differ significantly, and this can partly explain why a very low concentration of one pesticide, relative to another, can produce a much higher knockdown and mortality in tested mosquitoes. It has been reported that pesticides formulated as suspension concentrates have a greater degree of longevity/toxicity to mosquitoes than those of emulsion of oil in water, or emulsion concentrate (Cilek and Hallmon 2006), as was previously demonstrated for deltamethrin when compared with permethrin on treated surfaces (Ware and Whitacre 2004). In our study, resmethrin was used as an unformulated active ingredient. This likely had a significant impact on its residual lifetime in the field.

This study has demonstrated and confirmed that treatment of leaves with a water-based permethrin product provides efficacy against *Cx. quinquefasciatus* for >1 wk postapplication.

The adulticides d-phenothrin and resmethrin lose their effectiveness much more rapidly as measured by mortality. The application of insecticides to plants/vegetation as barrier sprays, when used in conjunction with other control measures, may become an integral part of a broad management scheme for mosquito control. However, to be feasible, additional studies are needed to examine various factors involved with this approach and how these influence the outcome as a function of efficacy and duration. One of these factors is that the most appropriate period of barrier treatment during a mosquito season within a district or areas will have to be determined. Another parameter, the duration of control or effectiveness for each insecticide on leaves against adult mosquitoes, will also need to be determined. The effectiveness may vary from area to area depending upon the vegetation size, shape, leaf integument, and environmental conditions, such as temperature, precipitation, sunlight, and dust. In the selection of an insecticide and treatment protocol for barrier treatment, care must be taken to avoid a situation where mosquitoes develop resistance to specific insecticides from prolonged or repeated exposure to highly residual insecticides (Georghiou and Saito 1983). Rather than limiting barrier treatments to the use of pyrethroids only, the use of several different classes of insecticides with short- to medium-term residual effects, in intermittent rotation, may decrease the ease of development of insecticidal resistance by mosquitoes.

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