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ASSESSMENT OF *ANOPHELES QUADRIMACULATUS* RESPONSE TO PERMETHRIN AND RESMETHRIN BY TOPICAL APPLICATION[1]

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ABSTRACT. Topical applications with field-captured mosquitoes provided LD_{50} estimates ranging from 13.3 to 20.0 ppm for permethrin and 8.5 to 18.9 ppm for resmethrin in the Stuttgart, AR, area in 1993. Mosquitoes collected from the abatement area appeared to be more susceptible to resmethrin than those from outside the area (8.5 ppm vs. 18.9 ppm). The observed difference was not great enough to be of concern from an economic or control standpoint. The data provided a firm baseline for future assessment of changes in susceptibility to these 2 materials. Mosquitoes collected in Walnut Ridge, AR, were susceptible to permethrin and resmethrin within the same range as the Stuttgart populations, but the limited number of observations preclude firm LD_{50} estimates.

Synthetic pyrethroids are currently the most commonly used adulticides against pest mosquitoes in the rice-growing region of eastern Arkansas. Both permethrin and resmethrin compounds are part of the synthetic arsenal against *Anopheles quadrimaculatus* Say in the Stuttgart, AR, rice-growing region. Until recently, malathion has been used as an adulticide for control of this species in the region. Efird et al. (1991) implied that significant resistance to malathion has accumulated in the resident population of *An. quadrimaculatus*.

This study continued to monitor the susceptibility of mosquitoes located in and around Stuttgart, AR, and Walnut Ridge, AR, to permethrin and resmethrin.

Mosquitoes were collected from the treatment zone at Garrison's barn in Stuttgart and beyond the treatment zone at the Hargrove barn, several kilometers south of Stuttgart. Resting mosquitoes were collected at noon with a hand-held aspirator powered by rechargeable battery packs (Sandoski et al. 1986). The collection tubes, capped with screened covers, were placed on a dry cloth in a small cooler containing a quart-sized bag of ice. Several hundred mosquitoes, in tube lots of 35–70, were collected each test day and transported to the laboratory at the University of Arkansas Rice Research and Extension Center for treatment.

Three separate collections were made at Garrison's barn (inside the spray zone) and 3 at Hargrove's barn (beyond the spray zone), the same sites that were utilized in the 1992 study (Dame, unpublished data). In addition, a single morning collection in Walnut Ridge was made with a backpack aspirator developed by USDA, Medical and Veterinary Entomology Research Laboratory (MVERL), Gainesville, FL; these mosquitoes were flown to Stuttgart in the original collection cups inside a dry cooler (without ice). They were exposed to topical applications in midafternoon of the same day.

Topical solutions were prepared on site by dilution in acetone of stock solutions of technical grade insecticide prepared by the staff of MVERL. Treatment solutions were replenished as needed and held at 23–25°C during the test period. The treatments were delivered with a 25-µl Hamilton microsyringe equipped with a repeating dispenser calibrated to deliver 0.5 µl per application. Both insecticides were used daily. To equalize variability due to differential holding periods for the mosquitoes, the sequence of chemicals was changed daily. For a given chemical the lowest concentration was used first. After treating the 10 subjects, the remaining solution was expelled from the microsyringe, which was then triple washed by filling it 3 separate times with acetone and rinsing it externally with acetone. The same cleansing process was repeated between chemicals.

Accuracy in delivering the proper volume of test material was controlled in the following manner: a stationary magnifying glass was positioned above the test subjects to enhance observation of the placement of the 0.5-µl treatment droplet. When a portion of the treatment droplet rolled off the thorax and onto the filter paper, or was improperly placed, the subject was discarded. To assure that evaporation at the syringe tip did not affect droplet volume, a pretreatment droplet was expelled from the microsyringe onto absorbent paper immediately before application of the treatment droplet.

To obtain maximum assurance of the quality

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Table 1. Toxicity of permethrin and resmethrin to susceptible populations of *Anopheles quadrimaculatus* inside and outside of the abatement region in Stuttgart and Walnut Ridge, Arkansas.

Insecticide	City	Location	*n*	LD_{50} (95% CL)[1]	LD_{90} (95% CL)	Slope ± SE
Permethrin	Stuttgart	Inside	170	13.3 (11.0–15.7)	29.6 (24.0–40.5)	3.7 ± 0.50
		Outside	180	20.0 (16.7–24.1)	51.5 (39.7–79.4)	3.1 ± 0.46
	Walnut Ridge	Inside	80	13.6 (8.9–19.0)	37.2 (25.3–87.4)	2.9 ± 0.70
Resmethrin	Stuttgart	Inside	180	8.5 (5.3–11.7)	57.1 (34.6–160.9)	1.6 ± 0.31
		Outside	190	18.9 (14.1–26.4)	110.2 (62.5–348.0)	1.7 ± 0.31
	Walnut Ridge	Inside	90	29.3 (20.9–54.0)	84.4 (48.3–592.5)	2.8 ± 0.84

[1] Confidence limits.

of the test subjects, similar exposures to acetone (0.5 μl) were conducted before initiating the insecticide series, between chemicals, and again after completing the day's treatments. This provided on-going controls and made it possible to assess test subject quality for each chemical series. The results of series associated with control mortality exceeding 20% were discarded, except for the Walnut Ridge collections, which could not be replicated.

To prepare the mosquitoes for topical application the tubes or cups were placed individually, as needed, inside a prechilled 3.8-liter (1-gallon) thermos jug situated inside a chest freezer. After approximately 5 min of chilling, the container was removed from the thermos jug, and the immobilized mosquitoes were placed and subsequently treated on dry filter paper on a cold table (0–2°C). Gravid females were selected for exposure; males, unblooded females, and recently engorged females were discarded. Gravid females were selected due to uniformity and ease of handling. Ten individuals were treated in each exposure replicate. The filter paper was replaced after each insecticide series or sooner when warranted. Precautionary measures were also used to prevent exposure of the test subjects to the other toxicants and to minimize exposure to cold temperature.

Following exposure, the 10 treated mosquitoes were transferred from the filter paper to clean paper cups (237 ml) and covered with screened lids. Small cotton pads containing 10% sugar water were placed on the screened lids. Mortality was recorded the following day, providing a holding period of at least 22 h. Means of mortality were corrected by Abbott's formula (Abbott 1925) and subjected to a probit analysis (PROC PROBIT) (SAS Institute 1990).

These LD_{50} estimates indicated a 2-fold difference between inside and outside populations with the 1993 resmethrin data (Table 1). This result was consistent with the 1992 findings (Dame, unpublished data), and the difference is so small that no significance can be attached to the finding. Less than a 2-fold difference in susceptibility of mosquitoes from inside and beyond the spray zone was detected for permethrin. LD_{50} (ppm) estimates generated from the probit procedure are presented in Table 1.

Estimates of LD_{90} and slope ± SE are also reported (Table 1). The estimates of the chi-square goodness of fit test associated with permethrin from both inside and beyond the spray zone in Stuttgart were 54.5 ($P > 0.0001$) and 46.5 ($P > 0.0001$), respectively. Estimates of chi-square inside and outside the spray zone for resmethrin in Stuttgart were 25.2 ($P > 0.0001$) and 28.9 ($P > 0.0001$), respectively. For Walnut Ridge, permethrin and resmethrin were 17.4 ($P > 0.0001$) and 11.1 ($P > 0.0009$), respectively. These results indicate that the log-dose probit data do conform to the Probit model.

The 2-fold observed difference between the 2 zones in 1993 is reasonably consistent with that observed in 1992 (4-fold) (Dame, unpublished data) but is probably not important relative to control operations. These data tend to confirm a 5- to 10-fold difference in susceptibility from that observed by Efird et al. in 1991 (the LD_{50} for both pyrethroids was *ca.* 2.3 ppm). It is likely that this was a result of treating only gravid females in the present study. No real differences were detected between the 1992 and 1993 results.

In summary, in 1993 the findings showed no important differences in susceptibility to the pyrethroids when comparing mosquitoes from inside to those beyond the spray zone.

The data from the unreplicated tests with mosquitoes from Walnut Ridge suggest that their susceptibility levels to the pyrethroids may fall within the same range as the Stuttgart mosquitoes although the analysis may not permit this assumption. Because these exposures were not rep-

licated, no firm conclusions can be drawn from the results, but they provide guidelines for subsequent assessments.

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