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LABORATORY BIOASSAY STUDIES TO DETERMINE METHOPRENE SUSCEPTIBILITY IN A NATURAL POPULATION OF *OCHLEROTATUS TAENIORHYNCHUS* FROM THE FLORIDA KEYS

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ABSTRACT. Larvae of Florida Keys *Ochlerotatus taeniorhynchus* collected from No Name Key were colonized and evaluated against technical *S*-methoprene in laboratory beaker tests. Glassware was treated with a silanizing reagent before testing to prevent methoprene attachment to the glassware. The No Name Key strain was compared with a susceptible laboratory strain of *Oc. taeniorhynchus*. Five serial dilutions (0.0100, 0.0050, 0.0010, 0.0005, and 0.0001 µg/ml) and an untreated control were evaluated. Tests were conducted in water baths with a constant water temperature of 27 ± 1°C and 250 ml of 3‰ salt water. Twenty-five late 3rd-stage larvae were placed in each beaker. Bioassay samples were analyzed by probit analysis and the median lethal concentration (LC_{50}), 90% lethal concentration (LC_{90}), and 95% lethal concentration (LC_{95}) values; confidence limits; χ^2 value; slope; and standard error were determined. The Florida Keys No Name Key strain exhibited no significant differences at the LC_{50}, LC_{90}, and LC_{95} levels from the laboratory strain in these studies.

KEY WORDS Florida Keys, methoprene, Altosid, *Ochlerotatus taeniorhynchus*, susceptibility, resistance, tolerance

INTRODUCTION

Methoprene applied as various Altosid® formulations has been used in the Florida Keys Mosquito Control District for more than 20 years to control the salt-marsh mosquito *Ochlerotatus taeniorhynchus* (Wied.) (Fussell, personal communication). Mosquito control failures have occurred in the past and often were attributed to influxes of offshore adult *Oc. taeniorhynchus*, inadequate applications or formulation problems with the product. The product was replaced upon occasion and resulted in improved control, but control failures occurred in subsequent years. Recently, concern has risen about resistance or tolerance to methoprene. Dame et al. (1998) demonstrated methoprene resistance in a barrier island strain of *Oc. taeniorhynchus* in Lee County, Florida. They determined a more than 14-fold tolerance in the indigenous barrier island strains (Captiva and Lover's Key) when compared to native Flamingo *Oc. taeniorhynchus*. Studies between a mainland strain (the Burnt Store) and the Flamingo strain showed no significant differences. They concluded that resistance might have been restricted to the barrier islands.

This study evaluated the efficacy of technical *S*-methoprene in laboratory beaker tests against a susceptible *Oc. taeniorhynchus* laboratory colony (Flamingo Key) and a natural Florida Keys population (No Name Key) suspected to have a tolerance toward methoprene. The median lethal concentration (LC_{50}), 90% lethal concentration (LC_{90}) and 95% lethal concentration (LC_{95}) mortality levels were determined, emergence differences were recorded (Floore et al. 1990), and tolerance ratios were obtained (Boike et al. 1985).

MATERIALS AND METHODS

Larvae of *Oc. taeniorhynchus* (approximately 8,000 1st through 3rd instars) were collected on No Name Key and shipped in 1-gal plastic wide-mouth jars containing water from the collection site to the John A. Mulrennan, Sr. Public Health Entomology Research and Education Center (PHEREC) by FedEx overnight air service. These larvae were reared to adults, which were bloodfed, and eggs were collected. A strain of *Oc. taeniorhynchus* (No Name Key strain) was established at the PHEREC. Subsequently, larvae of this strain were reared to the 3rd instar and laboratory beaker tests were conducted. The insecticide-susceptible PHEREC laboratory colony strain of *Oc. taeniorhynchus* was used as the standard for comparison. The PHEREC colony was established in 1995 from the USDA Center for Medical, Agricultural, and Veterinary Entomology Laboratory, University of Florida, Gainesville, FL, colony. This colony was originally established from Flamingo Key, FL.

Bioassays were conducted with 600-ml Pyrex® beakers (Fisher Scientific Products, Atlanta, GA) containing 250 ml of 3‰ salt water. Beakers were suspended in a water bath that used a Haake® immersion circulator (Fisher Scientific Products) to regulate and maintain a water temperature of 27 ± 1°C. The laboratory procedure was to transfer 25 3rd-stage larvae from rearing pans into 49 ml of well water, which was transferred into 200 ml of well water in 600-ml Pyrex beakers. Treatment was done by pipetting 1 ml of an appropriate methoprene dilution into the beaker, making a total of 250 ml of solution. Rearing water and testing water was deoxygenated well water with

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enough salt (sodium chloride, Fisher Scientific Products) added to make 3‰ salt water. Wellmark International (Schaumburg, IL) provided the technical *S*-methoprene (94.4% active ingredient, L950213519) used in preparing the serial dilutions, as well as the Sigmacote® (Sigma Biochemical & Reagents, St. Louis, MO) used in silanizing the glassware used in the study. The silanizing procedure followed that of Dame et al. (1998). Serial methoprene dilutions were 0.0100, 0.0050, 0.0010, 0.0005, and 0.0001 µg/ml, and an untreated control completed the testing regime. Stock and serial dilutions were formulated with reagent grade (ACS) acetone (Fisher Scientific Products). Treatments and controls were replicated 4 times per test and the tests were replicated 2 times. Larvae were fed daily approximately 3 ml of larval food slurry of 3 parts liver powder and 2 parts brewer's yeast (ICN Biomedical, Inc., Costa Mesa, CA) per beaker until pupation. Dead larvae were removed daily. Pupae were collected daily and placed in styrofoam cups (Dart 16J1; Dart Container Corp., Mason, MI) with approximately 30 ml of 3‰ salt water until they died or emerged.

Analysis: Percent emergence inhibition (Floore et al. 1990) was adjusted by Abbott's formula (Abbott 1925). This accounted for cast pupal exuviae, dead pupae, partially emerged adults, and dead adults. Probit analysis was used to determine the LC_{50}, LC_{90}, and LC_{95} mortality levels of both strains. Pearson chi-square was used to determine goodness-of-fit (SAS Institute 2001). A heterogeneity factor was used for correction when the value of χ^2 was greater than the appropriate tabular value (Robertson and Preisler 1992). A tolerance ratio also was determined, following Boike et al. (1985):

$$\text{tolerance ratio} = \frac{LC_{50},\ LC_{90},\ \text{or}\ LC_{95}\ \text{of No Name Key strain}}{LC_{50},\ LC_{90},\ \text{or}\ LC_{95}\ \text{of colony strain}}.$$

RESULTS AND DISCUSSION

A marked difference (more than 3 times) in mortality or emergence of adults between the Florida Keys and the PHEREC colony strains would be an indication of a difference between the Keys strain of *Oc. taeniorhynchus* and the susceptible colony strain. Conversely, if little difference (less than 3 times) was exhibited between the 2 strains, tolerance would be expected to be slight or not present (Boike et al. 1982).

Two tests were conducted to compare the Keys strain with the susceptible colony strain. No significant differences were observed between the 2 strains. The LC_{50}, LC_{90}, and LC_{95} values were calculated and tolerance ratios were developed (Table 1). In addition, the χ^2 value, slope, and standard error were given. The highest tolerance ratio was 1.3, thus indicating little differences between tol-

Table 1. Comparison of median lethal concentration (LC_{50}), 90% lethal concentration (LC_{90}), and 95% lethal concentration (LC_{95}) values of No Name Key *Ochlerotatus taeniorhynchus* to methoprene in laboratory beaker studies, 1999. The 95% lower and upper fiducial limits of the dose as determined by SAS (2001) are enclosed in parentheses.

Test locality	Slope ± SE	LC_{50} (µg/ml)	LC_{90} (µg/ml)	LC_{95} (µg/ml)	χ^2	*P* value	Tolerance ratio[1]		
							LC_{50}	LC_{90}	LC_{95}
No Name Key 1	1.37 ± 0.23	0.00131 (0.00054–0.00300)	0.01131 (0.00468–0.17694)	0.02083 (0.00679–0.65561)	32.99	<0.0001	1.3	1.1	1.0
Colony 1	1.19 ± 0.19	0.00120 (0.0004852–0.00263)	0.01414 (0.00531–0.18245)	0.02845 (0.00884–0.071891)	39.51	<0.0001			
No Name Key 2	1.19 ± 0.19	0.00120 (0.00048–0.00263)	0.01414 (0.00531–0.18245)	0.02845 (0.00884–0.71891)	39.80	<0.0001	0.9	0.1	0.08
Colony 2	1.5 ± 0.30	0.00145 (0.0004085–0.00350)	0.0995 (0.00397–0.21399)	0.01719 (0.00592–0.87752)	25.54	<0.0001			

[1] Tolerance ratio = (LC_{50}, LC_{90}, or LC_{95} of No Name Key strain)/(LC_{50}, LC_{90}, or LC_{95} of colony strain).

erances of the colony and No Name Key strains to methoprene in the laboratory study.

These results indicated that the No Name Key strain of *Oc. taeniorhynchus* was no more tolerant to methoprene than the PHEREC colony strain. Larvicide tolerance in the Florida Keys might be more difficult to establish and assess than in other areas in the State because of the almost daily influx of offshore adults. This occurrence and the dilution of the residence population would decrease the likelihood of development of resistance or tolerance. Personal communication with Florida Keys mosquito control personnel indicated constant migration of adult *Oc. taeniorhynchus* onto the inhabited islands from nearby offshore, mostly uninhabited islands. This natural occurrence would reduce development of tolerance in the Keys mosquito strains because of the constant mixing of susceptible breeding stock with adults that might exhibit some tolerance to methoprene treatment as larvae (Georghiou and Taylor 1977). Provost (1952, 1957) showed that flights by *Oc. taeniorhynchus* greatly exceeded the distance between offshore islands and the inhabited Florida Keys islands. Rathburn and Boike (1967) stated that natural variation or "vigor tolerance" might result in 1 strain of mosquitoes being more difficult to control than the same species in another area. The suggestion was made by Keys mosquito control personnel that the offshore island mosquitoes might be more vigorous than those on the main islands. However, this behavioral attribute was not investigated in this study. Lastly, only 1 Florida Key strain was evaluated and other Keys populations of *Oc. taeniorhynchus* might respond differently. If methoprene concerns continue in the Florida Keys, other island strains should be evaluated under similar laboratory settings as used in this study.

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