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MOSQUITO (*AEDES TAENIORHYNCHUS*) RESISTANCE TO METHOPRENE IN AN ISOLATED HABITAT

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ABSTRACT. Salt-marsh mosquitoes (*Aedes taeniorhynchus*), collected on 2 barrier islands in Lee County, Florida, that had been treated from 1989 to 1994 with 150-day methoprene briquets, were bioassayed with technical *s*-methoprene in the laboratory. Susceptibility of the indigenous Captiva strain (median lethal concentration [LC_{50}] estimate, 6.71 ppb) collected from Captiva Island was 14.9-fold lower than the naive Flamingo strain (LC_{50} estimate, 0.45 ppb) from Everglades National Park. The Lover's Key strain (LC_{50} estimate, 6.66 ppb) was 14.8-fold less susceptible than the naive strain. Determinations of the susceptibility of nearby foci of the mainland mosquitoes exposed in the past several years to methoprene have not been completed, but probit analysis of laboratory exposures revealed that the only mainland strain tested (Burnt Store) was no less susceptible (1.06-fold) than the naive Flamingo strain. These findings support the theory that the observed resistance might be restricted to the barrier islands. The known resistance foci (generated with briquet formulations) are located west of the mainland where there is minimal likelihood of inflow of genome from the mainland. On the other hand, the mainland mosquitoes, which were exposed to liquid formulations of methoprene from 1987 to 1994, are believed to have substantial gene flow between exposed and nonexposed populations and thus a reduced likelihood of selection for resistance.

KEY WORDS Briquet formulation, insect growth regulator, IGR, insecticide, restricted gene flow, selection, susceptibility, salt-marsh mosquito

INTRODUCTION

Operational use of methoprene in Florida extends back to the first applications of Altosid SR10® (Zoecon Corp., Palo Alto, CA) in Lee County in 1974. Several mosquito control programs in Florida have used methoprene for control of immature mosquitoes for more than 20 years. This study originated from an investigation into factors related to observed reduction in the efficacy of operational use of methoprene to control *Aedes taeniorhynchus* (Weid.) on the east coast of Florida (G. A. Curtis, D. A. Dame and G. F. O'Meara, unpublished). Difficulties in controlling *Ae. taeniorhynchus* on the barrier islands in Lee County prompted a request to include the Captiva strain in the east coast studies in which susceptibility analyses were being conducted.

The findings of the preliminary observations warranted additional studies to confirm and, if possible, determine the nature and distribution of the observed reduction in susceptibility of *Ae. taeniorhynchus* on Captiva Island. This report covers laboratory studies conducted on strains subsequently collected in Lee County to initiate an assessment of the geographical extent and possible habitat relationship of resistance to methoprene within the county. Although these studies are not yet completed, the results to date are considered sufficiently important to release the findings.

MATERIALS AND METHODS

Test insects: Adult female *Ae. taeniorhynchus* were collected early in the summer of 1995 from salt-marsh habitats on 2 barrier islands that had received applications of methoprene 150-day briquets annually from 1989 to 1994. The first-generation progeny of these barrier island collections are referred to as the Captiva and Lover's Key strains in this report.

Known methoprene-susceptible females were collected in 1995 and again in 1996 at Flamingo, FL, in Everglades National Park, which had no history of methoprene usage. First-generation eggs from these females are referred to as the Flamingo strain.

In 1996, collections were conducted on the Lee County mainland from an area that had received multiple applications of liquid formulations of methoprene (1987–94). First-generation eggs from this strain are known as the Burnt Store strain.

The parental Captiva, Lover's Key, and Burnt Store mosquitoes were trapped in the natural habitat at locations separated by 15–20 km (Fig. 1) and transported to the Lee County Mosquito Control laboratory in Ft. Myers, where they were transferred to holding cages and subsequently received blood meals from young chickens or through membranes. The resulting eggs were collected on cheesecloth pads moistened with water and placed on top of the cage outside the screen, through which the females oviposited. These eggs were stored and then hatched as needed to provide larvae for testing. Flamingo collections were taken to either the Lee County Mosquito Control laboratory or the Florida Medical Entomology Laboratory, where they were handled in the manner described above.

To provide larvae for testing, each day portions of egg pads were immersed for 2–5 h in deoxygenated reverse-osmosis (RO) water to which larval food had been added. The resulting larvae were

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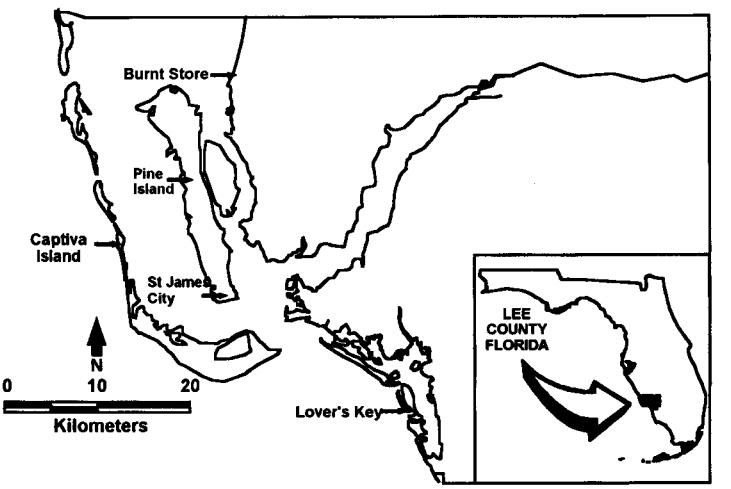

Fig. 1. Location of test strain collections (Captiva Island, Lover's Key, Burnt Store).

manually transferred to rearing trays (10 × 12.5-in. photographic developing pans) at a density of 125 per tray. Equipment was color coded to assure separation of the concurrently reared strains. The larvae were maintained at an ambient temperature of 28.6 ± 0.2°C. Finely ground liver powder (Bacto Liver Powder, Difco Laboratories, Detroit, MI) was sprinkled onto the surface of the rearing water at the rate of 50 mg/tray for the 1st and 2nd days and 150 mg/tray daily thereafter. Larvae were transferred to exposure dishes when they reached late 3rd or early 4th stage.

Treatment containers and solutions: The larvae were exposed to methoprene in culture dishes (3.5-in. diam) holding 100 ml of water. To prevent distortion of results by the known affinity of methoprene to adsorb to glass surfaces, each test container was pretreated with Sylon™ CT silanizing reagent (Sepulco Inc., Belleforte, PA) and rinsed to remove water-soluble and acetone-soluble Sylon CT residues.

The stock solution containers were preconditioned by first filling each container with a solution of the same concentration that it would ultimately hold and after ca. 24 h the conditioning solution was replaced with a freshly prepared solution. Stock solutions were prepared with technical *s*-methoprene (Lot 950531749, 95.63% purity, Zoecon Corp., Dallas, TX) and reagent grade acetone. Test solutions were prepared from these stock solutions using marked reusable pipettes, which were not rinsed between uses in order to maintain their preconditioned status.

These procedures were followed to minimize the number of attachment sites available on the glassware for adsorption of methoprene molecules in the preparation and during the actual experiment. Such attachment reduces the amount of methoprene available to the test insects. Stock solutions were refrigerated when not in use during the 1-month-long testing periods. Test solutions were prepared daily, as needed, and discarded the same day.

Larval exposures: Larvae were transferred from the rearing trays into holding cups before being placed in the test containers. To reduce the physiologic shock resulting from the direct transfer of larvae from rearing water to clear water, a standardized quantity of water from the rearing trays was mixed with the RO water in both the holding medium and the final test medium. In the holding cups the ratio of RO water to rearing water was approximately 1:1 and 3–4 drops of food slurry (10 mg liver powder/ml) were added to the mix. The test medium routinely consisted of 95 ml RO water and 5 ml rearing water, to which 4 drops of food slurry were added just prior to introduction of the larvae.

Twenty-five late 3rd- or early 4th-stage Flamingo and Captiva and/or Lover's Key larvae were exposed at each concentration in each replicate in the 1995 test series. A wide range of concentrations was used to assure that the observations would include the results of several exposures above and several below the 50% mortality level. With Burnt Store larvae reared in 1997 from the 1996 mainland site collections, triplicate sets of 15–28 larvae each were exposed on test days. Because of limited availability of Burnt Store larvae, exposures in that test series were limited to 5 concentrations that were expected to produce midlevel mortality of the naive Flamingo strain.

To minimize the toxic effect of the small amount of acetone in the test medium, larvae were placed in the treatment dish no sooner than 30 min after application of up to 1 ml of prepared solution and immediately after addition of a slurry containing 0.9 mg of liver powder per larva. The level of acetone was reduced to a maximum of 0.01 ppm in the exposure medium because of its observed detrimental affect on the larvae (an alternative solvent,

Table 1. Results of POLO PC probit analysis of the effect of *s*-methoprene on emergence of adults of barrier island (Captiva, Lover's Key) and naive (Flamingo) strains of *Aedes taeniorhynchus* exposed as late 3rd- and early 4th-stage larvae (95% confidence limits and standard error in parentheses for median lethal concentration [LC_{50}] and slope, respectively).

	Flamingo	Captiva	Lover's Key
No. subjects	1,117	343	550
No. controls	181	72	95
Slope	0.981 (0.103)	0.868 (0.134)	1.387 (0.157)
LC_{50} (ppb)	0.45 (0.25–0.73)	6.71 (2.52–16.0)	6.66 (3.20–13.4)
LC_{50} ratio[1]		14.9	14.8

[1] Ratio of barrier island strain to naive strain.

ethanol, was not utilized because it was also found to be deleterious). Daily series of the Captiva and Lover's Key tests included an untreated control containing 0.01 ppm acetone with each strain in addition to the untreated control without acetone.

Following introduction of larvae in tests with Captiva or Lover's Key strains, the culture dishes were secured with 100-mm petri dish covers to reduce evaporation; however, this step was bypassed in the 1997 tests with Burnt Store larvae. Pupae that developed within 6 h of introduction were removed and discarded due to possible inadequate exposure time (the record of the number of introduced larvae was adjusted accordingly when this occurred). Dead larvae were removed daily and pupae were transferred to covered holding cups containing fresh water. The larval exposure dishes and pupal holding cups were maintained at an ambient temperature of 28.5 ± 0.2°C. Adult emergence was observed 2 or 3 days after pupae were collected; complete separation from the pupal exuvium was considered to be adequate evidence for successful emergence.

Larval survival in this type of test is highly variable for 2 primary reasons. First, a minute amount of harmful acetone is introduced into each treatment dish as diluent; but without the solvent the technical material could not be serially diluted satisfactorily because the maximum solubility in water is ca. 1 ppm. Second, the duration of exposure in insect growth regulator tests varies from 1 to 4 days, the range of time required before the last larva pupates; this period is dependent on the specific age of the introduced larvae and the resulting nutritional and biological conditions within the treatment dish. These conditions in turn are dependent on the number of survivors that remain in the dish each day and the resulting utilization of the available nutritional materials and buildup of excreta and both beneficial and pathogenic microorganisms.

Because of these factors, each test series included at least one untreated control unit with 0.01 ppm acetone, in which survival from larva to adult ranged from 0 to 100%. Test series in which the survival to the adult stage among the untreated controls fell below 50% and individual tests in which larval mortality exceeded 44% were excluded from the probit analyses. The exclusion of those replicates in which the control mortality exceeds 50% overall or 44% in the larval stage (vs. 20% maximum for 24-h Abbott's formula adjustments [Abbott 1925]) represents an adjustment for test methodology and duration. The selected data were submitted to probit analysis (POLO PC) derived from Finney (1971) and based on the number of adult survivors compared to the initial number of larvae.

RESULTS

Barrier island strains: The results provided in Table 1 reveal the naive Flamingo strain to be 14.9-fold and 14.8-fold more susceptible than the Captiva and Lover's Key strains, respectively, based on the median lethal concentration (LC_{50}) estimates. The analysis for 10% lethal concentration (LC_{10}) and 90% lethal concentration (LC_{90}) levels provided similar estimates of relative susceptibility at the 95% level of confidence. These findings confirmed the results of the 1994 study (G. A. Curtis, D. A. Dame and G. F. O'Meara, unpublished) in which the difference between Captiva and Flamingo strains was estimated to be ca. 10-fold.

The mainland strain: The LC_{50} of the mainland Burnt Store strain was not significantly different from that of the naive Flamingo strain at the 95% level of confidence, producing a comparison ratio of 1.06. However, the LC_{50} estimate fell above the actual exposure level, so additional estimates were used for interpreting the analyses. The LC_{10} and 20% lethal concentration (LC_{20}) estimates both fell within the actual exposure range and the strains were not significantly different at the 95% level of confidence. The 30% lethal concentration (LC_{30}) and 40% lethal concentration (LC_{40}) estimates fell outside the exposure range, but here also the strains were not significantly different at the 95% level of confidence.

DISCUSSION

No previous studies have revealed significantly increased tolerance to methoprene among natural mosquito populations, although resistance has been observed in laboratory mosquitoes selected for many generations by methoprene exposure (e.g.,

Culex spp., Brown and Brown 1974, Georghiou 1974).

The data generated in this study confirm the existence of resistance in both the Captiva and the Lover's Key strains. Presumably, in both cases the resistance is due to extended exposure and selection in a genetically isolated site. Both strains had been exposed to applications of 150-day briquets for 5–6 seasons (1989–94), which had given satisfactory operational results until 1993–94. These strains presumably had been fully susceptible to methoprene prior to 1989, when they probably were genetically indistinct from the population at St. James City on nearby Pine Island, Florida (Fig. 1), where early experimental studies had been conducted with methoprene (Dame et al. 1976).

However, it is impossible to draw conclusions from these barrier island data concerning the susceptibility of mainland mosquitoes. Both of the barrier island resistant strains are located west of the mainland (Fig. 1), a factor which may tend to reduce migration from the mainland into the affected barrier island populations and thereby enhance selection. Earlier studies (Provost 1952, 1957) demonstrated long range *Ae. taeniorhynchus* flights oriented from the Lee County barrier islands eastward onto the mainland. As a result of the geographical orientation, and probable isolation from influx from the mainland, selection pressure may materially exceed that which would occur on the mainland where more genetic mixing would be expected to occur. Thus, there would appear to be a good possibility that selection towards resistance would be much less intense on the mainland than on the barrier islands.

The limited study conducted on the mainland Burnt Store strain revealed no indication of resistance or tolerance. Unlike the barrier island strains, for which the LC_{10} estimates in 1995 were significantly different following exposure to briquet formulations for several seasons than the naive strain at the 95% level of confidence, the Burnt Store mainland strain was not significantly different from the naive strain at any of the 1997 estimated LC levels and the slopes were similar. This strain had been exposed in nature to multiple applications of liquid formulations of methoprene from 1987 to 1994. Although the availability of a susceptible genome on the mainland may account for the lack of resistance in the Burnt Store strain, we are unable to rule out the possibility that the type of formulation and application technique might also be important factors.

Nevertheless, the resistance observed on the 2 separate barrier islands has not yet been detected on the mainland. Insofar as is possible, further studies will be conducted to elucidate the dynamics of methoprene resistance in *Ae. taeniorhynchus* in Lee County.

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