LABORATORY EVALUATION OF FORMULATIONS OF BACILLUS THURINGIENSIS VAR. ISRAELENSIS COMBINED WITH METHOPRENE OR A MONOMOLECULAR SURFACE FILM AGAINST ANOPHELES ALBIMANUS AND AN. STEPHENSI¹

M. J. PERICH, J. T. ROGERS, L. R. BOOBAR AND J. H. NELSON

U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, Frederick, MD 21701-5010

Bacillus thuringiensis var. israelensis (B.t.i.), methoprene, and Arosurf® MSF (monomolecular surface film) have been proven to be effective against various mosquito species (Laird and Miles 1985, Perich et al. 1987). These larvicides, based on their varied modes of actions, are limited in their efficacy against various mosquito immature life stages. The purpose of this study was to evaluate formulations of Duplex® (B.t.i. and methoprene) at two application concentrations, Arosurf MSF combined with Teknar® (B.t.i.), and Teknar alone, against each larval instar (1st-4th) and pupal stage of Anopheles albimanus Wiedemann and Anopheles stephensi Liston.

Ten larvae or pupae from laboratory colonies of An. albimanus and An. stephensi were placed into 400 ml sterilized beakers containing 250 ml of deionized water with a dissolved oxygen content of 4.8 mg/liter. All larvae and pupae were newly molted individuals except for the late 4th instar larvae which were 48 hr post-molt. Prior to application of the formulation, larvae were fed five drops of finely ground hog chow-distilled water suspension. All bioassays were replicated three times and maintained in an environmentally controlled room at $27 \pm 1^{\circ}\mathrm{C}$ and $80 \pm 5\%$ RH.

All formulations were prepared as agitated, water-based suspensions. The application concentrations were 0.24 (0.2 pint/acre) and 0.59 liters/ha for Duplex, 2.43 liters/ha (2.08 pints/acre) for Arosurf MSF combined with 0.59 liters/ha (0.5 pint/acre) Teknar, and 0.59 liters/ha (0.5 pint/acre) for Teknar alone. The potency of the B.t.i. in both the Duplex and Teknar was 3,000 Aedes aegypti International Toxic Units/mg.

Table 1. Efficacy of water-based formulations of Duplex®, Arosurf® MSF combined with Teknar® and Teknar alone against larvae and pupae of *Anopheles albimanus* and *An. stepheni*.

| Formulations and species of <i>Anopheles</i> | Mean percent mortality of each larval instar and pupal stage ^{a,b} | | | | | |
|--|---|------|------|---------------------------|--------------------------|-------|
| | 1st | 2nd | 3rd | Early 4th ^c | Late 4th ^d | Pupal |
| An. albimanus | | | | | | |
| Duplex (0.24 liters/ha) | 100a | 100a | 100a | 100a | 73a | 3a |
| Duplex (0.59 liters/ha) | 100a | 100a | 100a | 100a | 83a | 30b |
| Arosurf + Teknar | 100a | 100a | 100a | 100a | 100b | 100c |
| Teknar | 100a | 100a | 100a | 100a | 40c | 7a |
| Control | 3b | 0b | 0b | 0b | 0 d | 0a |
| An. stephensi | | | | | | |
| Duplex (0.24 liters/ha) | 100a | 100a | 100a | 100a | 77a | 0a |
| Duplex (0.59 liters/ha) | 100a | 100a | 100a | 100a | 80a | 20b |
| Arosurf + Teknar | 100a | 100a | 100a | 100a | 100b | 100c |
| Teknar | 100a | 100a | 100a | 100a | 40c | 0a |
| Control | 3b | 0b | 0b | 0b | 3 d | 0a |

^{*}Means within a column followed by the same letter are not significantly different ($P \le 0.01$; Chi Square test [SAS Institute 1985]).

¹ The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or reflecting the views of the Department of the Army or the Department of Defense. Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

^b All surviving test specimens became adults.

c>12 hr postmolt.

d <48 hr postmolt.

The percent mortality of larvae and pupae, along with the percent adult emergence, was recorded at 24 hr intervals and continued until all surviving pupae emerged as adults. The mean percent mortalities of treatments were compared against each other using Chi-square test (SAS/STAT 1985).

All formulations tested were found to produce 100% mortality in both An. albimanus and An. stephensi 1st-early 4th instar larvae (Table 1). An expected low mortality was found in late 4th instar larvae of both species when treated with Teknar alone, due to discontinuance of feeding. Significant mortality of late 4th instar larvae and a low adult emergence occurred with both species in water treated with each concentration of Duplex. These results can be explained by the methoprene component of Duplex, a juvenoid insect growth regulator sensitive to 4th instar larvae mosquito larvae that can be either absorbed through the cuticle or ingested by the larvae.

In conclusion, all formulations (Duplex, Arosurf MSF combined with Teknar, and Teknar) are effective against the actively feeding larval instars (1st-early 4th) of both An. albimanus and An. stephensi. Duplex at either application concentration (0.24 or 0.59 liters/ha) is effective against the late, nonfeeding 4th instar larvae of both species. Only Arosurf MSF combined with

Teknar produced significantly high mortalities in both species for all larval instars and pupae (Table 1). The additive effect of this formulation was due to the physical control effects of Arosurf MSF which increase with larval development (Levy et al. 1984, Perich et al. 1987), and the toxic effects of Teknar against the actively feeding stages. Although the Arosurf MSF combined with Teknar formulation is not commercially available and it cannot be stored for over 8 h once mixed, this study has shown it to be highly effective.

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