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EVALUATION OF DIVERSE FORMULATIONS OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AGAINST *ANOPHELES ALBIMANUS* IN HONDURAS[I]

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ABSTRACT

FourbioTationallarvicide formulations, Duplex® (melhoprene combined with B.t.i.), Teknar® *(B.t.i.),* Arosurf® MSF (Monomolecular Surface Film), and Arosurf MSF combined with Teknar, were evaluated against naturally occurring populations of *Anopheles albimanus* Wiedemann in Honduras. All formulations reduced the mean number of larvae per sample area to 0 within 48 hr posttreatment, and gave significant *(P*≤ 0.05) control through 240 hr posltreatment. The potential for large scale use of these formulations for vector suppression in Honduras is discussed.

INTRODUCTION

Malaria is the primary arthropod-borne communicable disease in Honduras, with over 30,000 cases reported in 1985 (PAHO, 1986). The disease is a major deterrent of socioeconomic development in the tropical Americas. Historically, vector control has been the major component of any malaria control program in endemic areas (Sloff, 1987).

Anopheles albimanus Wiedemannis a major vector of malaria in Central America (Clyde, 1987). This species has become physiologically resistant to many of the conventional insecticides (Brown, 1986). In addition, behavioral modifications (exophagic behavior) in *An. albimanus'*s blood feeding activity, has made standard domiciliary spraying with residual insecticides ineffective (J.C. Stivers, personal communication). These factors, along with the potential insult to the environment by synthetic organic insecticides (Matsumura, 1975), has created the need for biorational alternative control strategies.

Larviciding with biorational formulations has been proposed as one alternative control strategy against malaria vectors as part of an integrated vector control program (Sloff, 1987). Various commercial formulations of *Bacillus thuringiensis* var. *israelensis (B.t.i.*) alone, and combined with a monomolecular surface film (Perich et al., 1987) or methoprene (Perich et al., 1988), have been found to be efficacious under laboratory conditions against *An. albimanus.* The purpose of this study was to evaluate these biorational larvicide formulations against *An. albimanus* in various breeding habitats in the Comayagua Valley of Honduras.

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MATERIALS AND METHODS

The treatment areas were located within a 3 km radius of the city of Comayagua, in the Comayagua Valley, the principal agricultural area of southcentral Honduras. This region, historically an endemic malaria area, contains many drainage/irrigation ditches, small ponds (<0.5 ha), rice fields, and slow moving streams, known sites of *An. albimanus* breeding (Breeland, 1972; Rozeboom, 1941). Treatment applications were randomly assigned to these sites.

The larvicide formulations evaluations were: Teknar®, a commercial monomolecular organic surface film; Arosurf® MSF combined with Teknar (4:1), a noncommercially available formulation; and Duplex®, a commercially available formulation of Teknar and methoprene combined. All larvicide formulations were applied as technical grade solutions using a 7.6 liter hand-pumped compression sprayer. A separate sprayer was used with each formulation. The application rates in the study were 1.17 liters/ha for the Teknar and Duplex larvicides, and 4.67 liters/ha for the Arosurf MSF larvicide. A homogeneous suspension of the combined formulation of Arosurf MSF and Teknar was ensured by vigorous hand shaking throughout the spray operation.

Sites were selected based on pretreatment sampling of previously known positive *An. albimanus* breeding sites (L.A. Rivera, personal communication). Once a site was verified to contain *An. albimanus* larvae, three, 400 ml dipper samples were taken at three sample points, at a minimum of 5 m apart, prior to treatment. Sample points were marked to ensure that the same points would be sampled posttreatment. Treatments were applied with a fan spray to the sampling point and surrounding water for a 100 m radius in all directions using a sweeping pattern. Posttreatment samples (3 dips/sample point) were taken at 24, 48, 72 and 240 hr.

The mean number of larvae at each posttreatment sampling interval for all formulations was used as the criteria in evaluating their efficacy against *An. albimanus*. A completely randomized experimental design was used; the data were analyzed by an analysis of variance (ANOVA [SAS/STAT, 1985]). The means of the pre- and posttreatment samples were separated by use of Duncan's (1955) multiple range test ($P \leq 0.05$).

RESULTS AND DISCUSSION

All formulations evaluated reduced the number of *An. albimanus* larvae (1st–4th instar) significantly within 24 hr posttreatment (Tables 1 and 2). No statistical difference ($P \leq 0.05$) was found between any two of the formulations. Each formulation provided significant reduction in the number of *An. albimanus* 1st–4th instar larvae through 240 hr posttreatment. Ramoska et al. (1982), van Essen and Hembree (1982), Margalit and Bobroglio (1984), and Ohana et al. (1987) have shown that large amounts of organic matter in the water significantly reduce the field efficacy and persistence of *B.t.i.*. Since organic matter in the treatment area water was relatively low, the high level of initial control and persistance provided in this study was expected.

At 24 hr posttreatment, the Arosurf MSF alone did not provide 100% reduction in the 1st and 2nd larval instar populations as did the other three formulations although 100% mortality was obtained at 48 hr (Table 1). This lower sensitivity of an early *An. albimanus* instars to Arosurf MSF corroborates previous findings described by Perich et al. (1987). Although no significant pupal data were collected in this study (only one pupa for entire test period), the pupicidal activity of Arosurf MSF can be a significant component in the suppression of *An. albimanus* (Perich et al., 1987).

The combined formulation of Arosurf MSF + Teknar has been shown to produce a joint larvicidal/pupicidal action against *An. albimanus* (Perich et al., 1987). This additive effect along with the induced spreading action afforded by Arosurf MSF (Levy et al., 1984), makes this formulation highly efficious against *An. albimanus*, as shown in Tables 1 and 2. A major operational advantage of using combined formulations (Arosurf MSF + Teknar, Duplex) rather than single component larvicides is that they can be used against a broader range of immature mosquitoes (1st

TABLE 1
Mean number of *Anopheles albimanus* 1st and 2nd larval instar after application of larvicide formulations in Comayagua, Honduras[a,b]

Larvicide Formulation	0 hr Pre-trt	24 hrs Post-tr	48 hrs Post-tr	72 hrs Post-tr	240 hrs Post-tr
Teknar	23.7 a	0 a	0 a	0 a	1.5 a
Arosurf MSF	11.7 a	1.0 a	0 a	0 a	0 a
Arosurf MSF + Teknar	11.0 a	0 a	0 a	0 a	0 a
Duplex	17.3 a	0 a	0 a	0 a	1.0 a
Control	18.0 a	16.0 b	12.0 b	17.7 b	11.7 b

[a]Mean number based on three, 400 ml dipper samples per three sample points per sample area.
[b]Means within a column followed by the same letter are not significantly different ($P \leq 0.05$; Duncan's [1955] multiple range test).

larval instar-pupal stage). A broad, efficious application range against *An. albimanus* immatures is necessary because of their rapid stadia and continuous breeding in the tropics (Breeland, 1974; Del Carmen et al., 1984).

Certain limiting factors are cosmopolitan for any large scale use of *B.t.i.* and/or monomolecular organic surface film formulations for mosquito control. These limiting factors are: short field persistence by either formulation in areas treated; *B.t.i.* formulations (excluding combined formulation with Arosurf MSF) not remaining suspended in treated water; little canopy penetration when aerially applied; and high cost of treatment. These same factors are then potential influences on the efficacy of all formulations evaluated in this study, when used in large area treatments against *An. albimanus* throughout its geographical distribution.

Short persistence in mosquito breeding habitats is a major disadvantage for both *B.t.i.* (Margalit and Dean, 1985) and monomolecular organic surface films (Levy et al., 1981). In a subsequent control operation in the Comayagua Valley, all formulations were found to provide significant control for only 12 days posttreatment. This limited persistence by *B.t.i.* can, in part, be attributed to its ready adsorption to organic particles (Ohana et al., 1987) and gravity which prevent it from remaining suspended in the water and therefore available for larval ingestion.

TABLE 2
Mean number of *Anopheles albimanus* 3rd and 4th larval instars after application of larvicide formulations in Comayagua, Honduras[a,b]

Larvicide Formulation	0 hr Pre-trt	24 hrs Post-tr	48 hrs Post-tr	72 hrs Post-tr	240 hrs Post-tr
Teknar	1.8 a	0 a	0 a	0 a	0 a
Arosurf MSF	1.3 a	0 a	0 a	0 a	0 a
Arosurf MSF + Teknar	1.3 a	0 a	0 a	0 a	0.3 a
Duplex	1.3 a	0 a	0 a	0 a	0.3 a
Control	5.3 b	5.0 b	3.3 b	2.3 b	2.0 b

[a]Mean number based on three, 400 ml dipper samples per three sample points per sample area.
[b]Means within a column followed by the same letter are not significantly different ($P \leq 0.05$; Duncan's [1955] multiple range test).

Canopy penetration was not a factor in this study because applications were made at ground level ≤30 cm from the water surface. Canopy penetration becomes a limiting factor for these formulations when aerial applications are used in treating large areas. Thus, it becomes necessary to add suitable canopy penetrating carriers to the active ingredients (*B.t.i.*, Arosurf MSF, and methoprene) in order to get through the dense vegetation associated with *An. albimanus* larval habitat (Elliott, 1969).

The cost of using any of the formulations is high (Table 3) when considering the potential need for retreatment at 10–12 day intervals. Such high application costs make large area treatment with these formulations prohibitive for most areas in Honduras at the present time.

TABLE 3
Approximate costs for the amount of four larvicide formulations necessary to treat one hectare

Larvicide formulation	Cost per ha (US$)
Teknar	6.18
Arosurf MSF	22.66
Arosurf MSF + Teknar	28.84
Duplex	8.90

In conclusion, all formulations evaluated against naturally occurring populations of *An. albimanus* larvae in the Comayagua Valley of Honduras provided significant control ($P \leq 0.05$) within 24 hr and continued to provide such control through 240 hr posttreatment. Results from this study indicate that these formulations (Teknar, Arosurf MSF, Teknar + Arosurf, and Cuplex), costs notwithstanding, offer excellent potential as biorational alternative control strategies against this important malaria vector in Honduras.

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