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Field evaluation of four biorational larvicide formulations against *Anopheles albimanus* in Honduras

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Abstract. Four biorational larvicide formulations: Teknar® (*B.t.i.*), Arosurf® MSF (Monomolecular Surface Film), Arosurf MSF combined with Teknar, and SAN-809-I ([s]-methoprene combined with *B.t.i.*), were evaluated against naturally occurring populations of the malaria vector mosquito *Anopheles albimanus* Wiedemann in Honduras. All formulations reduced the mean number of larvae per sample area to 0 within 48 h after treatment, and gave significant ($P<0.05$) control when compared with similar untreated areas for at least 10 days after treatment. It is concluded that each of these four formulations can be used effectively to control the larvae of *An.albimanus* in Honduras.

Key words. *Anopheles albimanus*, larvicides, methoprene, monomolecular surface film, *Bacillus thuringiensis* var. *israelensis*.

Introduction

Anopheles albimanus Wiedemann is 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, behavioural changes (exophagic behaviour) in the blood-feeding activity of *An.albimanus* have made standard domiciliary spraying with residual insecticides ineffective as reported by Rachou *et al.* (1965) in El Salvador, and Elliott (1969) in Jamaica and Venezuela. These factors, along with the potential risk to the environment by synthetic organic insecticides, have created the need for 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 programme (Slooff, 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 (s)-methoprene (Perich *et al.*, 1988), were found to be active against *An.albimanus* under laboratory conditions. The purpose of this study was to evaluate these biorational larvicide formulations against *An. albimanus* under field conditions in 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 south-central Honduras. This region, historically an endemic malaria area, contains many irrigation ditches, small ponds (<0.5 ha), rice fields, and slow-moving streams, which are sites of *An.albimanus* breeding (Breeland, 1972). Five of each breeding site type containing *An.albimanus* were selected, from a before treatment survey of suspected breeding areas, making a total of twenty sample sites. Four larvicide formulations and a control were randomly assigned to each breeding site type. The twenty sample sites were further divided into three sample points. Sample points were located a minimum of 5 m apart and marked to ensure that the same points would be sampled after treatment. Three 400 ml dipper subsamples were collected at each of three sample points before treatment for a total of nine dips per sampling site, the number of larvae recorded, and all larvae returned to their respective sample point.

The larvicide formulations evaluated were: Teknar®, a commercial *B.t.i.* formulation; Arosurf® MSF, a commercial mono-molecular organic surface film; Arosurf MSF combined with Teknar (4:1); and SAN-809-I (Zoecon Corp.), a formulation of *B.t.i.* and (s)-methoprene combined. The potency of the *B.t.i.* in both the Teknar and SAN-809-I was 3000 *Aedes aegypti* International Toxic Units/mg, and the percent of active ingredient (*B.t.i.*) was 1.6 and 1.5 for each formulation, respectively. The SAN-809-I contained 0.2% (s)-methoprene. All larvicides were formulated from commercial suspensions and were applied using Hudson® 7.6 litre hand-pumped compression sprayers, fitted with a pressure gauge. A separate sprayer was used with each formulation. The application rates in the study were 1.17 litres/ha for the Teknar and SAN-809-I larvicides, 4.67 litres/ha for the Arosurf MSF larvicide, and 5.84 litres/ha for the combined formulation of Arosurf MSF and Teknar. A homogeneous suspension of the combined formulation was assured by vigorous manual agitation throughout the spray operation and maintenance of 50 psi tank pressure. Treatments were applied by spraying in a fan-shaped pattern which extended 100 m in all directions from the sample point. Larval samples were collected 24, 48, 72 and 240 h after treatment. The mean numbers of larvae in each sample, before and after treatment, were compared by use of Duncan's (1955) multiple range test (ANOVA [SAS/STAT, 1985]) ($P<0.05$).

Results and Discussion

There was no statistical difference between sites ($P<0.05$) in the *An.albimanus* larval population (instars I–IV) before treatment application (Table 1). Within 24 h after treatment, the numbers of *An.albimanus* larvae of all instars were significantly reduced by all four formulations compared to untreated areas, but with no statistical difference between any of the formulations. Each formulation provided significant reduction ($P<0.05$) in the numbers of *An.albimanus* larvae of all instars, up to at least 10 days post-treatment (Table 1).

Twenty-four hours after treatment all four products gave complete control of all third and fourth instar larvae. Teknar, SAN-809-I and Arosurf + Teknar also gave complete control of all first and second instars. Arosurf MSF alone did not provide 100% reduction of the first and second larval instar populations, although 100% mortality was obtained at 48 h (Table 1). This lower sensitivity of early *An.albimanus* instars to Arosurf MSF corroborates previous laboratory bioassay results of Perich *et al.* (1987) which reported a range of 16.7–20% mortality at 24 h after treatment. This observed lower sensitivity of younger larval instars probably results from their lower oxygen demand (Clements, 1963).

Two operational advantages of using Arosurf MSF and Teknar, SAN-809-I combined are expanded effectiveness against immature *An. albimanus* from first larval instar through the pupal stage (Perich *et al.*, 1988) and improved treatment area coverage due to the spreading effect of Arosurf MSF (Levy *et al.*, 1984). The first advantage is important because of this species' rapid stadia and continuous breeding in the tropics (Breeland, 1974; Del Carmen *et al.*, 1984).

Certain limiting factors influence the practicability of the formulations evaluated for mosquito control in this study. These limiting factors

Table 1. Mean number per dip of *Anopheles albimanus* larvae by instar after application of larvicide formulations in Comayagua, Honduras.*†

Larvicide formulations and larval instar groupings	0 h pre-trt	24 h post-trt	48 hr post-trt	72 h post-trt	240 h post-trt
First and second larval instars					
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]
SAN-809-I	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]
Third and fourth larval instars					
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]
SAN-809-I	1.3[a]	0[a]	0[a]	0[a]	0.3[a]
Control	2.3[a]	5.0[b]	3.3[b]	2.3[b]	2.0[b]

* Mean number based on three 400 ml dipper subsamples per three sample points per sample area.

† Means within a column followed by the same letter are not significantly different ($P<0.05$; Duncan's (1955) multiple range test).

are: short-term persistence of treatment effectiveness; *B.t.i.* formulations (excluding the formulation combined with Arosurf MSF) not remaining suspended in treated water; limited canopy penetration (Mulla, 1985) when applied aerially; and the high costs of treatments.

Short-term persistence in mosquito breeding habitats is a major disadvantage of both *B.t.i.* (Mulla, 1985; Garcia & Sweeney, 1986) and monomolecular organic surface films (Levy *et al.*, 1981). The limited persistence of *B.t.i.* can, in part, be attributed to the density of the formulation which prevents it from remaining suspended in the water and therefore unavailable for larval ingestion, and to its ready adsorption to organic particles (Ohana *et al.*, 1987). Other studies have shown that large amounts of organic matter in the water significantly reduce the field efficacy and persistence of *B.t.i.* (Ramoska *et al.*, 1982; van Essen & Hembree, 1982; Margalit & Bobroglio, 1984). Since organic matter in the water at the treatment sites was relatively low, the high level of initial control (100%) was expected. Due to logistical limitations, evaluation of the persistence of the four formulations in this study was limited to a maximum of 10 days after treatment. In a subsequent control operation in the Comayagua Valley, the four formulations were found to provide effective control for only 12 days after treatment. A gradual settling of the *B.t.i.* to a level below the primary feeding zone of *An.albimanus* larvae may explain this loss of effective control after 12 days.

Canopy penetration was not a factor in this study because applications were made at ground level approximately 30 cm from the water surface. When aerial applications are used in large areas, it becomes necessary to reformulate the larvicides (*B.t.i.*, Arosurf MSF, and [s]-methoprene) with suitable carriers such as sand, plaster-based pellets, and other materials to penetrate the dense vegetation often associated with *An.albimanus* larval habitat (Elliott, 1969). However, such canopy penetrating carriers may affect the efficacy of the larvicides after the formulations have entered the water.

In conclusion, the four formulations, evaluated against naturally occurring populations of *An.albimanus* larvae in the Comayagua Valley of Honduras, provided significant control ($P<0.05$), as compared with similar untreated areas, within 24 h after treatment and continued to provide control for at least 10 days after treatment. Results from this study indicate that these formulations (Teknar, Arosurf MSF, Teknar + Arosurf, and SAN-809-I) offer excellent potential as biorational alternative control

strategies against this important malaria vector in Honduras.

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