EFFICACY OF FLOWABLE CONCENTRATE FORMULATIONS OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AGAINST BLACK FLIES (DIPTERA:SIMULIIDAE)[1]

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ABSTRACT. Seven flowable concentrate formulations of *Bacillus thuringiensis* (H-14), Teknar® wdc, autodispersible Teknar, Teknar 2X aqueous concentrate, Teknar 2X oil base concentrate, Vectobac® AS, Bactimos® FC and Skeetal® F, were evaluated in small streams against *Simulium vittatum*. There was no significant difference in efficacy among the formulations with the exception of the Teknar 2X aqueous concentrate, which required considerably less formulation (5 mg/liter/1 min) than the others to produce 95% mortality in penultimate instars of *S. vittatum*. The field determined LC-95 for the other formulations ranged from 10.6 to 15.9 mg/liter/1 min. There was no significant difference between the efficacy of excessively diluted and undiluted formulations.

INTRODUCTION

Initial field trials with *Bacillus thuringiensis* var. *israelensis* (serotype H-14) de Barjac against black flies indicated high levels of larvicidal activity with little or no effect on non-target organisms (Undeen and Colbo 1980, Colbo and Undeen 1980). Where problems of resistance to conventional chemical larvicides exist or where chemical larviciding is prohibited due to legal constraints or environmental concerns, *B. thuringiensis* (H-14) can provide effective and selective alternative control of vector and pestiferous black flies. Relative to chemical larvicides, however, there are significant disadvantages encountered with the use of *B. thuringiensis* (H-14) as currently formulated: it is considerably bulkier and provides shorter effective downstream transport (carry) of larvicidal activity than its chemical counterparts.

Effective carry of *B. thuringiensis* (H-14) was shown to be strongly correlated with stream discharge (Undeen and Lacey 1982) and profile (Undeen et al. 1984). Formulation can also have a major effect on efficacy and effective carry. Tests conducted under laboratory and simulated field conditions indicated superior activity of primary powders and wettable powder formulations over that of experimental and commercially available liquid formulations (Guillet and Escaffre 1979; Molloy et al. 1981, 1984). They, nevertheless, exhibit abbreviated carry when compared with flowable concentrates under field conditions (Guillet et al. 1982a, Lacey and Undeen 1984). The larger particle size of powder formulations results in rapid settling in still water (Guillet et al. 1980, Molloy et al. 1984) and apparently facilitates increased filtration of the active moiety from the water through contact with the substratum. By contrast, spores and parasporal inclusions in flowable concentrate formulations that have been produced from wet fermentation slurry tend to suspend individually and settle less rapidly (Guillet and Escaffre 1979, Guillet et al. 1980, Molloy et al. 1984).

The quantity of formulation that is required to achieve effective control represents a major limitation on the efficient application of *B. thuringiensis* (H-14), especially aerial treatment of large rivers. Compounding this problem is the fact that most formulations require some dilution with water in order to be totally effective (Guillet et al. 1982b). Reduction of the amount of formulation required for effective control via concentration of the active moiety, with concomitant improvement of miscibility of undiluted product, would facilitate increased use of *B. thuringiensis* (H-14) in black fly control programs.

It was the objective of this investigation to evaluate new formulations of *B. thuringiensis* (H-14) against *Simulium vittatum* Zetterstedt in small streams using both diluted and undiluted formulations and to provide background information on their physical properties that could affect application and carry.

METHODS AND MATERIALS

Seven flowable concentrate formulations of *B. thuringiensis* (H-14) were evaluated in small streams at the Holston Army Ammunition Plant, Kingsport, Tennessee, against penultimate instars of *S. vittatum* using the field exposure-laboratory holding technique described in earlier publications (Lacey and Un-

[1] Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the U.S. Department of Agriculture.

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deen 1984, Undeen et al. 1984). The technique consisted of application of diluted formulation to the streams, waiting 1 hr to ensure adequate exposure and subsequent voiding of the *B. thuringiensis* (H-14) from the larvae, and then collecting larvae on natural substrates 95 to 100 m below the treatment points. Penultimate instars were then held in waxed paper cups (356 ml) in simulated current (3 cups/test; 25 larvae/cup) in the laboratory for 24 hrs before assessing mortality. Depending on the degree of larvicidal activity, 3-4 concentrations ranging from 1.0 to 15.0 mg of formulation/liter of stream water during a 1 min. application were evaluated for each formulation except the autodispersible Teknar which was only evaluated at 10 mg/liter/1 min. We selected the particular concentrations for each in an attempt to generate a range of mortalities between 50 and 95%. Three replicate tests for each concentration were run in three different streams. Comparisons between the formulations were made with excessively diluted material, i.e., mixed with sufficient water to bring the total amount of liquid to be applied to 7.2 liters. Successively working upstream in 100 m increments it was possible to make 16 separate treatments in the four available streams per field trip against previously unexposed larvae. All other procedures for the evaluation of larvicidal activity were identical to those described by Lacey and Undeen (1984) and Undeen et al. (1984). Using the same procedures, tests were also conducted with undiluted material at 10 mg/liter/1 min applied to the streams with a large syringe for all of the formulations except the Teknar 2X which was applied at a rate of 5.0 mg/liter/1 min.

The formulations, their factory determined potencies in International Toxicity Units (ITU) and concentration range used in our tests were as follows: Teknar® wdc (Zoecon, Inc.) (600 ITU/mg; lot no. 21501; 2.5-15 mg/liter); Teknar 2X aqueous concentrate, (1200 ITU/mg; San 402 SC 61, lot no. Bti 52 and San 402 SC93 lot no. Bti 71; 1.0–7.5 mg/liter); Teknar 2X oil base concentrate (1200 ITU/mg; San 402 SC 78, lot no. 8321; 2.5–10.0 mg/liter); Teknar autodispersible (600 ITU/mg; lot no. W-85-27; 10 mg/liter); Bactimos® FC (Biochem Products) (1000 ITU/mg; lot. K83004NF; 5.0–15.0 mg/liter; Vectobac® AS (Abbott Laboratories) (600 ITU/mg; ABG-6145; lot no. 63-007-BA; 2.5—10.0 mg/liter); and Skeetal F® (Tate and Lyle Industries, Ltd) (1400 ITU/mg; lot no. ST8; 2.5–15 mg/liter).

The average discharge and temperature during the course of our studies was 31.8 m^3/min and 21.9°C, respectively. Detailed descriptions of the streams and methods for measurement of velocity and discharge are presented in Lacey and Undeen (1984). Probit analyses were performed on all mortality data from tests on diluted formulations, and analyses of variance and Duncan's New Multiple range test (Duncan 1955) were performed on arcsine-transformed mortality data at the discriminating dosage of 5 mg/liter/1 min for diluted formulations. Linear correlation analysis was also performed on the mortality data (inverse of LC-95) and ITU level for each formulation. *T*-tests were used to analyze data comparing diluted with undiluted formulations.

Physical characteristics of the formulations that could influence application and carry were studied under laboratory conditions. Relative viscosity was measured by weighing the amount of each formulation that was delivered at room temperature through 25 ml Kimax® glass pipettes with tips cut to leave a uniform opening of 3 mm in diameter. Three samples of each formulation were delivered through 3 separate 25 ml pipettes in either 3, 4 or 5 seconds, leaving at least 2 ml of formulation in the pipette. To assure uniformity in assessment, the same 3 pipettes were used for all 7 formulations after cleaning and drying. The weight of formulation delivered/sec of the most viscous formulation, i.e., the one delivering the least g/sec, was divided by the individual weights of the other 6 to yield relative viscosities.

Optical density was used as a criterion for degree of miscibility of all of the formulations except the Teknar oil base. Samples (10 g) of each formulation were added to each of three glass 500 ml graduated cylinders containing 490 ml of deionized water. The exact weight of each sample was determined by placing the graduated cylinder plus water on a Mettler top loading balance and gently adding the formulation just above the surface of the water. After the addition of formulation, each cylinder was either vigorously agitated by shaking for 60 sec, minimally agitated by inverting the cylinder once and returning it to the upright position, or was not agitated. Five ml samples were pipetted from 2 cm below the surface of the water of each cylinder after 1 min and measured for optical density in a Bausch & Lomb® Spec 20 spectrophotometer set at a wavelength of 625 μ.

RESULTS

Table 1 presents mortality data on six of the flowable formulations. Probit analyses of the data generated from all of the treatment rates indicate a pronounced disparity in efficacy between the aqueous Teknar 2X and the other five formulations. Roughly half as much Tek-

Table 1. Efficacy of six commercially available and experimental liquid formulations of *Bacillus thuringiensis* (H-14) against penultimate instars of *Simulium vittatum* under field conditions at 5.0 mg of formulation/liter of stream discharge for 1 min and the calculated LC-95 for 1 min application.

Formulation	Mean % mortality ± s.e.[a]	LC-95 (mg/liter/min)[b]
Teknar 2x	96.0 ± 1.35 a	5.5 (2.75–7.00)[c]
Teknar oil base	83.6 ± 6.72 ab	10.6 (8.95–13.55)
Vectobac	80.8 ± 2.98 ab	10.7 (6.86–14.54)
Teknar wdc	76.0 ± 12.47 ab	13.1 (9.76–23.95)
Skeetal F	75.0 ± 12.17 ab	13.0 (8.35–22.44)
Bactimos	60.0 ± 6.65 b	15.9 (13.60–18.20)
Control	2.6 ± 0.6 c	—

[a] Means in the same column followed by the same letter are not significantly different at the 0.05 level.
[b] LC-95 values calculated from mortality data (corrected for control mortality) for 3–4 concentrations; 3 replicate tests/concentration.
[c] 95% fiducial limits.

nar 2X would be required to produce 95% mortality in *S. vittatum,* relative to the next most efficacious formulations. Comparison of the formulations using Duncan's New Multiple range test on mortality data generated at a discriminating dosage was more conservative in determining relative ranking. The Bactimos formulation was significantly less efficacious than Teknar 2X ($P>0.05$) but not significantly different from the others. The similarity of larvicidal activity of all of the formulations except the Teknar 2X is also displayed in Table 2. The potencies (ITU) of the formulations (determined by bioassay with *Aedes aegypti* (Linn.) larvae under laboratory conditions by the manufacturer) do not correlate well with activity against *S. vittatum* ($r^2 = 0.25$). Our results corroborate similar observations by Guillet et al. (1982a) and Molloy et al. (1984).

The results of comparative tests with diluted and undiluted *B. thuringiensis* (H-14) formulations are presented in Table 2. The greatest contrast was observed with the oil base formulation of Teknar, although *t*-tests revealed no significant difference. The oil formulation was also the most difficult to mix with water for application and to clean from application devices after use. Data on the relative viscosities of the formulations are also presented in Table 2. A wide range of viscosity was observed for the group, with the Teknar oil and Teknar dispersable being the most and least viscous, respectively. Interestingly, the Teknar 2X concentrate was one of the less viscous materials.

Good miscibility of the formulations with minimal agitation was observed for the group as a whole (Fig. 1). The Teknar dispersible and Teknar 2X formulations, however, suspended far more readily without agitation than the other four tested. The Teknar oil base was omitted from the miscibility studies because of problems associated with the extremely high optical density of the emulsifiers even though little mixing of the bulk of the formulation occurrred with minimal agitation. Optical density of the other six formulations was due to suspension of particulates as evidenced by slow to rapid settling of formulation constituents after 1 hr to 8 days with a concomitant accumulation of material at the bottom of each cylinder and a drop in optical density readings from samples taken at the top of each cylinder.

Table 2. The comparative efficacy of diluted and undiluted formulations of *Bacillus thuringiensis* (H-14) against penultimate instars of *Simulium vittatum* under natural conditions.

Formulation	Concentration mg/liter/min	Mean % mortality ± s.e.		Relative viscosity
		Diluted[a]	Undiluted	
Teknar wdc	10	91.3 ± 3.9	90.7 ± 2.1	0.42
Teknar oil base	10	94.6 ± 0.8	84.7 ± 7.9	1.00
Teknar dispersible	10	88.5 ± 3.8	96.0 ± 0.8	0.24
Teknar 2x	5	96.0 ± 1.4	90.2 ± 0.5	0.31
Bactimos	10	92.2 ± 0.8	86.2 ± 3.8	0.77
Vectobac	10	93.1 ± 5.0	94.7 ± 2.3	0.36
Skeetal F	10	94.2 ± 4.5	91.1 ± 2.7	0.32
Control	0	2.4 ± 0.6	—	—

[a] Mixed with water to produce a total of 7.2 liters.

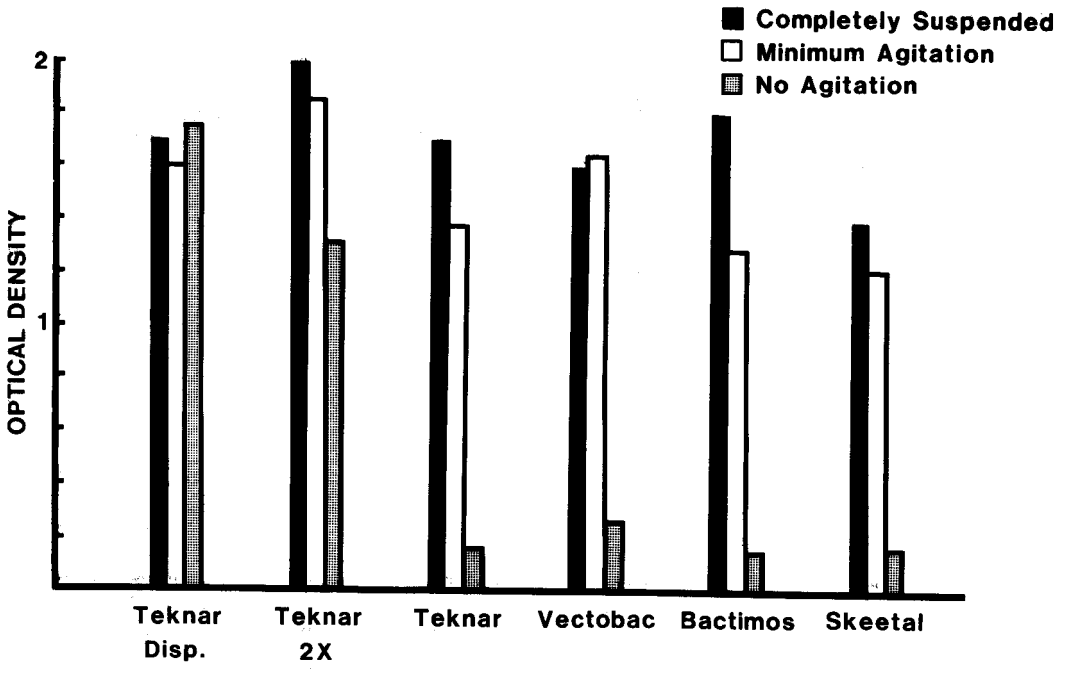

Fig. 1. Relative miscibility of six formulations of *Bacillus thuringiensis* (H-14) determined with a spectrophotometer.

DISCUSSION

Formulation may affect the activity of *B. thuringiensis* (H-14) against black flies by direct effect of the formulation process on particle size, the effect of diluents on the stability and suspendibility of the toxic moiety or indirectly by the effect of formulation constituents on normal black fly feeding (Molloy et al. 1981). The data indicate that the level of field efficacy of *B. thuringiensis* (H-14) against black flies does not parallel laboratory derived ITU against *Ae. aegypti*. Formulation clearly has more effect on the activity of each of the products against black flies than does the level of ITU alone.

The formulations evaluated in this study represent advancements in efficacy and/or handling relative to their precursors of just three years ago. The earlier flowable concentrates that were made with primary powders performed less well than their wettable powder analogues (Guillet and Escaffre 1979, Molloy et al. 1981, Lacey and Undeen, unpublished data). Teknar wdc, the one formulation made with wet fermentation residue at that time, was selected for use in the Onchocerciasis Control Programme (OCP) in West Africa because it represented a compromise between efficacy and physical characteristics that enabled operational aerial application (Guillet et al. 1982a, 1982b). Application of undiluted Teknar wdc, however, rendered the material ineffective against *S. damnosum* s.l. even at higher dosages (Guillet et al. 1982b). However, Teknar wdc and the other formulations investigated in this study all appear to be reasonably efficacious when applied without prior dilution and their larvicidal activities are on par with or better than those reported for prediluted Teknar wdc produced between 1981 and 1982 (reported by Lacey and Undeen 1984). Good miscibility with minimal agitation provides an indication that the formulations should remain evenly suspended in the lotic environment during the effective exposure period.

The formulation that represents the greatest improvement in terms of both efficacy and the physical characteristics that would permit undiluted aerial application is the Teknar 2X aqueous concentrate. Despite the concentration of active ingredients, its viscosity and miscibility are comparable to or more desirable than those observed for Teknar wdc.

Ostensibly, the Teknar oil base was formulated to maintain as much active moiety as possible near the surface of the water and thus provide better carry. Regardless of the high ITU rating of the oil base formulation, it did not perform as well as the Teknar 2X aqueous concentrate. Considering the decreased ease of handling, high viscosity and lack of increased efficacy, despite the doubling of ITU in this

formulation, it is unlikely this will provide a viable alternative to Teknar wdc for black fly control.

Continued improvement in *B. thuringiensis* (H-14) formulations is warranted if they are to be competitive with conventional chemical larvicides. Further concentration of the active moiety through improved production of toxin and more efficient refinement procedures will complement the advances already made in formulation.

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