SIMULATED FIELD STUDIES WITH FOUR FORMULATIONS OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AGAINST MOSQUITOES: RESIDUAL ACTIVITY AND EFFECT OF SOIL CONSTITUENTS

FRANK W. VAN ESSEN[1] AND STEPHEN C. HEMBREE[2, 3]

US Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD 21701

ABSTRACT. A study was undertaken to determine the effects of suspended and dissolved soil constituents on the residual larvicidal effectiveness of *Bacillus thuringiensis* var. *israelensis* in large outdoor artificial containers. Mortality decreased progressively with time although one formulation was still effective 4 days posttreatment. The presence of soil constituents was associated with a lowering of larval mortality. In the absence of soil constituents, stirring the medium tended to enhance the duration of effective control, while in the presence of soil constituents stirring tended to reduce the duration of effective control. Settling patterns of the *Bti* formulations were also affected by the presence of soil constituents. *Bti* tended to settle out faster in those troughs containing soil, probably because of adsorption to the soil particles.

INTRODUCTION

Since the discovery of *Bacillus thuringiensis* var. *israelensis* (*Bti*) in Israel in 1977, (Goldberg and Margalit 1977) numerous studies have been conducted with this bacterial agent against various species of mosquitoes and blackflies in both the laboratory and field (De Barjac and Coz 1978, Frommer et al. 1980, Garcia and Desrochers 1979, Hembree et al. 1980, Undeen and Berl 1979, Undeen and Nagel 1978, Van Essen and Hembree 1980). As would be expected, results varied with the test habitat. One factor which may influence the results is the amount of dissolved solids present in the water. Since this could affect the amount of material needed for effective control, a study was undertaken to determine the effect of soil constituents on the mortality of *Aedes aegypti* (Linn.) larvae treated with 4 formulations of *Bti* under simulated field conditions. The experimental design also allowed for studies relating to the persistence of the materials over an extended period of time during which additional larvae could be added daily to plots that were treated only once. Because differences in residual activity among the materials tested were noted, an additional study was undertaken to determine the settling rates of those materials under the same conditions.

MATERIALS AND METHODS

All experiments for this study were carried out at Fort Detrick, Frederick, MD, during the summer of 1980. Tests were conducted outside in large galvanized cattle watering troughs (1.2 × 0.61 by 0.61 m deep) holding approximately 330 liters of water. During testing, laboratory-reared third instar *Ae. aegypti* larvae (72 hr old) were held in cylindrical containers made from PVC pipe (28 cm long × 10 cm diam) fitted with screened bottoms and 6 (2.5 cm diam) screened openings to allow for water circulation (Fig. 1).

[1] Present address: 5014 N.W. 41 Street, Gainesville, FL 32601.

[2] Present address: Department of Immunology, Walter Reed Army Institute of Research, Washington, DC 20012.

[3] Opinions, assertions, and product names contained herein are the private views of the authors and are not to be construed as official or as reflecting the views or endorsements of the Department of the Army or the Department of Defense.

Fig. 1. Simulated field trial system showing several 330 liter capacity troughs with cylindrical holding containers in place.

Four formulations of *Bti* were studied: 2 powder formulations supplied by Abbott Laboratories (Lot #6406-125, 400-600 ITU, and #6478-194, 1000 ITU); a liquid formulation provided by Sandoz, Inc. (Lot #402 WDC, PH-6, 1500 ITU), and a powder formulation donated by Biochem Products (Bactimos® LRB 676, 3500 ITU). Based on previous laboratory data, all 4 materials were tested at a rate of 0.9 ppm.

To ascertain the effect of soil constituents on the ability of *Bti* to control mosquitoes, each formulation to be tested was subjected to 5 treatment regimes depending on the presence or absence of soil and on the stirring schedule. All troughs were filled with 320 liters of tap water followed by 6 liters of clear pond water. For each formulation, 1 liter of soil was added to 3 troughs while no soil was added to the remaining 2 troughs. Two additional troughs served as controls. One received 1 liter of soil and the other none; both received pond water. All troughs were then stirred thoroughly and allowed to sit 4 days to simulate field conditions. All were completely exposed to environmental conditions. At the end of the 4-day period, the *Bti* was added to the 20 treatment troughs. Those troughs which received no added soil constituents were either stirred at treatment or stirred at treatment then daily thereafter. Those troughs which did receive soil were subjected to one of the following 3 regimens: 1) stirred initially only (when soil was added), 2) stirred initially and at treatment (when *Bti* was added), 3) stirred initially, at treatment then daily thereafter.

Three test cylinders (PVC pipe) containing 20 larvae were placed in all tanks each day of each experiment until effective control was no longer obtained. Mortality was recorded at 24 and 48 hr intervals and averaged for the 3 replicates run each day for each treatment. The entire experiment was then repeated twice and all data averaged.

A second experiment was undertaken

to determine the settling rates of the materials tested. Ten troughs were utilized: 2 for controls and 2 for each of the 4 *Bti* formulations to be tested. Soil, which had been autoclaved at 120° C for 30 min, was added to 1 of the controls and to 1 of each set of 2 troughs to be used for each formulation of *Bti*. The remaining troughs received no soil. *Bti*, at the rate of 0.9 ppm, was then added to each trough and all were stirred thoroughly.

Water samples were taken the first day at 2 hrs posttreatment and at 24 hr intervals thereafter. Troughs were sampled at 3 levels in the water column, designated upper, middle and lower. Each layer was sampled at 3 locations within each tub (10 ml each). The 3 samples from each layer were pooled and then stored at 4° C until processed.

In the laboratory, samples collected from the outdoor troughs were first heat shocked for 30 min at 60° C to eliminate bacterial vegetative stages just prior to being plated. Three 1 ml aliquots were taken from each 30 ml sample. Each aliquot was either serially diluted or poured directly into petri dishes to which 15 mls of tryptose blood agar base (Difco Laboratories) would be added immediately thereafter. Dilutions were prepared such that 30 to 300 colonies would be produced per agar plate in 24 hrs. Each collected sample, therefore, was replicated 3 times on agar plates to determine the relative number of *Bti* spores present in each layer of the troughs at 24 hr intervals. Counts for the 3 replicates of each sample were averaged and plotted against time to produce a distribution pattern of the settling of the various *Bti* materials as affected by the presence or absence of soil constituents.

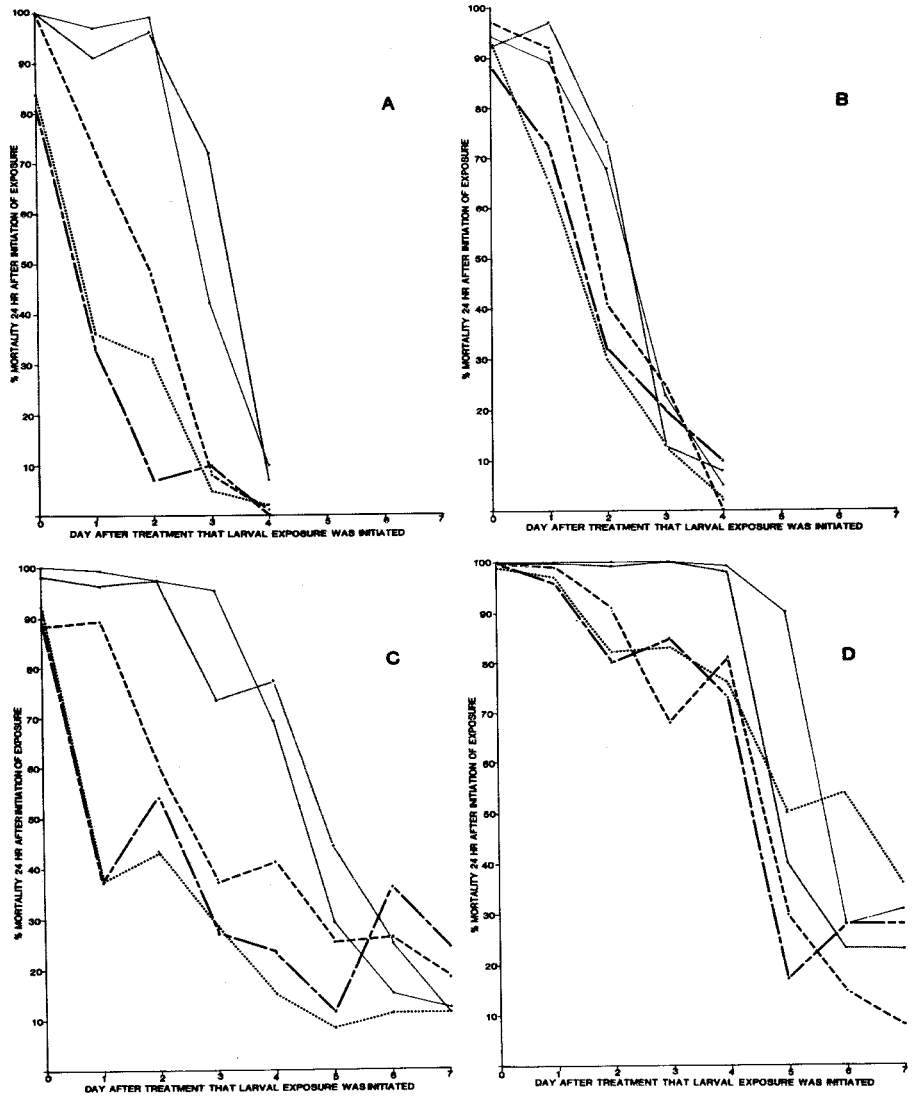
RESULTS AND DISCUSSION

The results of the first experiment are summarized in Fig. 2. As expected, the rate of mortality decreased progressively with time regardless of the material tested or the treatment employed. For example, the Abbott 125 material killed 100% of the larvae added on day 0 after 24 hr of exposure but only 10% of the larvae added on day 4 after 24 hr of exposure.

A second general conclusion is that the presence of soil constituents affected larval mortality. In most cases, percent mortality in troughs that contained no soil constituents was consistently higher than the same treatment with soil constituents added, especially in the early treatment days. This can be seen clearly in Fig. 2, especially 2A. On any given day, those treatments without soil constituents generally had higher mortality rates than ones which contained soil. Residual activity is also greater with treatments without soil as can be seen by a generally longer duration of larval mortality. Ramoska and Rodriquez, (personal communication 1981), in laboratory tests, noted that the presence of clay, soil and sand lowered larval mortality. The more material that was added the more detrimental was the effect. Ignoffo et al. (1981) reported similar results in laboratory experiments using pond water sediments.

In Fig. 2, if one draws a line at the 80% control level, the duration of control (in days) for the various treatment regimes can be compared. Data for the 4 formulations studied can then be averaged to produce the following results: a) soil constituents not present, medium stirred at treatment and daily thereafter, 3.125 days of control; b) soil constituents not present, medium stirred at treatment only, 2.85 days of control; c) soil constituents present, medium stirred initially, at treatment and daily thereafter, 1.1 days of control; d) soil constituents present, medium stirred initially and at treatment,

Fig. 2. Effects of presence or absence of soil constituents and of different schedules of stirring on the larvicidal potential of 4 formulations of *Bti* for third stage *Aedes aegypti* larvae in large artificial containers. Mortality determined after 24 hr exposure. A - Abbott 125; B - Sandoz WDC; C - Abbott 194; D - Biochem.


A
B
C
D
% MORTALITY 24 HR AFTER INITIATION OF EXPOSURE
DAY AFTER TREATMENT THAT LARVAL EXPOSURE WAS INITIATED
SOIL CONSTITUENTS NOT PRESENT. MEDIUM STIRRED AT TREATMENT & DAILY THEREAFTER.
SOIL CONSTITUENTS NOT PRESENT. MEDIUM STIRRED AT TREATMENT ONLY.
SOIL CONSTITUENTS PRESENT. MEDIUM STIRRED INITIALLY ONLY.
SOIL CONSTITUENTS PRESENT. MEDIUM STIRRED INITIALLY & AT TREATMENT.
SOIL CONSTITUENTS PRESENT. MEDIUM STIRRED INITIALLY, AT TREATMENT& DAILY THEREAFTER.

1.0 days of control; e) soil constituents present, medium stirred initially only, 1.8 days of control. In the absence of soil constituents, stirring the treated medium tended to enhance the duration of effective larvicidal potential of *Bti,* perhaps by countering settling. In the presence of dissolved and suspended soil constituents, stirring at treatment and stirring daily tended to reduce the duration of effective larvicidal control.

It should be emphasized that the intent of the study was not to compare formulations directly but to determine if similar trends in residual activity and effect of soil constituents occurred with several available materials. It is evident, however, that residual control in excess of 4 days was produced by one of the materials under several different treatment regimes. *Bti* is generally believed not to recycle in nature and its residual activity is probably related to the rate of settling of the particular formulation being used.

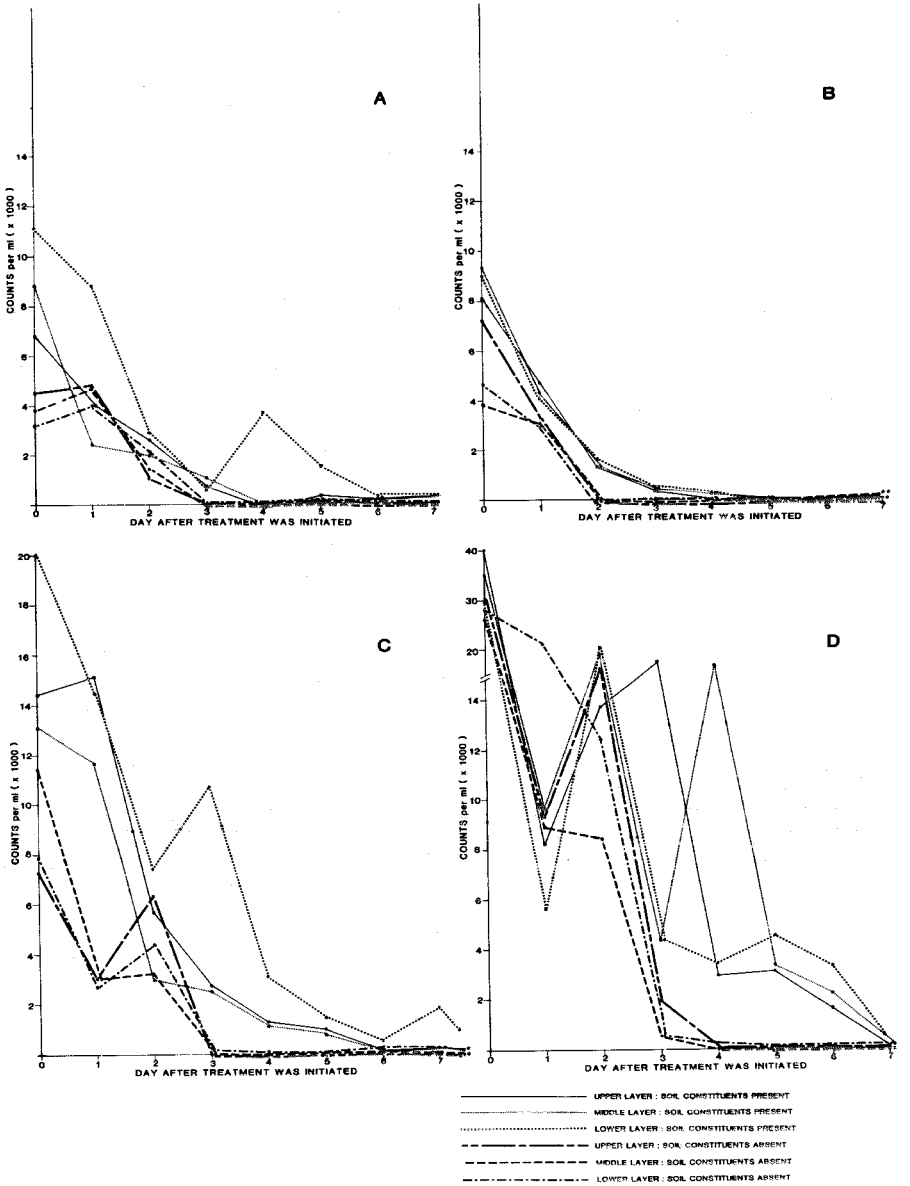
The results of the second experiment are presented graphically in Fig. 3 where plate counts are plotted against time. Settling patterns are the important thing to look for and not exact count comparisons. As expected, spore counts diminished with time. Although fluctuations did occur, in general the trend was toward lower activity as time went on, regardless of the material tested or treatment employed.

Another trend can be seen by comparing the number of counts at one level with that of another level on a given day with one material. The Abbott 125 formulation illustrates one point. Looking at the treatment that contained soil, it can be seen that the highest number of counts occurred in the lower level on day zero after only 2 hr settling time. Whereas, without soil present, the highest number of counts occurred in the upper layer on day 0 and day 1. It appears, therefore, that the presence of soil constituents is associated with an increased settling rate of *Bti*. The same is true with the Abbott 194 material although the difference is not as great. Again the highest number of counts occurred in the lowest level on day 0 with soil present. The settling was delayed somewhat in the treatment without soil, as the highest counts were in the middle level on day 0. The Sandoz material was primarily in the upper layer without soil present but in the middle layer with soil present (day 0).

In the laboratory tests, Ignoffo et al. (1981) reported that while significant settling of *Bti* suspension occurred after only 1 hr, high mortality was still obtained after 24 hrs exposure. In the present study, a significant amount of one material was still in suspension in the upper layer of the troughs on day 3.

The mechanism by which soil constituents reduce the larvicidal effectiveness of *Bti* has not been determined. Several possibilities exist: 1) chemical deterioration of the toxin, 2) physical adherence to and sedimentation of toxin-containing sporangia with soil particles and 3) interference with feeding behavior. In this study, any chemical associated with the soil was not responsible for any larval mortality since controls with dirt produced 1.5% mortality and those without dirt 1.2% after 24 hr. At 48 hr the rate for both was identical at 3.6%. Data from the second experiment reported here lend support to the second mechanism. With soil constituents present, the larvicidal effectiveness of *Bti* was lowered. And, with soil constituents present, *Bti* settled out faster—probably because of adsorption to soil particles. In addition, the material with the longest residual activity also had the slowest settling rate. Ramoska and Rodriquez (personal communication 1981) reported that smaller clay particles produced a more deleteri-

Fig. 3. Settling patterns of 4 formulations of *Bti* in the presence or absence of soil constituents. A - Abbott 125; B - Sandoz WDC; C - Abbott 194; D - Biochem.


A
B
C
D
COUNTS per ml (x 1000)
DAY AFTER TREATMENT WAS INITIATED
UPPER LAYER : SOIL CONSTITUENTS PRESENT
MIDDLE LAYER : SOIL CONSTITUENTS PRESENT
LOWER LAYER : SOIL CONSTITUENTS PRESENT
UPPER LAYER : SOIL CONSTITUENTS ABSENT
MIDDLE LAYER : SOIL CONSTITUENTS ABSENT
LOWER LAYER : SOIL CONSTITUENTS ABSENT

ous effect on the larvicidal ability of *Bti* than larger soil particles presumably due to more surface area at a given concentration and thus greater opportunity for adsorption to take place.

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