A SIMPLE TECHNIQUE FOR DETERMINING RELATIVE TOXICITIES OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* FORMULATIONS AGAINST LARVAL BLACKFLIES (DIPTERA: SIMULIIDAE)

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ABSTRACT. A laboratory system for assaying the potency of *Bacillus thuringiensis* var. *israelensis* formulations against larval blackflies was developed. An orbital shaker was used to create a water current in 250-ml Erlenmeyer flasks containing the test larvae. This system produced dose-mortality relationships with acceptable statistical parameters.

Clemson University has been involved in field evaluations of various *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) formulations (Vectobac®) against larval blackflies for Abbott Laboratories (North Chicago, IL) since 1986. In-stream evaluation gives an accurate representation of 2 critical characteristics of a particular formulation, namely potency and carry. In these field trials, formulated material is suspended in approximately 13 liters of stream water and administered across the stream treatment site over 1 min. After 2–3 h, stream substrate (submerged leaves, twigs, trailing vegetation, plastic streamers) with 150 attached larvae is collected and placed in 2-liter plastic containers filled with ca. 1.75 liters of stream water (enough to cover the substrate) at approximately 100-m intervals downstream. The containers are transported in an insulated cooler on ice from the stream to the laboratory. Once in the laboratory, the containers are aerated with aquarium pumps and stones for 24–48 h. Typically, mortality is determined after 24 h; however, 48-h mortality determinations may be made should such data be desired on any given formulation.

Field evaluation of *B.t.i.* formulations does have several disadvantages. Replication of formulations in streams is difficult when large numbers of samples are to be evaluated. Further, the number of suitable streams within a reasonable travelling distance is limited in most areas. Also, stream conditions can vary significantly from week to week and often from day to day. Stream blackfly larval fauna also changes seasonally. Approximately 9 h of labor are required for a thorough field evaluation of each *B.t.i.* formulation. This figure includes the time required for stream treatment, travel, collection and mortality determinations in the laboratory.

In an effort to maximize our evaluation efficiency by applying only the best formulations in the field and replicating these formulations over time, we began, in conjunction with Abbott Laboratories, to search for methods whereby we could evaluate formulations in the laboratory. The primary objective of these evaluations would be simply to relate potencies of various experimental formulations with a known standard.

Any laboratory system for evaluating *B.t.i.* formulations against blackflies must create a water current sufficient for stimulation of normal feeding behavior. Several methods of creating a water current in the laboratory have been proposed and evaluated. Colbo and Thompson (1978) used a magnetic stirring bar to create a current for rearing *Simulium verecundum* Stone and Jamnback. Undeen and Berl (1979) used a portable version of this system in assaying *B.t.i.* against *S. damnosum* Theobald larvae. Lacey and Mulla (1977) used a stream of air bubbles while a trough system was utilized by Gaugler et al. (1980). The system of Hembree et al. (1980) created water current in 16-oz test containers by plastic bottles attached to a rotating shaft. Each of these systems was effective but was complex and required a good deal of maintenance. In searching for a simpler system, an orbital shaker was chosen as a means of creating a water current in a laboratory setting that would stimulate larval feeding.

Blackfly larvae were exposed to the various *B.t.i.* formulations in 250-ml Erlenmeyer flasks positioned on an orbital shaker (G10 Gyratory Shaker, New Brunswick Scientific, Edison, NJ). Larvae were collected the morning of the trial from a stream near the Clemson University campus. The larval fauna used in the trials listed in Table 1 were identified as *Simulium tuberosum* (Lundstrom) cytospecies A (76.4%), *S. notiale* Stone and Snoddy (13.2%), *S. verecundum* cytospecies AA-AC (9.4%) and *S. aureum* Fries cytospecies A (1.0%). Larval collection procedures were as described previously. Once in the laboratory, the containers were aerated by

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Table 1. Toxicity of *Bacillus thuringiensis* var. *israelensis* formulations against larval blackflies using an orbital shaker.

Formulation (lot no.)	Date	LC_{50} (ppm)	95% FL	LC_{90} (ppm)	95% FL	Chi-square	DF	$P >$ chi-square	Slope
26-261-BD	July 17	0.068	0.050–0.089	0.366	0.243–0.707	5.00	4	0.29	1.748
26-261-BD	July 18	0.094	0.078–0.113	0.315	0.241–0.465	4.80	3	0.19	2.450
26-261-BD	July 20	0.096	0.077–0.117	0.513	0.367–0.847	4.67	3	0.20	1.756
26-261-BD	Sep. 13	0.094	0.074–0.117	0.588	0.404–1.041	0.32	3	0.96	1.611
12-158-BA	Sep. 12	0.475	0.393–0.587	2.009	1.427–3.330	0.65	3	0.89	2.046
29-679-BD	Aug. 18	0.327	0.261–0.413	1.924	1.272–3.679	2.66	3	0.45	1.666
31-110-BR	Sep. 7	1.221	1.044–1.425	3.620	2.906–4.875	1.60	3	0.66	2.715

aquarium pumps until the larvae were seeded into the exposure flasks.

Blackfly larvae were exposed to *B.t.i.* formulations in 250-ml flasks containing a total volume of 150 ml distilled water. The control (no *B.t.i.*) flask was initially filled with 150 ml of water while the treatment (with *B.t.i.*) flasks were initially filled with 149 ml of distilled water. Containers brought from the field were emptied into white enamel pans from which 15 late-instar larvae were taken with flexible forceps and placed in each exposure flask.

After all flasks had been seeded with larvae, the *B.t.i.* suspension was added. Serial dilutions of the *B.t.i.* formulations were prepared so that 1 ml of the appropriate dilution placed in a flask with 149 ml of water resulted in the desired ppm concentration in that flask. One milliliter of each concentration was placed in each of 4 seeded flasks, yielding a total of 60 larvae per concentration. After treatment, the flasks were spun on an orbital shaker at 160 rpm for 5 h. The flasks then were removed from the shaker, and the contents emptied into a white enamel pan to determine mortality. Larvae were declared dead when no physical response was detected when probed. Larvae that had pupated or that were beginning to pupate (prepupae) were disregarded. Approximately 3.5 h of labor were required to evaluate one formulation using this technique.

The statistical parameters of 4 Vectobac formulations evaluated in the laboratory using an orbital shaker [as determined by a probit analysis of the mortality data (SAS Institute 1985)] are found in Table 1. Mortality in the control flasks consistently remained below 5%. No significant differences in LC_{50}s and LC_{90}s (overlap of the 95% fiducial limits) and slopes (t-test, Steel and Torrie 1980, $\alpha = 0.05$) for evaluations of lot 26-261-BD (standard wettable powder) on 4 dates indicated an acceptable level of precision for the orbital shaker technique. The data in Table 1 demonstrated that lot 26-261-BD was significantly more toxic than 12-158-BA (1988 aqueous standard), 29-679-BD (1989 aqueous standard) and 31-110-BR (an early *B.t.i.* fermentation). Lot 31-110-BR was the least toxic formulation while 12-158-BA and 29-679-BD had essentially equal toxicities.

The information in Table 1 indicates that the use of an orbital shaker can provide statistically valid dose-mortality relationships. The ideal for any bioassay system is to predict field efficacy of a particular compound based on laboratory results. With the complexity of the larval blackfly environment, this has proven difficult. The orbital shaker system just proposed cannot be directly correlated with field results because stream water differs greatly from distilled, and larvae in this system are exposed to the *B.t.i.* for 5 h. Exposure time in the stream would be much less. Water properties and exposure time could be altered to better approximate stream conditions. Preliminary data suggest that a 30-min exposure to *B.t.i.* does not significantly alter the results. However, the present protocol for this orbital shaker system provides a simple and efficient way of quickly determining relative potencies of *B.t.i.* formulations against larval black-flies.

Dispersion and carry of *B.t.i.* formulations presently can only be determined by stream trials. Use of the orbital shaker technique to eliminate less potent formulations would greatly reduce the number of formulations applied in the field and save time, money and other resources (i.e., stream populations of simuliids).

We thank Peter Adler, Clemson University, for cytotaxonomic identification of the blackfly larvae, and Elmer Gray, Sam Hudson and Doug Bidlack for physical assistance with this study. This manuscript is Technical Contribution No. 3058 of the South Carolina Agricultural Experiment Station, Clemson University, Clemson, SC.

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