A SYSTEM FOR RECOMMENDING DOSAGE OF *BACILLUS THURINGIENSIS* (H-14) FOR CONTROL OF SIMULIID LARVAE IN SMALL STREAMS BASED UPON STREAM WIDTH[1]

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ABSTRACT. All available data on the treatment of simuliid larvae with *Bacillus thuringiensis* var. *israelensis* (H-14) in small streams were evaluated for relationships between stream width, discharge and carry. The observed correlations suggest that width is equal to discharge as a predictor of carry. Application rates based upon width have the advantage in small streams of being self-correcting for variability in stream morphology and would not require time-consuming calculations.

INTRODUCTION

Bacillus thuringiensis var. *israelensis* (serotype H-14) is currently available as a safe, effective alternative to chemical insecticides for the control of larval simuliids (Gaugler and Finney 1982). Dosage recommendations for its use against mosquito larvae are given in terms of formulation weight per unit area, whereas black fly dosages are expressed in terms of the formulation weight per unit volume of stream water and the length of time over which the dosage should be applied to the stream. Applications, therefore, currently require calculation of stream discharge, including measurements of width, depth and velocity.

Measurement of discharge is time consuming and requires trained personnel and, for maximum accuracy, expensive instrumentation (Amrine 1983). For the most accurate discharge measurements used in black fly larvicide experimental trials, velocity and depth measurements are taken at measured intervals across the stream and the total discharge is obtained from the sum of the interval discharge measurements. This method requires the use of a stream velocity meter. A quicker but less accurate method uses a floating object, timed over a measured segment of the stream, to obtain the velocity. In small streams, either of these methods is difficult to apply.

Jamnback and Collins (1955) recommended DDT dosages based only upon depth and width measurements over a wide range of stream sizes. A similar approach could be applied to *B. thuringiensis* (H-14) applications. Considering its nontoxicity to humans and its limited potential for environmental damage, the consequences of overdosage would affect only the cost of treatment. Treatments based upon stream width, measured at the actual black fly breeding sites, might therefore be a practical alternative to the more complex procedure of discharge calculation. This would be analogous to the treatment of larval mosquitoes on a surface area basis.

This paper reports the relationships between stream width, discharge and carry based upon all available field test data. Based upon this work and results of recent field dosage experiments using a commercial liquid formulation of *B. thuringiensis* (H-14), a relatively simple system of dosage determination for small streams was developed.

METHODS AND MATERIALS

DOSAGE-MORTALITY TESTS. This work was conducted during two visits, one between June 7–10, 1983, and the other from June 28-July 1, 1983. One hundred meter segments of 4 streams at the Holston Army Ammunition Plant in Kingsport, Tennessee, were treated for 1 min by the method of Undeen and Colbo (1980) with various concentrations of the Teknar® formulation of *B. thuringiensis* (H-14). Working upstream in successive 100 m increments, it was possible to make 15 separate treatments, at dosages between 1 and 20 mg/liter, against previously unexposed larvae of *Simulium vittatum* Zetterstedt. One hour after each application of inoculum the larvae were collected 100 m below the treatment point and transported to the laboratory within 10-15 min. Twenty-five penultimate instar larvae from each site were then placed in each of 3 cups containing 355 ml of untreated aerated stream water. Untreated control larvae were set up in an identical manner each day that tests were conducted. Mortality was assessed 24 hr later. Details of this method are available in Lacey and Undeen (1984). Three replicate tests for

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[1] This paper represents the results of research only. Mention of a pesticide, commercial or proprietary product in this paper does not constitute a recommendation or an endorsement of this product by the U.S. Department of Agriculture.

each concentration were run in 3 different streams with the following discharge rates: 16–19 m^3/min, 20–21 m^3/min, and 38–40 m^3/min. Water temperature was between 18.6 and 25°C (mean 22°C).

Stream velocity was measured with a March-McBirney Model 201 portable water current meter. Discharge was calculated by measuring stream velocity and depth every 0.3 m across each stream. The widths of the 4 streams at the point where discharge was measured were 2.7, 3.2, 3.2 and 4.7 m. Further description of the streams and materials used are given by Lacey and Undeen (1984).

Stream width correlations. Data on discharge, stream width and carry from all available sources, published and unpublished (Table 1), were used to establish relationships between stream width, discharge and carry. Studies in which stream widths were not given or from which carry could not be deduced were excluded from the analysis. Carry is defined, for the purpose of this study, as the farthest point downstream at which 80% mortality was achieved. The 80% point was chosen because usually when mortality falls to this level it continues to drop rapidly thereafter. This point was sometimes estimated by interpolation from the available data. Regression analysis of these data was conducted after logarithmic (base 10) transformation of carry, discharge and width. All analyses were conducted using Statistical Analysis System (version 79.6) from SAS Institute, Cary, NC, at the Northeast Regional Data Center, University of Florida. The analyses were performed on all available data regardless of treatment dosage or treatment time and, separately, on data from tests which uniformly used a 1-min treatment duration, also at a variety of dosages. Sample tables of formulation weight were constructed based upon stream widths and a calculated dosage of 20 mg/liter (20 g/m^3) in the stream.

RESULTS

The 1-min concentrations required to kill 50% (LC_{50}) and 90% (LC_{90}) of the penultimate instar larvae of *S. vittatum* 100 m downstream in the Tennessee streams were 2.7 mg/liter (1.95, 3.4) and 11.47 mg/liter (9.03, 15.92), respectively (Fig. 1).

Using all the field test data available (Table 1), the correlation between carry and discharge is poor (Fig. 2, $r = 0.55$). A better correlation exists between carry and width (Fig. 3, $r = 0.73$). There is good correlation between discharge and width (Fig. 4, $r = 0.83$). When only the results from the 1-min treatments are considered, even though dosage was not constant,

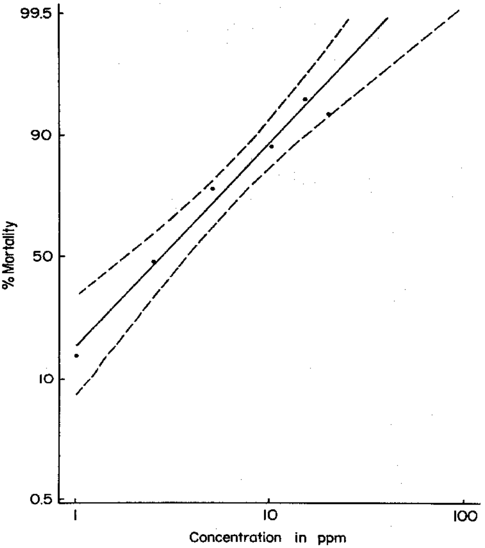

Fig. 1. *Simulium vittatum* mortality in streams treated with *Bacillus thuringiensis* (H-14) at an average 100 m below the treatment point. Probit (percent mortality) = 4.12 + 0.884 (log dose).

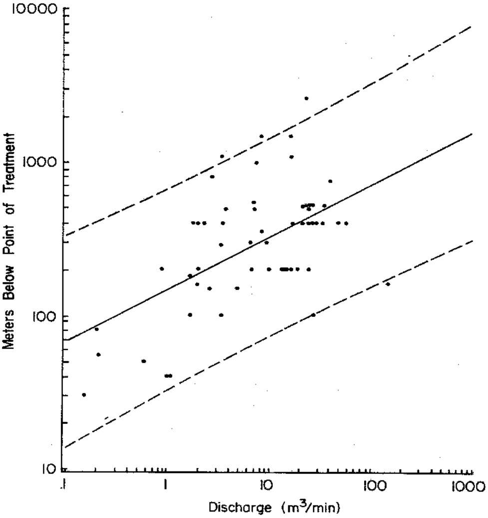

Fig. 2. Correlation between estimated discharge in all streams treated with *Bacillus thuringiensis* (H-14) and estimated carry (distance through which a minimum of 80% mortality was observed). Log carry = 2.17 + 0.34 (log discharge), $r = 0.55$.

Table 1. Sources of data used to evaluate the relationships between width, discharge and carry.

Location	Species	Number of treatments	Dose rate range (mg/liter)	Treatment times (min)	Author
Guatemala	*Simulium ochraceum* *Simulium metallicum*	2	0.2–5.0	1.0, 10.0	Undeen et al. 1981
Mexico	*Simulium ochraceum* *Simulium metallicum* *Simulium callidum*	2	7.5–45.0	0.5, 1.0	Gaugler et al. 1983
Canada (Newfoundland)	*Prosimulium mixtum* *Cnephia ornithophilia* *Simulium venustum* *Simulium verecundum*	4	$0.77–2.2 \times 10^5$ spores/ml	1.0	Undeen and Colbo 1980
	Simulium venustum *Simulium verecundum*	2	10.0	1.0	Finney (pers. commun.)[1]
	Prosimulium mixtum *Simulium venustum* *Simulium verecundum*	8	10.0	1.0	Colbo (pers. commun.)[1]
New Zealand	*Australosimulium laticorne* *Australosimulium multicorne*	3	0.2–10.0	15.0	Chillcott, Pillai and Kalmakoff 1982
USA					
Idaho	*Simulium vittatum*	4	1.0–3.0	15.0	Stoltz (pers. commun.)[2]
New York	*Prosimulium* spp. *Simulium* spp.	1	0.5	15.0	Molloy and Jamnback 1981
	Simulium tuberosum	8	0.5–5.0	15.0	Molloy (pers. commun.)[3]
South Carolina	*Prosimulium mixtum* *Simulium* spp.	3	$2.5, 2.5, 1.8 \times 10^4$	5.0, 10.0, 15.0	Horosko and Noblet 1983 and pers. commun.[4]
Tennessee	*Simulium vittatum*	35	0.5–20.0	1.0, 20.0	Lacey and Undeen 1984

[1] Colbo, M. H. and J. Finney. Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1C 5S7. Personal communication, April 1982.

[2] Stoltz, R. L. Cooperative Extension Service, College of Agriculture, University of Idaho, 1330 Filer Avenue East, Twin Falls, ID 83301. Personal communication, 27 July 1982.

[3] Molloy, D. Science Service, Biological Field Station, Cambridge, NY 12816. Personal communication, 1 April 1983.

[4] Horosko, S., III, and R. Noblet. Department of Entomology and Economic Biology, Clemson University, Clemson, SC 29631. Personal communication, 27 April 1983.

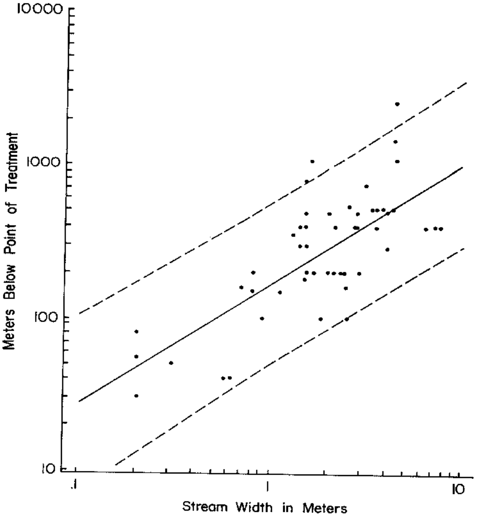

Fig. 3. Correlation between width in all streams treated with *Bacillus thuringiensis* (H-14) and estimated carry (distance through which a minimum of 80% mortality was observed). Log carry = 2.22 + 0.79 (log width), r = 0.73.

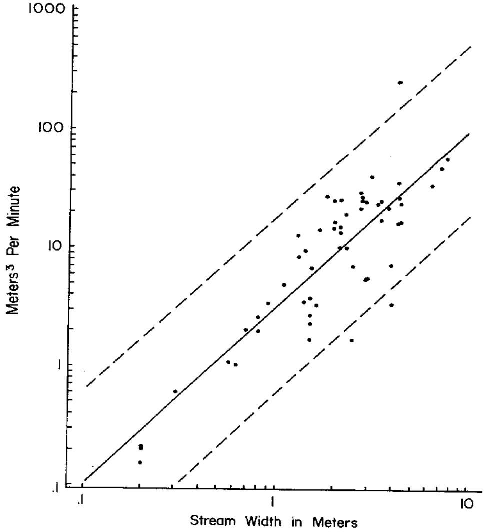

Fig. 4. Correlation between width and estimated discharge in all streams treated with *Bacillus thuringiensis* (H-14). Log discharge = 0.505 + 1.49 (log width), r = 0.83.

the correlations between carry and discharge and carry and width are much better, with carry correlating only slightly better with discharge ($r = 0.89$) than with width ($r = 0.86$) (Figs. 5 and 6). Limiting the analysis to 1 min treatment results, even though the treatments had no bearing upon these parameters, produced a better correlation between width and discharge ($r = 0.97$) than when all data were used (Fig. 7).

DISCUSSION

Dosages of *B. thuringiensis* (H-14) applied to streams must be sufficient to achieve 100% or nearly 100% mortality just below the treatment point, where the formulation is adequately mixed with the stream water and its concentration is highest. It is assumed that the particulate toxin settles out of the stream and, probably of greater significance, is filtered from the water by organisms at the water-substrate interface, and by the stream floor sediments as well. Some particles which are not lost may be slowed by temporary entrapment in vortices and upon surfaces, causing the treatment to be spread over a greater length of the stream than to which the treatment was initially applied. The concentration of toxin is also diluted in impoundments. The net result is that, although the initial dosage is high enough for 100%

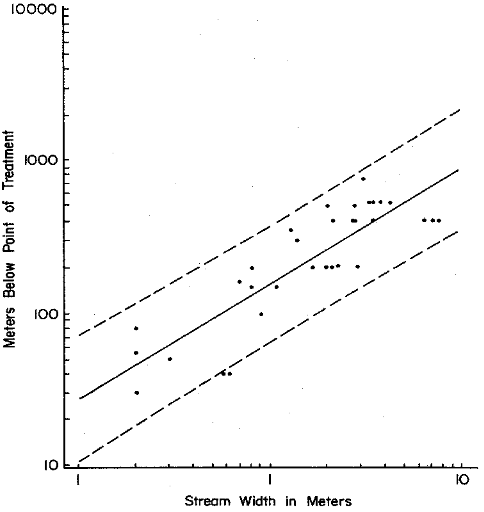

Fig. 6. Correlation between width in streams treated for 1 min with *Bacillus thuringiensis* (H-14) and estimated carry (distance through which a minimum of 80% mortality was observed). Log carry = 2.19 + 0.75 (log width), $r = 0.87$.

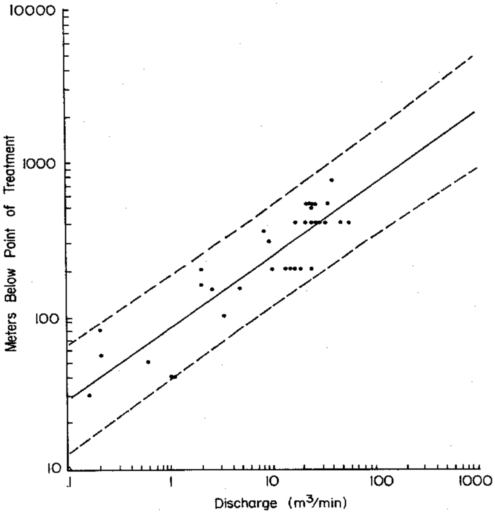

Fig. 5. Correlation between estimated discharge in streams treated for 1 min with *Bacillus thuringiensis* (H-14) and estimated carry (distance through which a minimum of 80% mortality was observed). Log carry = 1.92 + 0.465 (log discharge), $r = 0.89$.

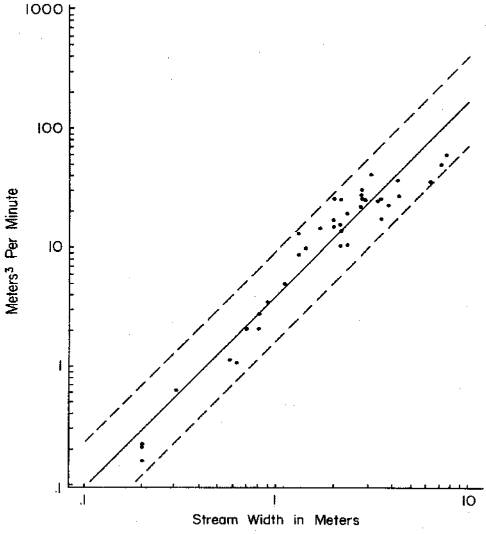

Fig. 7. Correlation between width in streams treated for 1 min with *Bacillus thuringiensis* (H-14) and estimated discharge. Log discharge = 0.58 + 1.62 (log width), $r = 0.97$.

mortality, eventually it is reduced to a level too low to achieve larval control. The distance below the treatment point at which control is no longer achieved is called "carry." Carry can be increased by increasing the initial dosage but this is costly. For example, doubling the dosage results in less than twice the carry (Lacey and Undeen 1984).

A treatment time of 1 min appears to be optimal in terms of both efficacy and efficiency of application. Using the same total amount of material over a longer period of time fails to produce either higher initial mortality or greater carry (Lacey and Undeen 1984).

The major factors regulating carry are those over which the applicator has no control. These are stream discharge and stream morphology. The latter term refers to the stream width, depth, number and size of pools, amount of vegetation, and so forth. In deep fast streams with clear channels, particulate material will be carried downstream with minimal opportunity for settling out or coming into contact with an obstruction. Under these conditions carry will be maximized. Shallow streams and streams choked with vegetation provide a greater amount of filtering capacity and reduced carry. Pools and slowly flowing areas promote dilution and settling out of particles which are more dense than water.

Over a wide range of discharges, carry is greater in the larger streams (Undeen and Lacey 1982). Discharge has also been the essential parameter used for dosage calculation in all tests of *B. thuringiensis* (H-14) against black fly larvae. The dosage used by these researchers has been one which provides 100% mortality near the treatment site without being wasteful of material. Undoubtedly some variation in carry is due to the different dosages used. Other important variables are formulation, species susceptibility and stream morphology.

For the size range of the streams reviewed in this study, the correlation between discharge and carry is not very good when all streams are considered regardless of other treatment parameters. The correlation between width and carry is better. Confining the analysis to those streams treated for 1 min, regardless of dosage, improves the correlation considerably, and the width correlates with carry almost as well as does the discharge. The improved correlation is probably as much a result of reduced variability in stream types and measurement procedures as of the reduced variability in treatments, probably because a high percentage of the 1-min treatments were done by a few workers (Table 1). These results suggest strongly that precision would not suffer if stream width were substituted for discharge as the parameter upon which treatment intervals were based in these small streams.

Wider streams can logically be expected to have higher discharge than narrower streams. Figures 4 and 7 show these relationships for the streams included in this study. The amount of a *B. thuringiensis* (H-14) formulation used has always been established by the discharge. Using these discharge/width correlations we can substitute width for discharge as the parameter to tabulate quantity of formulation to use in a stream.

Table 2 is an example of the type of dosage table which could be provided for users of *B. thuringiensis* (H-14) formulations. The amount of *B. thuringiensis* (H-14) to be applied was estimated by multiplying a hypothetical dosage of 20 mg/liter times discharge calculated from the regression analyses used in Figs. 4 and 7.

Swifter or deeper than average streams would receive lower actual dosages and slower or shallower ones would receive higher dosages using a table such as Table 2. This is not particularly a disadvantage, however. As previously stated, carry is better in the swifter, deeper streams, meaning that the insecticide maintains a tighter front as it moves down the stream and, consequently, a higher concentration over a greater length of stream. Under these conditions, a lower dosage, as long as it provides 100% mortality near the treatment site, is acceptable. In shallow or slow streams the insecticide is spread and lost over a shorter length of stream. The tabulated quantity of formulation would provide an overdosage needed to help overcome the rapid loss.

Measuring the stream width in the areas where black fly larvae are found provides a guarantee that the stream is measured where it is flowing and not at impoundments. Furthermore, the velocity range preferred by simuliid larvae, although broad and dependent to an extent upon species preferences, establishes a velocity range for the stream. Careful selection of width measurement points is, therefore, an important factor in the precision of dosage calculation.

Treatment recommendations for future formulations must be based upon their individual field dosage-response relationships. This report described the rationale for using the stream width as the sole parameter upon which to base the quantity of a *B. thuringiensis* (H-14) formulation and the treatment spacing required in practical applications against black fly larvae. The table which would finally appear on a product package should be constructed using accurately width-measured discharge data from a large number of streams which could support black fly populations. The mid-line dosage

Table 2. Estimated dosage requirements and treatment intervals* based upon widths of all streams treated with *B. thuringiensis* (H-14).

	Grams of formulation		Expected carry in meters	
Width (m)	All streams	1 min treatments	All streams	1 min treatments
0.25	8	8	56	54
0.50	23	25	97	91
0.75	42	48	133	124
1.00	64	76	167	154
1.25	89	109	199	182
1.50	117	147	230	208
1.75	147	189	260	234
2.00	180	234	289	258
2.25	214	283	317	282
2.50	250	336	345	305
2.75	289	392	372	328
3.00	329	452	398	350
3.25	370	515	424	372
3.50	413	580	450	393
3.75	458	649	475	414
4.00	504	721	500	434
4.25	552	795	524	455
4.50	601	872	549	474
4.75	651	952	573	494
5.00	703	1035	596	513

* This table was based upon an 80% mortality as the carry end-point, whereas under actual abatement conditions, >90% would be required. Therefore, treatments should be made at shorter intervals.

must be obtained from field assays of the particular formulation in the package. Carry is probably not as formulation-dependent as dosage but would still need to be derived, as a function of stream width, for each formulation. Since there are indications that some simuliid species are more susceptible to *B. thuringiensis* (H-14) than others (Undeen and Berl 1979), separate dosage estimates or correction factors might be necessary for different groups of simuliid larvae.

This report deals specifically with small streams. Large streams should still be treated according to their discharge. The cost of a dosage error could be high enough in a large stream to make the extra expense of the discharge measurement worthwhile. The distinction between large and small streams is arbitrary. A width of 5 m is suggested here because of the physical difficulty for 1 person to apply a uniform treatment to a stream larger than this.

In conclusion, the width of a small stream appears to be at least as good a criterion as its discharge in establishing quantity of *B. thuringiensis* (H-14) needed for treatment and the treatment interval, thereby limiting the need for time consuming velocity and depth measurements and dosage calculations to large streams.

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