in the vicinity of Marquette, Michigan during the summer of 1959. Ill. State Acad. Sci. Trans. 53: 46–47.
41. Wood, D. M. 1977. Notes on the identities of some common nearctic *Aedes* species. Mosquito News. 37: 71–82.
42. Zaim, M. 1978. Impact of wastewater irrigation on the population of the tree-hole breeding mosquito, *Aedes triseriatus*. Ph.D. Thesis, Mich. State Univ.
43. Zaim, M., H. D. Newson and G. D. Dennis. 1977. *Psorophora horrida* in Michigan. Mosquito News. 37:763.
44. Zavortink, T. H. 1972. Mosquito studies (Diptera: Culicidae) XXVII. The New World species formerly placed in *Aedes (Finlaya)*. Contr. Amer. Entomol. Inst. 8(3):1–206.
45. Zorka, T. J. 1975. Seasonal distribution of human feeding mosquitoes in a sewage spray irrigation complex. M. S. Thesis, Mich. State Univ.

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# EFFECT OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* ON NON-TARGET INSECTS IN STREAM TRIALS FOR CONTROL OF SIMULIIDAE

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ABSTRACT. The mortality of non-target organisms during efficacy tests of *Bacillus thuringiensis* var. *israelensis* against simuliid larvae was evaluated in a small stream in Newfoundland. Three applications of about $1 \times 10^5$ cells/ml were made during the spring and summer of 1979. Non-target insects occurring on stones with simuliids were sampled within 24 hr prior to treatment and from 3 to 7 days post treatment. The results indicated there were no significant decreases in the major groups of non-target insects that could be directly accounted for by the treatments. On the other hand the simuliids in the last 2 treatments had an overall mortality of more than 93% in the zone sampled.

## INTRODUCTION

Recently, Undeen and Nagel (1978), and Undeen and Berl (1979) showed that *Bacillus thuringiensis* var. *israelensis* de Barjac was an effective larvicide against Simuliidae in the laboratory. Preliminary laboratory trials against Hydropsychidae and *Leptophlebia cupida* indicated these were not affected by the dosage required to kill simuliid larvae. In addition previous studies with *B.t.* strains have indicated that they are relatively specific, and one would not expect a broad spectrum effect. Therefore permission was obtained from the Newfoundland Government for a field test in a section of a small stream below a lake outflow. The results of these tests on the simuliid species have been reported by Undeen and Colbo (1980), and this paper reports the findings with regard to other stream insects.

## MATERIAL AND METHODS

The application point for the bacterial suspension was 30 m below a lake outflow, at the head of a rubble riffle which constituted the treatment zone. A single 1 min application, calculated to provide a concentration of $1 \times 10^5$ bacterial spores/ml, was made using a precalibrated 4 liter sprinkling can (Undeen and Colbo 1980).

Due to the difficulties of stream sampling it was decided to use an arbitrary method and examine only the fauna associated with simuliids on stones in the stable riffle. These stones were all rough and angular. Samples were collected 24 hr before treatment and within 3–7 days after treatment with *B.t.* var. *israelensis*. Stones were collected from 10–80 m below the dose site, a stable riffle. The upper section had a high percentage of gravel but the 5 stones from here were all of greater than 300 sq cm surface area while the remaining 10 stones, in the lower section with little gravel, ranged from 500 to 1000 sq. cm. Stones selected were set into the bed with at least one surface clean and exposed to a current suitable for simuliids. Each stone was quickly removed, placed into a plastic tray and scrubbed with a brush in water. The material from each stone was poured into a plastic freezer carton and the length, width and depth of each stone recorded. Upon returning to the laboratory the water was sieved through a plankton net and residue preserved in ethanol within 5 hr of collection.

The preserved residue was examined by eye and under a dissecting microscope, removing all insects for identification. The level of identification depended on availability of information, ease of identification and familiarity of the authors with these groups. Chironomidae were numerous and several different forms were present but, because of the difficulty encountered in identification to genus using available keys, we simply divided them into two groups, the mainly predacious Tanypodinae and the remainder.

For comparative analysis the surface area of each stone was corrected to a standard size of 1000 sq cm and the number of specimens collected multiplied by the correction factor. These values were then ranked and tested by the Mann-Whitney statistic (Steel and Torrie 1960, Elliott 1971) for significance.

## RESULTS

The mean numbers in each insect group, corrected to number per 1000 sq cm, before and after the 3 treatments with *B.t.* var. *israelensis*, are shown in Table 1. Table 2 shows the Mann-Whitney statistics for the most numerous taxa present for each experiment. Only in treatment 2 and 3 were very highly significant ($P = 0.001$) differences noted, and these were both with the *Simulium venustum* complex samples which were almost eliminated in this zone as shown by Undeen and Colbo (1980). In the non-target group, treatment 2, *Hydropsyche sparna* was reduced to a level that was significant at $P = 0.05$ which we believe may have been due to pupation which was occurring during the 6-day period between samples. This is supported by the lack of any depression of numbers seen in the August treatment between samples. The other taxon was the Baetidae which showed a drop in numbers both in June and August treatments with the latter significant at $P = 0.01$ level. This, however, could be due to real effects or error as the treatment samples were processed rapidly in order to obtain results for a report and the Baetidae were primarily in the 1 mm size range and easily overlooked in the debris. However, they are important members of the stream fauna and this result will be pursued in future tests to determine the toxicity of *B.t.* var. *israelensis* to them.

## DISCUSSION

The results clearly show that *B.t.* var. *israelensis* does not cause a marked reduction in the stream fauna associated with simuliid larvae and especially those with similar method of feeding, namely, the Hydropsychidae and Philopotamidae. The only other report of the effect of *B.t.* var. *israelensis* on stream fauna was made in 1979 by Dejoux, (WHO/BC/79.721 unpublished report) who found the same results as reported here using drift from troughs placed in a river system in West Africa. He found little drift of filter feeding trichopterans, (Hydropsychidae, Philopotamidae), nor mayflies of the family Baetidae and his overall conclusion was that there was practically no effect on non-target invertebrates. There were however a few minor taxa such as Chironomini which showed an appreciable drift (38%) and these taxa should be further evaluated.

When comparing these results with

Table 1. The numbers of various insects recovered from stones collected before and after treatments of a riffle with *B.t. israelensis* at $1 \times 10^5$ cells/ml. The value given is the mean number per 1000 sq cm of stone surface derived from 15 stones collected along the riffle. Treatment occurred within 24 hours after each first date.

| Insects | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | |
| Simuliidae | April 16 | April 23 | June 5 | June 11 | Aug 6 | Aug 10 |
| *Cnephia ornithophilia* | 141.6 | 94.7 | — | — | — | — |
| *Stegopterna mutata* | 7.3 | 12.7 | — | — | — | — |
| *Prosimulium mixtum* | 30.4 | 24.1 | — | — | — | — |
| *Simulium vittatum* | 0.1 | 2.4 | 1.3 | 1.9 | 1.3 | 0.5 |
| *S. venustum* complex | — | — | 148.9 | 11.1 | 64.3 | 0.8(15.6)* |
| *S. tuberosum* complex | — | — | — | — | 0.9 | — |
| Other Diptera | | | | | | |
| Tanypodinae | 26.8 | 20.6 | 28.6 | 19.7 | 14.5 | 26.3 |
| Other Chironomidae | 48.0 | 63.4 | 209.1 | 279.3 | 359.6 | 372.7 |
| Ceratopogonidae | 0.1 | 0.1 | 0.7 | 0.1 | — | 0.5 |
| Empididae | 0.6 | 1.5 | 0.1 | — | 0.2 | 0.7 |
| Tipulidae | — | — | — | — | 0.2 | 0.2 |
| Trichoptera | | | | | | |
| *Rhyacophila* | — | 0.1 | — | 0.1 | 0.2 | 0.2 |
| *Diplectrona modesta* | 2.5 | 2.6 | 1.7 | 0.6 | 9.9 | 3.9 |
| *Cheumatopsyche pettiti* | 3.4 | 1.0 | 2.8 | 2.0 | 11.0 | 11.7 |
| *Hydropsyche sparna* | 19.8 | 27.7 | 17.5 | 9.3 | 42.0 | 43.1 |
| *H. bretteni* | — | 0.3 | 0.4 | 1.5 | — | 0.1 |
| *Chimarra* spp. | 3.2 | 6.5 | 8.4 | 4.5 | 51.3 | 60.1 |
| *Dolophilodes* sp. | — | — | — | 1.1 | 1.9 | 1.3 |
| *Polycentropus* spp. | 0.7 | 0.3 | — | 0.2 | 0.2 | 1.1 |
| Leptocercidae | — | 0.1 | — | — | 0.2 | 0.5 |
| Limnephilidae | — | 0.3 | — | 0.6 | — | — |
| Hydrophilidae | — | 0.1 | 0.2 | 1.3 | 0.5 | 0.1 |
| Coleoptera | | | | | | |
| Elmidae | 0.1 | — | 0.1 | 0.1 | 0.5 | 0.5 |
| Plecoptera | | | | | | |
| *Isoperla* sp. | 0.7 | 0.9 | — | — | 0.8 | 0.9 |
| *Nemoura* sp. | 0.9 | 0.9 | — | — | — | — |
| *Leutra* sp. | — | — | 0.1 | 0.6 | — | — |
| Ephemeroptera | | | | | | |
| *Ephemerella (Ephmerella)* sp. | 5.7 | 8.0 | 1.7 | 2.7 | 4.1 | 4.9 |
| *E (Eurylophella)* spp. | — | — | — | — | 0.5 | 1.2 |
| *E. (Drunella)* sp. | 1.4 | 1.3 | 0.1 | 1.1 | — | — |
| *Stenonema* sp. | 0.1 | 1.3 | 0.3 | 1.1 | 1.7 | 2.1 |
| *Epeorus* sp. | 0.3 | 0.3 | — | 0.1 | — | — |
| *Paraleptophlebia* sp. | — | + | — | — | 1.5 | 3.7 |
| *Habrophlebia vibrans* | 0.1 | — | — | — | — | 0.2 |
| Baetidae | 0.5 | 1.2 | 1.3 | 0.5 | 7.5 | 1.8 |
| Odonata | | | | | | |
| *Aeschna* sp. | — | — | 0.1 | 0.1 | 0.3 | 0.7 |

* 1st and 2nd instars hatching after dosage.

those of temephos and methoxychlor (Wallace et al. 1973, Wallace and Hynes 1975, Dejoux and Elouard 1977 and Helson and West 1978, Flannagan et al. 1979) it is clear that the *B.t.* var. *israelensis* has far less effect on the associated fauna and would therefore be preferable as an agent for simuliid control projects. Indeed if further safety testing continues to support the present pattern, it should be possible to extend black fly control to areas not considered for control because the available chemicals are unacceptably toxic to many non-target organisms.

Table 2. A comparison of the before and after dosing samples using the Mann-Whitney statistic for the major groups of insects found.

| | U-value | Z-value | Significance from Z |
|---|---|---|---|
| **Field test 1 April 1979** | | | |
| *C. ornithiphilia* | 96.5 | 0.66 | NS |
| *St. mutata* | 62.5 | 2.07 | * |
| *P. mixtum* | 101.5 | 0.46 | NS |
| *S. venustum* complex | 85.0 | 1.14 | NS |
| Tanypodinae | 100.5 | 0.50 | NS |
| Other Chironomidae | 86.0 | 1.10 | NS |
| Hydropsychidae | 92.5 | 0.83 | NS |
| *H. sparna* | 81.0 | 1.31 | NS |
| *D. modesta* | 103.0 | 0.39 | NS |
| *Chimarra* sp. | 91.5 | 0.93 | NS |
| Ephemeroptera | 93.0 | 0.81 | NS |
| *E. (Ephemerella)* sp. | 91.5 | 0.87 | NS |
| *E. (Drunella)* sp. | 96.0 | 0.68 | NS |
| **Field test June 1979** | | | |
| *S. venustum* complex | 29.5 | 3.44 | *** |
| Tanypodinae | 92.0 | 0.85 | NS |
| Other Chironomidae | 73.5 | 1.62 | NS |
| Hydropsychidae | 73.0 | 1.64 | NS |
| *H. sparna* | 68 | 1.85 | * |
| *C. pettiti* | 110.0 | 0.10 | NS |
| *Chimarra* | 85.0 | 1.14 | NS |
| Ephemeroptera | 86.0 | 1.10 | NS |
| **Field test Aug. 1979** | | | |
| *S. venustum* complex | 40.0 | 4.50 | ***[3] |
| Tanypodinae | 80.0 | 1.37 | NS |
| Other Chironomidae | 95.0 | 0.73 | NS |
| Hydropsychidae | 86.5 | 1.08 | NS |
| *H. sparna* | 87.5 | 1.04 | NS |
| *D. modesta* | 85.0 | 1.22 | NS |
| *C. pettiti* | 100.0 | 0.52 | NS |
| Ephemeroptera | 111.5 | 0.04 | NS |
| Baetidae | 50.5 | 2.57 | ** |

1. U-values of ≤ 64 are significant in these samples where $n_1 = n_2 = 15$.

2. Z-values of ≥ 1.65 are significant at P = 0.05; Z ≥ 2.33 is significant at P = 0.01 and Z ≥ 3.08 is significant at P = 0.001.

3. This does not include larvae hatching after dosing, i.e., 1st and 2nd instars.

*References Cited*

Dejoux, C. and J. M. Elouard. 1977. Action de l'Abate sur les invertébratés aquatiques cinétique de décrochement a court et moyen terme. Cah. ORSTOM. Ser. Hydrobiol. 11:217–230.

Elliott, J. M. 1971. Statistical analysis of samples of benthic invertebrates. Scient. Publs. Freshwater Biol. Assoc. 25:1–144.

Flannagan, J. F., B. E. Townsend, B. G. E. de March, M. K. Friesen and S. L. Leonhard. 1979. The effects of an experimental infection of Methoxychlor on aquatic invertebrates: Accumulation, standing crop and drift. Can. Entomol. 111:73–89.

Helson, B. V. and A. S. West. 1978. Particulate formulations of Abate® and Methoxychlor as blackfly larvicides: Their selective effects on stream fauna. Can. Entomol. 110:591–602.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw Hill, New York. 481 pp.

Undeen, A. H. and D. Berl. 1979. Laboratory studies on efficacy of *Bacillus thuringiensis* var. *israelensis* de Barjac against *Simulium damnosum* (Diptera: Simuliidae) larvae. Mosquito News 39:742–745.

Undeen, A. H. and M. H. Colbo. 1980. The efficacy of *Bacillus thuringiensis* var. *israelensis* against black fly larvae (Diptera: Simuliidae) in their natural habitat. Mosquito News 40:181–184.

Undeen, A. H. and W. L. Nagel. 1978. The effect of *Bacillus thuringiensis* ONR-60A strain (Goldberg) on *Simulium* larvae in the laboratory. Mosquito News 38:524–527.

Wallace, R. R. and H. B. N. Hynes. 1975. The catastrophic drift of stream insects after treatments with Methoxychlor (1,1,1, - trichloro - 2,2 - Bis (p Methoxyphenyl) ethane). Environ. Pollut. 8:255–268.

Wallace, R. R., A. S. West, A. E. R. Downe and H. B. N. Hynes. 1973. The effects of experimental blackfly (Diptera: Simuliidae) larviciding with Abate, Dursban and Methoxychlor on stream invertebrates. Can. Entomol. 105:817–831.