THE EFFECT OF *BACILLUS THURINGIENSIS* ONR-60A STRAIN (GOLDBERG) ON *SIMULIUM* LARVAE IN THE LABORATORY

ALBERT H. UNDEEN AND WENDY L. NAGEL

Research Unit on Vector Pathology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1C 5S7

ABSTRACT. Laboratory tests conducted on lab reared black flies, *Simulium verecundum* and field collected *Cnephia ornithophilia, C. mutata, S. vittatum* and *Prosimulium mixtum* demonstrate that *Simulium* larvae are susceptible to a mosquito pathogenic strain of *Bacillus thuringiensis*. For exposure times of 30 minutes and 24 hours LC_{50}'s were between $4.4x10^2$ and $5.3x10^3$ viable cells/ml. One minute exposures of *S. verecundum* to $5x10^4$ and $1x10^5$ cells/ml resulted in 87 and 93% mortality respectively.

INTRODUCTION

Attempts to apply concepts of biological control to the Simuliidae have been frustrated by the paucity of pathogens in black flies which lend themselves to the mass cultivation necessary for field applications. In fact, although several species each of Microsporida, mermithid nematodes and fungi are known as widespread and relatively common pathogens of simuliid larvae, the total life cycles of most of them are still unknown. Laboratory transmission of the Microsporida has never been successful, and the natural mode of transmission is still only a conjecture. Life histories of Mermithidae are better understood, but because of the difficulties in rearing black fly larvae in the lab mass production *in vivo* is out of the question at this time. *In vitro* mermithid production is also still in the future. Transmission of simuliid pathogenic fungi is still not understood. As larval simuliids are filter feeders, a biocontrol agent acting after ingestion must be suspended in the appropriate size and concentration in order to be taken in with the food in an effective concentration. Overlying all these difficulties is the riverine habitat of the larval simuliids, an open system in which materials added are rapidly swept downstream.

Because of these difficulties it seemed reasonable to assay, against simuliids, the several agents in use or under study for potential use, against other insects. This approach has other adherents as well.

Lacey and Mulla (1977) tested several strains of *Bacillus thuringiensis* against *Simulium vittatum*. Although *B. thuringiensis* did kill the larvae, the dosages were too high for practical use. Similar tests at the Research Unit on Vector Pathology (RUVP) against laboratory reared *S. verecundum* yielded comparable results (unpublished data).

Recently, certain bacterial strains have been found to be effective against mosquito larvae (Ramoska et al. 1977, Goldberg & Margalit 1977). A black fly larval rearing system suitable for performing bioassay work was recently developed at RUVP (Colbo & Thompson 1978). These 2 advances were combined to produce the study reported here.

MATERIALS AND METHODS

Simulium verecundum larvae were reared in the lab from eggs collected the previous summer and stored on ice for ca. 8 months. All other species, *S. vittatum, Cnephia ornithophilia, C. mutata* and *Prosimulium mixtum* were obtained from the field. Larvae were placed, 30 per 500 ml of water, in plastic containers on the magnetic stirring apparatus of Colbo & Thompson (1978). Twenty-five mg of ground Tetra® fish food was added for nutrition. The magnetic bars were turned at 150 RPM for 24 hours then the larvae were counted, the water was changed, and 25 mg food and the bacterium was added.

The bacterium was ONR-60A/WHO 1897 a strain of *Bacillus thuringiensis* (Goldberg, pers. comm.) isolated by Dr. Leonard Goldberg from the bottom substrate of a mosquito larval pond (Goldberg & Margalit 1977). The bacterial spores for this study were grown on Tryptose blood agar base (TBA base) (Difco), scraped from the plates after 14+ days of growth at room temperature, suspended in water and refrigerated until use. Quantitation was accomplished by dilution and plating out of the aqueous suspension on TBA base plates and counting colonies after 12–18 hrs growth at 30°C. The original suspension was diluted to produce a standard solution of about 10^7 cells-ml to dose the larvae. Counts were again made on the day of dosage and it is these counts which were used for Tables 1 and 2.

Exposure times of 30 min and 24 hrs were used. For the 30 min dosing, dilutions of the bacteria and 25 mg Tetra^R were added to each container, 30 min later the water was drawn off by suction and fresh water added, producing a 50- to 100-fold dilution of the suspended bacteria. Water was changed and the larvae fed again after 24 hrs. At the end of the 24 hr exposure format the water was changed via the automatic water changing procedure (Colbo & Thompson 1978) flushing ca. 4 volumes of water through each container. One min exposures to 5 x 10^4 and 10^5 spores/ml were done in triplicate with the addition of only 5 mg of Tetra^R to better simulate stream conditions. This exposure was ended by 2 complete water changes. All water used was city tap water purified by a Culligan Reverse Osmosis system.

Percentage mortality was scored 48 hrs after the beginning of the bacterial exposure. Larvae which moved after prodding with a wood applicator stick were scored as live. Pupae and parasitized larvae were removed from the sample. Percentage mortality was corrected by Abbott's formula and analyzed using the log-probit method of Litchfield and Wilcoxon (1949).

RESULTS

A 30-min exposure to a sufficient quantity of ONR-60A/WHO 1897 appeared to be lethal to all species tested, and mortality among the mixed larvae was increased by extending the exposure time to 24 hrs (Table 1). Significant mortality was also caused by 1 min exposures to 5 x 10^4 and 1 x 10^5 cells/ml (Table 2). With *S. vittatum* no dose-response line could be drawn, but all test containers with above 3 x 10^4 cells/ml had 100% mortality.

One spore suspension used in several tests decreased in its viable cell count dur-

Table 1. The effect of *Bacillus thuringiensis*, ONR-60A on simuliid larvae

Spiecies	Exposure time	LC_{50}	95% Confidence interval	Standard error	LC_{90}	95% Confidence interval	Standard error	χ^2	Degrees of freedom
Cnephia ornithophilia	*30 min*	1.5×10^3	$1.19\times10^3-1.83\times10^3$	4.13	2.5×10^3	$2.02\times10^3-3.10\times10^3$	5.00	9.79	5
Simulium verecundum	30 min	2.5×10^3	$1.91\times10^3-3.22\times10^3$	1.92	5.6×10^3	$4.04\times10^3-6.8\times10^3$	1.78	22.22	16
Simulium verecundum	24 hrs	5.3×10^3	$3.65\times10^3-8.11\times10^3$	4.66	1.7×10^4	$1.24\times10^4-2.34\times10^4$	11.77	5.98	3
mixture of:	30 min	1.8×10^3	$1.61\times10^3-1.90\times10^3$	3.07	5.0×10^3	$4.56\times10^3-5.37\times10^3$	6.92	8.54	7
Cnephia ornithophilia									
Cnephia mutata									
Simulium vittatum									
Prosimulium mixtum									
" "	24 hrs	4.4×10^2	$3.79\times10^2-5.10\times10^2$	16.28	1.2×10^3	$1.10\times10^3-1.35\times10^3$	15.71	9.20	4

Table 2. Mortality of *S. verecundum* exposed for 1 min to *B. thuringiensis*/ONR-60A

Spore dose	Mean corrected %mortality	S.E.	n
0	20	.8	3
5×10^4	87.1	2.9	3
10^5	92.8	5.1	3

ing storage. The day after preparation it contained 4.8 x 10^6 cells/ml, 4 days later it had 3.5 x 10^6 cells/ml and after 18 days it had dropped to 2.0 x 10^6 cells/ml. In the 24 hr tests on *S. verecundum* (Table 1) all quantitation was done immediately after preparing test suspensions; in subsequent 30 min tests, counting plates were prepared no earlier than 24 hr after suspension of the bacteria.

DISCUSSION

A spore exposure of 30 min was chosen arbitrarily as an easily managed lab procedure which is of short enough duration for extrapolation to actual stream conditions. The water change ending the exposure, although not a complete elimination of the bacterium, achieved a 50-100 times dilution, a bacterial concentration below that which caused significant mortality in the 24 hr (24 hr - 4x dilution - 24 hr) procedure. The 1 min exposure was imprecise because of the time needed for water changes (1.5 min) but the results of such a test could be more representative of the situation occurring close to the dosing site in the stream.

By Dunn's Multiple Comparison Test, at the .05 level, none of the species differed significantly in susceptibility in the 30 min exposures. The 24 hr exposure produced higher mortality among the mixed species than did the 30 min exposure, or the 24 hr dosing of the *S. verecundum*. These comparisons between the species are suspect, however, because the relationship between the viable cell counts and insecticidal activity was not rigorously tested. The 24 hr assay on *S. verecundum* had a higher LC_{50} and LC_{90} than the other assays probably because the bacte-

rial plates for the counts were made immediately after the spore suspension was prepared when many vegetative, non-insecticidal, cells may have been still viable. In later tests, quantitation plates were prepared just before dosing, and at least 1 day after mixing the bacteria with water.

This strain of *B. thuringiensis* and also *B. sphaericus* have high levels of toxicity to mosquitoes and are seriously considered as biocontrol agents. A comparison of the results here with those of Goldberg & Margalit (1977) indicates for ONR-60A, an even higher level of activity against the simuliids than the culicids, especially when the much shorter exposure times used here are considered.

B. thuringiensis ONR-60A/WHO 1897 appears to offer very real promise for practical application for the control of larval simuliids. When compared with the lepidopterophilic strains of *B. thuringiensis* (Lacey & Mulla 1977) the ONR-60A strain IC_{50} is at least 1 log lower and the dose response slope much steeper. Continuous exposure of 3rd-5th instar *S. verecundum* larvae to a commercial preparation of *B. thuringiensis* (Dipel^R) gave an approximate IC_{50} of 10^5 spores/ml and increasing the food supply increased the dosage requirement even further (unpublished data).

Because of the spore size and the slowness with which they settle in water the bacterial suspension appears almost ideal as a formulation for a black fly larvicide. Small scale field tests are planned in local streams pending the assessment of pathogenicity towards local stream non-target organisms and the outcome of an application for a permit from the Newfoundland Department of Consumer Affairs and Environment.

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FAREWELL *fatigans*

Culex quinquefasciatus was described by Say in 1823. *Cx. fatigans* was described by Wiedemann in 1828 and has been recognized as a junior synonym by many specialists for many years. Alan Stone (1956. Proc. Entomol. Soc. Wash. 58:342–343) and Stone, Knight and Starke (1959. Synoptic Catalog . . . Thomas Say Foundation 6:1–258) considered *quinquefasciatus* to be the valid name. However, nomenclatural arguments have persisted. To promote nomenclatural stability, Sunthorn Sirivanakarn and G. B. White have designated a neotype for the southern (tropical) house mosquito (1958. Proc. Entomol. Soc. Wash. 80:360–372). There is no excuse for refusing to use the name *quinquefasciatus*. As T. E. McNeel once said, "If you can't say '*quinquefasciatus*' just call them kinkies." Mosquito workers are indebted to Sirivanakarn and White for a laborious and painstaking task.—W. E. Bickley.