Research Establishment Ottaws, DREO Tech. Note No. 74–28, 30 p.
Mulrennan, J. A., L. A. Lewis and R. H. Grothaus. 1975. Field tests with repellent treated wide-mesh netted jackets against the valley black gnat, *Leptoconops carteri*. Mosquito News 35:228–229.
Schiefer, B. A., R. W. Vavra, Jr., R. L. Frommer, and E. J. Gerberg. 1976. Field evaluation of several repellents against black flies (Diptera, Simuliidae). Mosquito News 36:242–247.
Schreck, C. E., I. H. Gilbert, D. E. Weidhaas and K. H. Posey. 1970. Spatial action of mosquito repellents. J. Econ. Entomol. 63:1576–1578.
Sholdt, L. L., R. H. Grothaus, H. K. Gouck and C. E. Schreck. 1975. Field studies using repellent-treated wide-mesh net jackets against *Glossina morsitans* in Ethiopia. East African Med. J. 52:277–283.

LABORATORY STUDIES ON THE EFFECTIVENESS OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* DE BARJAC AGAINST *SIMULIUM DAMNOSUM* (DIPTERA: SIMULIIDAE) LARVAE

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A variety of *Bacillus thuringiensis* pathogenic to mosquito larvae was isolated from a mosquito larval habitat in Israel (Goldberg and Margalit 1977). It was found to be a new serotype and given the name *Bacillus thuringiensis* var. *israelensis* (de Barjac, 1978). Aqueous suspensions of this *Bacillus*, cultured on agar from an inoculum provided by Dr. L. J. Goldberg of the Naval Biosciences Laboratory, Oakland, California, proved quite toxic to the larvae of several species of Newfoundland black flies (Undeen and Nagel 1978). Arata et al. (1978) suggested that the control potential of this microbe should be assayed against other simuliid species, especially *Simulium damnosum*, the vector of West African onchocerciasis.

MATERIALS AND METHODS.

A sample of *B. thuringiensis* var. *israelensis* powder, rated at 1,000 *Aedes aegypti* units per mg, was obtained from Dr. H. de Barjac of the Pasteur Institut and bioassayed for *Simulium verecundum* efficacy in Newfoundland (Undeen and Nagel 1978). The bacterial powder, as well as some of the aqueous suspension produced in Newfoundland was taken to Bouake, Ivory Coast, for testing against *S. damnosum*.

The tests were carried out in 2 systems. (1) A portable version of the Colbo and Thompson (1978) system used in Newfoundland so that parallel investigations of *S. damnosum* could be carried out using procedures and doses described in Undeen and Nagel (1978) and (2) a concrete trough system (Berl and Prud'hom, 1979) through which water is circulated by pumps.

The *S. damnosum* tests in the magnetic stirrer system differed from the *S. verecundum* tests in that the African water temperature was 26° compared to the Newfoundland 19°C. Tetra 4-in1[R] food

was fed the *S. damnosum* instead of the Tetra[R] fed to *S. verecundum*. The rest of the procedure was as described by Undeen and Nagel (1978) for Newfoundland black fly larvae.

For the trough trials *S. damnosum* eggs were placed in the 3 meter concrete troughs and the larvae were in the final 2 instars at the beginning of these tests. The only organisms in the troughs, other than the black fly larvae, were algae as a thin growth on the walls. Three trough experiments were conducted, 2 using the Pasteur Institut powder and 1 with the RUVP spore suspension. The flow rate was adjusted to between 0.5 and 0.8 liters/sec and measured by the filling of a calibrated container at the outflow. The larvae, either a sample, or all the larvae in the trough, were counted, then the test troughs were dosed with a single measured amount of bacterial spores suspended in water. The dose was applied from a vessel with a fixed aperture precalibrated to a 1-min emptying time. The 1-min flow of dosed water was collected in a large container at the trough outflow to prevent the contamination of the entire system.

There were no barriers to prevent larvae from detaching and circulating through the system to repopulate a dosed trough in the 24 hr between dosage and mortality assessment. To evaluate the effect of larval drift, unpopulated parallel troughs in the same system were monitored and the larvae in undosed control troughs were counted. As the unpopulated troughs remained free of larvae during the tests we feel that the effect of repopulation was negligible.

RESULTS AND DISCUSSION

The eye-fitted dose-mortality lines for *S. verecundum* at 19°C and *S. damnosum* at 26°C are very similar (Fig. 1). These data demonstrated that the stirring system efficacy results with *S. verecundum* were very useful in predicting the dose range for use in the *S. damnosum* trials using the same equipment.

The Pasteur Institut powder in the trough tests (Table 1) gave only 84% mortality at the highest dosage tested (5 million spores/ml for one minute). On the other hand the bacteria grown in Newfoundland killed 99% of the larvae at an effective concentration of 100,000 spores/ml for 1 min. The large effectiveness difference between the 2 formulations may be due to one of several factors. The RUVP material has a smaller particle size than the suspended powder which may result in the former being more readily ingested by the larvae. Perhaps vigorous mixing in a blender would increase the activity of the suspended powder by reducing particle size. Also, the RUVP bacteria were grown on agar plates for 2 weeks which might have produced more toxic spores or a higher spore to vegetative cell ratio than the liquid culture used for the de Barjac powder. Another possibility is that through an accumulation of dead, autolysed spores during the lengthy growth period on agar, a buildup of toxic material in excess of the numbers of countable cells may have occurred in the RUVP material. Work on the practical aspects of formulation and application for optimal effects against simuliid larvae is currently under way at the authors' laboratories in both St. John's and Bouaké.

One-minute dosages of the spore suspension made from agar plates have now been administered in 3 different circumstances, to *S. verecundum* in the magnetic

Table 1. Mortality of *S. damnosum* by *B. thuringiensis* var. *israelensis* in 1 min exposure times in the trough system.

	sp/ml de Barjac powder	Live larval counts before	Live larval counts after	Corrected mortality
Exp. 1	0	63	40	0
	1×10^5	50	21	37
	1×10^6	141	24	71
Exp. 2	0	50	54	0
	5×10^6 RUVP	977	158	84
Exp. 3	0	543	431	0
	5×10^4	537	22	95
	1×10^5	306	4	99

stirring system (Undeen and Nagel 1978), against *S. verecundum* in a small Newfoundland stream (Thompson and Undeen 1978) and against *S. damnosum* in this study. All of these tests, at dosages of 10^5 spores/ml, produced very similar results. In the stirring system 92.8% mortality was obtained with *S. verecundum* while in the stream, against the same host and at the same temperature 100% mortality was achieved down to 30 m below the dose site. At 50 m the mortality was 85%. This agreement between laboratory and field results is encouraging in that it

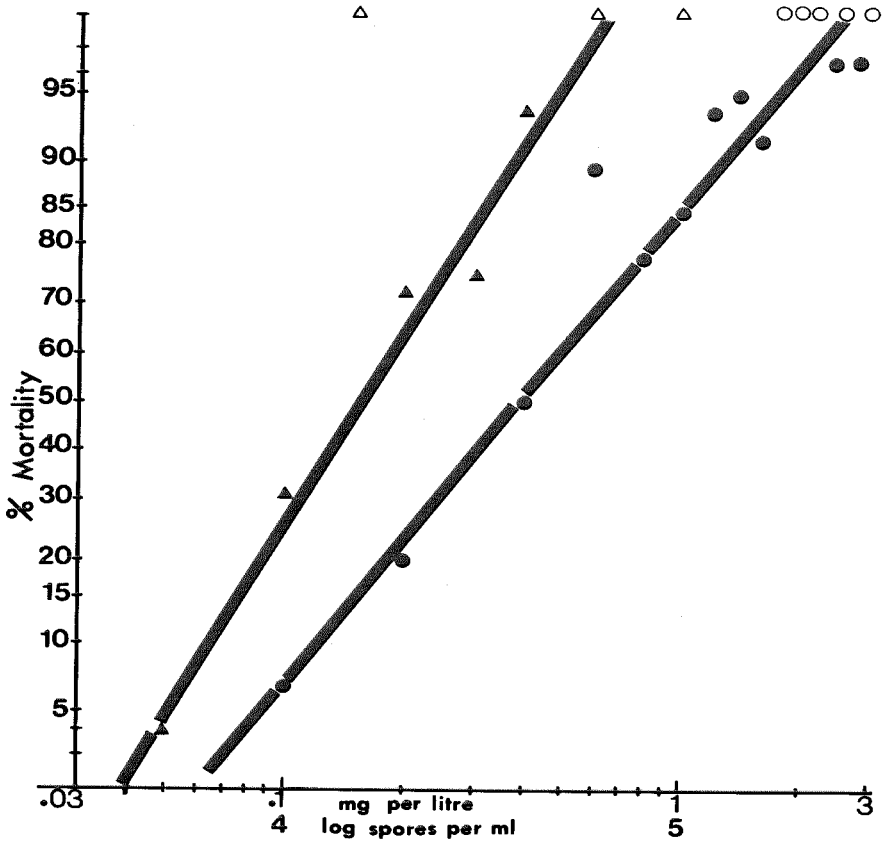

Fig. 1. Comparison between *Simulium verecundum* (●) and *Simulium damnosum* (▲) of the effect of *B. thuringiensis* var. *israelensis* spore powder from the Pasteur Institut (H. de Barjac). ● and ▲ = 100% mortality. *S. verecundum* tests done in St. John's, Newfoundland, Canada in reverse-osmosis filtered tap water at 19°C and the larvae fed in Tetra[R] fish food. *S. damnosum* tested at Bouaké, Ivory Coast in filtered tap water at 26°C with Tetramin 4-in-1[R] fish food for larval nutrition. Percentage mortality corrected by Abbott's formula. One mg spore powder = 10^8 bacterial spores.

demonstrates the effectiveness of both the pathogen used in these trials and the testing methodologies employed for their assessment. In support of this conclusion are the results of the evaluation of Altosid[R] against black flies (Thompson and Adams 1979), in which a close correlation between the magnetic stirring system and field mortalities and dosages was also demonstrated.

This work was supported in part by the U.S. National Institutes of Health and the International Development and Research Center, Ottawa, Canada. Thanks are also extended to Dr. H. de Barjac of the Pasteur Institut, Paris for supplying the spore powders used in these tests.

References Cited

Arata, A. A., H. C. Chapman, J. M. Cupello, E. W. Davidson, M. Laird, J. Margalit and D. W. Roberts. 1978. Status of biocontrol in medical entomology. News and Views, Nature 276:669–670.

Barjac, H. de. 1978. Une nouvelle variété de *Bacillus thuringiensis* très toxique pour les moustiques: *B. thuringiensis* var. *israelensis* sérotype 14. C.R. Acad. Sci. Paris 286:797–800.

Berl, D. and J. M. Prud'hom. 1979. Un nouveau systeme d'élevage de mass de *Simulium damnosum* s.l. II. Modifications et nouvelles experiences. N° 9/Oncho/Rap/79. (Report, Office de la Recherche Scientifique et Technique Outre-Mer.).

Colbo, M. H. and B. H. Thompson. 1978. An efficient technique for laboratory rearing of *Simulium verecundum* S. & J. (Diptera: Simuliidae). Can. J. Zool. 56:507–510.

Goldberg, L. J. and J. Margalit. 1977. A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii, Uranotaenia unguiculata, Culex univitattus, Aedes aegypti* and *Culex pipiens*. Mosquito News 37:355–358.

Thompson, B. H. and B. G. Adams. 1979. Laboratory and field trials with Altosid[R] insect growth regulator on blackflies (Diptera: Simuliidae) of Newfoundland. J. Med. Entomol. In press.

Thompson, B. H. and A. H. Undeen. 1978. Research on the control of blackflies with Altosid[R] and *Bacillus thuringiensis* var. *israelensis*. Proceedings, 18th Annual Meeting of the Northwest Mosquito and Vector Control Association, 10–12 October 1978, Boise, Idaho.

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.