RELATIVE POTENCY OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AND *BACILLUS SPHAERICUS* 2362 FOR *MANSONIA TITILLANS* AND *MANSONIA DYARI*

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ABSTRACT. *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*), HD-968-S-1983, and a primary powder of *Bacillus sphaericus* 2362 were assayed against *Mansonia titillans* and *Ma. dyari*. The susceptibility of the 2 species to the individual bacterial toxins was similar. The *B.t.i.* LC_{50} values were 50.5 μg/liter for *Ma. dyari* and 54.7 μg/liter for *Ma. titillans*; the *B. sphaericus* LC_{50} values were 339.0 μg/liter and 347.2 μg/liter, respectively. The log dose-probit mortality lines were parallel for both target species. The potency of *B. sphaericus* relative to *B.t.i.* was 0.15 for *Ma. dyari* and 0.16 for *Ma. titillans*.

Mosquitoes of the genus *Mansonia* are of concern worldwide because of their transmission of filarial nematodes, and in Florida because of their aggressive biting behavior and transmission of Venezuelan equine encephalitis. The larvae of *Mansonia* remain submerged while attached to the roots of aquatic vegetation. This characteristic greatly complicates control efforts.

The feasibility of using bacterial larvicides for control of *Mansonia* mosquitoes has been the subject of several investigations targeting Asian mosquito species. The results have been inconsistent and even contradictory. Pantuwatana and Youngvanitsed (1984) reported that 4th-instar *Ma. uniformis* (Theobald) and *Ma. indiana* Edwards were not susceptible to either *Bacillus thuringiensis* var. *israelensis* de Barjac (*B.t.i.*) or *B. sphaericus* Meyer and Neide, strain 1593. On the other hand, Foo and Yap (1982) reported an LC_{50} value of 169.6 ITU/liter (= parts/billion) for the international *B.t.i.* standard preparation, IPS-78, against *Ma. indiana*; and Cheong and Yap (1985) reported an LC_{50} value of 18.23×10^4 (ca. 600 μg/liter) spores/ml of B. sphaericus 1593 (ca. 600 μg/liter) against *Ma. uniforms*. Potential for control of *Mansonia* spp. under field conditions has been indicated for both bacteria. Foo and Yap (1983) reported 95% control of natural *Mansonia* spp. with 26 kg *B.t.i.*/ha (600 ITU/mg). In India, Pradeepkumar et al. (1988) reported 4 weeks of control of unidentified *Mansonia* with briquettes of an indigenous *B. sphaericus* applied at 15–30 kg AI/ha. In the present study, both bacilli were assayed against the only North American *Mansonia* representatives, *Ma. titillans* (Walker) and *Ma. dyari* Belkin, Heinemann and Page in order to gain a better understanding of their operational potential.

The U.S. standard of *B.t.i.*, HD-968-S-1983 (Dulmage et al. 1985), and primary powder of *B. sphaericus* 2362 (potency 0.99 relative to RB80) were assayed against mixed *Ma. titillans* and *Ma. dyari* larvae (ca. 58% *Ma. titillans*) collected from water lettuce *Pistia stratiotes* Linn. in an abandoned phosphate pit in Bartow, FL. Third- and 4th-instar larvae, in groups of 10, were placed in plastic cups with 5 ml of field water to provide protozoa as a food supplement and 1 ml of a 40 g/liter hog chow suspension. The cups were then filled to 100 ml with deionized water and the required amount of bacterial suspensions to provide nine 1:2 (suspension: water) serial dilutions. A piece of Styrofoam was placed in each cup for larval attachment. Eight separate assays were conducted with larvae collected on 4 dates from August to October 1989. Assays consisted of 5 cups for each concentration of bacterial suspension and 10 control cups. The tests were terminated after 36 h because mortality after longer periods did not increase relative to control mortality. Dead and living larvae from each concentration were placed in separate vials with ethanol and refrigerated until they could be identified to species.

For each replicate test the data for the cups of each dose were pooled for probit analysis with the Polo-PC program (Russell et al. 1977). Treatment mortality data were adjusted for control mortality by Abbott's formula (Abbott 1925). All variances were multiplied by heterogeneity factors for calculation of fiducial limits (Table 1). The *Ma. dyari* data for 3 of the 8 replicate assays were discarded because of control mortality exceeding 10%. *Mansonia titillans* mean control mortality was 2.8% but never exceeded 10%. A total of 3,867 *Ma. titillans* and 1,721 *Ma. dyari* were included in the analyses, exclusive of controls.

The susceptibilities of the 2 species to the individual bacterial toxins were remarkably similar (Fig. 1). The *B.t.i.* LC_{50}s were 50.5 μg/liter for *Ma. dyari* and 54.7 μg/liter for *Ma. titillans*; their *B. sphaericus* LC_{50}s were 339.0 μg/liter and 347.2 μg/liter, respectively (Table 1). The log

Table 1. Analysis of probit-log concentration toxicity tests of *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* strain 2362 against 3rd- and 4th-instar *Mansonia dyari* and *Mansonia titillans* larvae.

	Bacillus thuringiensis israelensis				*Bacillus sphaericus*				
	LC_{50}[a]	LC_{90}	Slope	h[b]	LC_{50}	LC_{90}	Slope	h	Relative potency
Ma. dyari	50.5 (39.64–62.78)	227.3 (166.47–357.50)	1.96	2.50	339.0 (275.25–416.41)	1,472.5 (1,065.97–2,373.77)	2.01	1.90	0.15 (0.113–0.194)
Ma. titillans	54.7 (46.98–64.81)	266.8 (197.94–396.97)	1.86	3.14	347.2 (305.18–397.85)	1,701.5 (1,328.69–2,335.13)	1.86	5.99	0.16 (0.130–0.192)

[a] LC_{50} and LC_{90} values are in mg bacteria/liter; values in parentheses are 95% confidence intervals.
[b] Heterogeneity factor = χ^2/degrees of freedom.

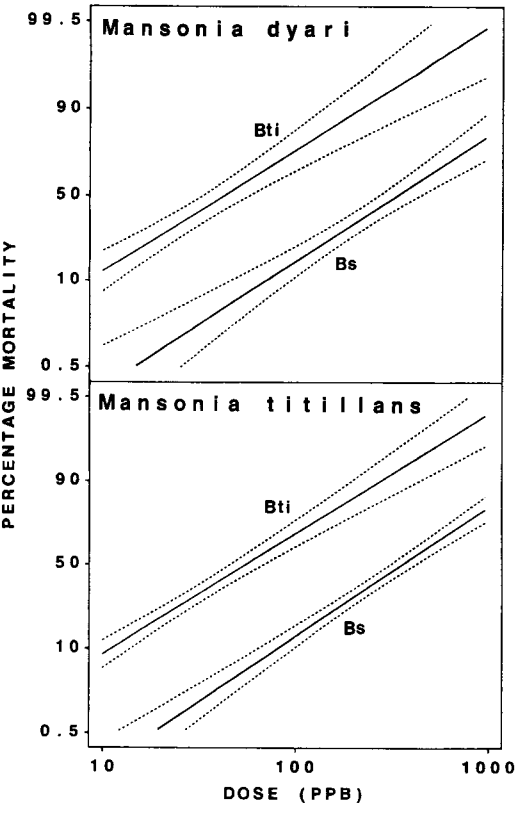

Fig. 1. Efficacy of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) and *Bacillus sphaericus* strain 2362 (Bs) against *Mansonia dyari* and *Mansonia titillans* larvae.

dose-probit mortality lines were parallel for both target species. The chi-square values for equal slopes were 0.058 for *Ma. dyari* and 0.02 for *Ma. titillans*.

The potency of *B. sphaericus* relative to *B.t.i.* was 0.15 for *Ma. dyari* and 0.16 for *Ma. titillans* (Table 1). Although the median lethal concentrations for strain 2362 reported here are approximately 2-fold lower than the value reported by Cheong and Yap (1985) for strain 1593 against *Ma. uniforms*, *B. sphaericus* would not be competitive with *B.t.i.* for development as a control agent for North American *Mansonia*. The activity of *B.t.i.* against both native *Mansonia* spp. was comparable to the activity of the same preparation against *Aedes aegypti* (Linn.) (Lord and Undeen, in press).

Exposing *Mansonia* larvae to adequate concentrations of *B.t.i.* in natural larval habitats remains a difficult problem. Operational *Mansonia* control with bacteria will require a delivery system that can penetrate the foliage of host plants.

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REFERENCES CITED

Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18:265–267.

Cheong, W. C. and H. H. Yap. 1985. Bioassays of *Bacillus sphaericus* (strain 1593) against mosquitoes of public health importance in Malaysia. S.E. Asian J. Trop. Med. Public Health 16:54–58.

Dulmage, H. T., R. E. McLaughlin, L. A. Lacey, T. L. Couch, R. T. Alls and R. I. Rose. 1985. HD-968-S-1983, a proposed U.S. standard for bioassays of preparations of *Bacillus thuringiensis* subsp. *israelensis*-H-14. Bull. Entomol. Soc. Am. 31:31–34.

Foo, A. E. S. and H. H. Yap. 1982. Comparative bioassays of *Bacillus thuringiensis* H-14 formulations against four species of mosquitoes in Malaysia. S.E. Asian J. Trop. Med. Public Health 13:206–210.

Foo, A. E. S. and H. H. Yap. 1983. Field trials on the use of *Bacillus thuringiensis* serotype H-14 against *Mansonia* mosquitoes in Malaysia. Mosq. News 43:306–310.

Lord, J. C. and A. H. Undeen. 1990. Inhibition of *Bacillus thuringiensis* var. *israelensis* toxin by dissolved tannins. Environ. Entomol. 19:(In press).

Pantuwatana, S. and A. Youngvanitsed. 1984. Preliminary evaluation of *Bacillus thuringiensis* serotype H-14 and *Bacillus sphaericus* strain 1593 for toxicity against mosquito larvae in Thailand. J. Sci. Soc. Thailand 10:101–108.

Pradeepkumar, N., S. Sabesan, M. Kuppusamy and K. Balaraman. 1988. Effect of controlled release formulation of *Bacillus sphaericus* on *Mansonia* breeding. Indian J. Med. Res. 87:15–18.

Russell, R. M., J. L. Robertson and N. E. Savin. 1977. POLO: a new computer program for probit analysis. Bull. Emtomol. Soc. Am. 23:209–213.