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EVALUATION OF LIQUID *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* PRODUCTS FOR CONTROL OF AUSTRALIAN *AEDES* ARBOVIRUS VECTORS

M. D. BROWN,[1] J. CARTER,[1] T. M. WATSON,[1] P. THOMAS,[2] G. SANTAGULIANA,[2] D. M. PURDIE[1] AND B. H. KAY[1]

ABSTRACT. Laboratory bioassay studies were conducted in southeast Queensland, Australia, on the efficacy of Teknar®, VectoBac®12AS, and Cybate® (active ingredient: 1,200 international toxic units *Bacillus thuringiensis* var. *israelensis* [*Bti*]) against 3rd instars of the arbovirus vectors *Aedes aegypti, Ae. notoscriptus, Ae. vigilax,* and *Ae. camptorhynchus*. Probit analyses were then used to determine LD_{50} (median lethal dose), LD_{95}, and lethal dose ratios (LDR). *Aedes aegypti* and *Ae. notoscriptus,* both container-habitat species, tolerated the highest *Bti* concentrations compared with saltmarsh *Ae. vigilax* and *Ae. camptorhynchus*. For example, the LDR for *Ae. vigilax* versus *Ae. notoscriptus* exposed to Cybate was 0.14 (95% confidence limit [CL] 0.03–0.61). Similarly, the Cybate LDR for *Ae. camptorhynchus* versus *Ae. notoscriptus* was 0.22 (95% CL 0.07–0.70). Teknar produced similar results with an LDR of 0.21 (95% CL 0.04–1.10) for *Aedes vigilax* versus *Aedes notoscriptus*. Differences in product efficacy were found when tested against the 2 container-breeding species. Cybate was less effective than Teknar with LDRs of 1.55 (95% CL 0.65–3.67) and 1.87 (95% CL 0.68–5.15) for *Aedes aegypti* and *Ae. notoscriptus,* respectively. The significant differences in susceptibility between mosquito species and varying efficacy between products highlight the importance of evaluating concentration-response data prior to contracting with distributors of mosquito control products. This information is crucial to resistance management strategies.

KEY WORDS *Aedes, Bti,* insecticides, susceptibilities, mosquito control

INTRODUCTION

Ross River virus (RR), Barmah Forest virus (BF), and Dengue virus (DEN) types 1–4 seriously impact human health in Australia (Russell 1998). Several *Aedes* species have been implicated in the transmission of these arboviruses. *Aedes camptorhynchus* (Thompson) has been identified as a vector of RR (Lindsay et al. 1997), *Ae. vigilax* (Skuse) (Kay 1982, Boyd and Kay 1999) and *Ae. notoscriptus* (Skuse) (Watson and Kay 1998, 1999) have been associated with the transmission of RR and BF. *Aedes aegypti* (Linn.) is the major vector of DEN in Australia (Watson and Kay 1999). In the absence of vaccines for these viruses, we are mainly dependent on insecticides to control the mosquito vectors.

Liquid formulations of *Bacillus thuringiensis* var. *israelensis* de Barjac (*Bti*), an entomopathogenic bacterium, can control mosquitoes with minimal environmental impact (Hershey et al. 1995, Brown et al. 1999). Accordingly, this agent is being applied with increasing frequency to saltmarsh, mangrove, and freshwater habitats in Australia. This broadscale use has stimulated evaluations aimed at delivering cost-effective *Bti* applications.

Interspecific differences in susceptibility to *Bti* occur (Mulla et al. 1982, Mahmood 1998). Mahmood (1998) found that much higher concentrations of *Bti* are required to kill anopheline larvae than *Ae. aegypti* (described as a very susceptible mosquito species). The surface-feeding behavior of anophelines (Nugud and White 1982) and the feeding rate of *Ae. aegypti* (Mahmood 1998) have been suggested as possible causes for these differences.

Crucial to cost-effective insecticide delivery is the development of concentration-response data for candidate insecticides and subsequent calculation of LD_{50} (median lethal concentration) and LD_{95} values (Roush 1987). Historical records of these values are invaluable to insecticide resistance management strategies (Roush 1987). For resistance management, Becker and Ludwig (1993) have recommended that the susceptibility of mosquito populations to *Bti* be checked every 3 years.

Consequently, this study was designed to provide information on the current susceptibility of 4 species of Australian *Aedes* mosquito to three 1,200 international toxic units (ITU) *Bti*/mg liquid products. The 3 products were Teknar®, VectoBac®12AS, and Cybate®. Based on the well-recognized fact that significant differences in susceptibility to insecticides occur between mosquito species, we hypothesized that 1) significantly different LD_{50} values would be determined for each of the 4 *Aedes* species, and 2) as the 3 products are all liquids and have 1,200 ITU *Bti*/mg active ingredient (AI), no significant differences in efficacy would occur. Using this data, lethal dose ratios (LDR) (LD_{50} species A/LD_{50} species B) with 95% confidence limits (CL) (Robertson and Preisler 1992) were calculated as a means of comparing efficacy between species and products.

[1] Queensland Institute of Medical Research and University of Queensland Tropical Health Program, Royal Brisbane Hospital PO, Brisbane, Queensland 4029, Australia.

[2] Redland Shire Council, Mosquito Control Unit, PO Box 21, Cleveland, Queensland 4163, Australia.

Table 1. Label rates in liters/ha for 3 liquid *Bti* products against 4 Australia *Aedes* spp.

	Cybate	Teknar	VectoBac12AS
Ae. aegypti	Not on label	Not on label	Not on label
Ae. notoscriptus	0.3–1.2	0.6–1.2	0.3–0.6
Ae. vigilax	0.6–1.2	Not on label	0.6–1.2
Ae. camptorhyncus	Not on label	1.2–1.4	Not on label

MATERIALS AND METHODS

Liquid *Bti* products evaluated

In order to evaluate the efficacy of 1,200 ITU *Bti*/mg products utilized in field applications, we tested 1) Teknar (Pacific Biorational, Scarborough, Queensland, Australia), 2) VectoBac12AS (Aventis Environmental Science, East Hawthorn, Victoria, Australia), and 3) Cybate (Cyanamid Agriculture Pty. Ltd., Baulkham Hills, New South Wales, Australia) (Table 1). All batches tested were less than 6 months old and had been stored indoors at 23°C.

Laboratory bioassays

Laboratory bioassays, based on standard methods for testing of larval susceptibility (World Health Organization 1981), were used to determine the concentration-response relationship between the selected *Bti* products and the 4 *Aedes* species. The *Ae. notoscriptus* (Brisbane colony, 1995, supplemented with wild-caught larvae from same locality in Brisbane in 1997) and *Ae. aegypti* (Townsville colony, 1990, supplemented with wild-caught larvae from the same locality in 1997) larvae used in these assays were derived from colonies maintained in the Queensland Institute of Medical Research Insectary (Brisbane, Queensland, Australia), using the methods of Watson et al. (2000a). We believe that these mosquitoes provided relevant baseline data on species susceptibility in the region since 1) neither population had ever been treated with *Bti* in the field and 2) both species rarely disperse more than a few hundred meters (Muir and Kay 1998, Watson et al. 2000b).

The saltmarsh mosquitos *Ae. vigilax* (Victoria Point, Queensland) and *Ae. camptorhynchus* (Gippsland Lakes, Sale, Victoria) were collected from the field as early 2nd instars in March 1999. While the *Ae. camptorhynchus* had never been treated with *Bti,* the *Ae. vigilax* population had received about 20 treatments with VectoBacG over the last 2 years.

In the bioassays, the 3rd instars were exposed to serial dilutions of *Bti* in water that had been filtered through a 130-μm mesh net. The salinity of the test water was 0 g/liter for the *Ae. notoscriptus* and *Ae. aegypti* bioassays and 33.5 g/liter habitat water for the *Ae. vigilax* and *Ae. camptorhynchus* tests. The various test salinities replicated the habitat water from which the respective mosquito species were collected. Five replicates each of twenty 3rd instars were introduced into 250-ml glass beakers containing 200-ml of test concentration. The various test concentrations were based on the surface area (0.0034 m^2) treated for each 250-ml glass beaker. Test specimens were individually removed from holding trays and distributed randomly among the test beakers. Five control beakers holding 20 test larvae each in water without insecticide were used in each bioassay.

Initially, a number of range-finding tests with widely spread exposure concentrations were conducted. Based on these tests, a narrow range of concentrations that straddle the effective range were evaluated. The numbers surviving were counted at 24 h. Death or the lack of reaction to gentle prodding with a glass pipette was the measured deleterious response. All assays were conducted at 25°C under a light:dark cycle of 12:12 h. The test larvae were not fed during the 24 h of testing to minimize variability due to nutritional and metabolic condition.

Statistical methods

Data were analyzed using probit analyses (PROC PROBIT, SAS Institute 1998). In order to compare *Bti* efficacy between species and products, LDRs, along with 95% CL, were calculated using the methods of Robertson and Priesler (1992). Zero concentrations were analyzed as concentrations of 0.000001 liters/ha to avoid infinite logarithmically transformed values. This method was adopted in favor of Abbott's formula (Abbott 1925) because it does not modify the exposure variable and thus has negligible impact on the probit curve.

RESULTS

Significantly different LD_{50} and LD_{95} values were determined for the 3 *Bti* products tested against the 4 *Aedes* species (Table 2, Fig. 1). *Aedes aegypti* and *Ae. notoscriptus* tolerated the highest *Bti* concentrations compared with saltmarsh mosquitoes *Ae. vigilax* and *Ae. camptorhynchus.* For example, the LDR for *Ae. vigilax* versus *Ae. notoscriptus* exposed to Cybate was 0.14 (95% CL 0.03–0.61). Similarly, the Cybate LDR for *Ae. camptorhynchus* versus *Ae. notoscriptus* was 0.22 (95% CL 0.07–0.70). Teknar produced similar results with an LDR of 0.21 (95% CL 0.04–1.10) for *Aedes vigilax* versus *Aedes notoscriptus.* Differences in product efficacy were found when tested against the 2 con-

Table 2. LD_{50} and LD_{95} probit values in liters/ha for 3 liquid *Bti* products against 4 *Aedes* species after 24-h exposure.

	Cybate[1]		Teknar		VectoBac12AS	
	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Ae. aegypti	0.42 (0.39, 0.44)[2], 0.72 (0.64, 0.85)		0.14 (0.12, 0.16)[2], 0.46 (0.39, 0.60)		0.20 (0.18, 0.22)[2], 0.40 (0.35, 0.47)	
Ae. notoscriptus	0.25 (0.23, 0.28)[3], 0.47 (0.41, 0.56)		0.11 (0.10, 0.12)[3], 0.25 (0.21, 0.31)		0.11 (0.10, 0.12)[3], 0.19 (0.17, 0.23)	
Ae. vigilax	0.04 (0.04, 0.05)[2], 0.06 (0.06, 0.07)		0.02 (0.02, 0.02)[2], 0.05 (0.04, 0.07)		0.03 (0.03, 0.03)[2], 0.05 (0.05, 0.07)	
Ae. camptorhyncus	0.07 (0.06, 0.07)[3], 0.10 (0.09, 0.11)		0.05 (0.05, 0.06)[3], 0.14 (0.12, 0.17)		0.04 (0.04, 0.05)[2], 0.14 (0.11, 0.18)	

[1] The values in parentheses are the 95% lower and upper confidence limits.
[2] The *P* value corresponding to the maximum likelihood chi-square statistic for goodness of fit of the model is >0.1.
[3] $P < 0.05$.

tainer-breeding species. Cybate was less effective than Teknar, with LDRs of 1.55 (95% CL 0.65–3.67) and 1.87 (95% CL 0.68–5.15) for *Aedes aegypti* and *Ae. notoscriptus,* respectively.

DISCUSSION

This study confirmed our hypothesis that there is significant variation in susceptibility to *Bti* between Australian *Aedes* species. There was a clear difference between the susceptibility of the container-habitat species compared with the saltmarsh (*Ochlerotatus*) species. In order to kill 95% of *Ae. aegypti,* 9.2 (95% CL 5.6–15) times more of the Teknar concentration was required than was needed to kill 95% of *Ae. vigilax.* However, it was also notable that, for the saltmarsh species, *Ae. camptorhynchus* required 2.8 (95% CL 2–8.5) times the concentration of Teknar required to kill 95% of *Ae. vigilax.*

In contrast with Mahmood (1998), we found *Ae. aegypti* was the most *Bti*-tolerant species evaluated. Although there are no published differences in foraging behavior between the 4 species, ingestion rates may differ. Based on our results, we recommend that, at a minimum, container habitats holding *Ae. aegypti* be treated with these products at a rate of at least 2 liters/ha. The LD_{50} and LD_{95} data developed for *Ae. aegypti* will prove useful to the respective distributors in Australia, as no label rate currently exists for this species.

Our hypothesis that the 3 products would all be equally effective against the 4 *Aedes* species was rejected. Cybate was consistently the least effective product, especially when applied against the container-inhabiting mosquito species. In fact, at the low label rate for Cybate, in clean water, we would expect between 60–90% of treated *Ae. notoscriptus* larvae to survive. Similarly, the label rate for VectoBac12AS applications against *Ae. notoscriptus* should be increased to 0.6–1.2 liters/ha, similar to that for Teknar. Increasing the rates for these products will provide a greater safety margin for application error and compensate for decreases in product efficacy resulting from environmental and biological influences in aquatic habitats (Becker et al. 1992, Nayar et al. 1999).

We can only hypothesize that these differences in efficacy are related to formulation characteristics. Although this information is commercially sensitive, one possibility is that the number of particles on which the *Bti* toxins are carried per milligram varies between products. Teknar and VectoBac12AS may be formulated with more particles than Cybate, which would be available for ingestion by feeding larvae. Also, it is commonly known that producers may increase potency to account for some losses that occur during formulation processes and changes that may take place during shipping and storage. Therefore, there may be variability in potency even from one batch to the next. Accord-

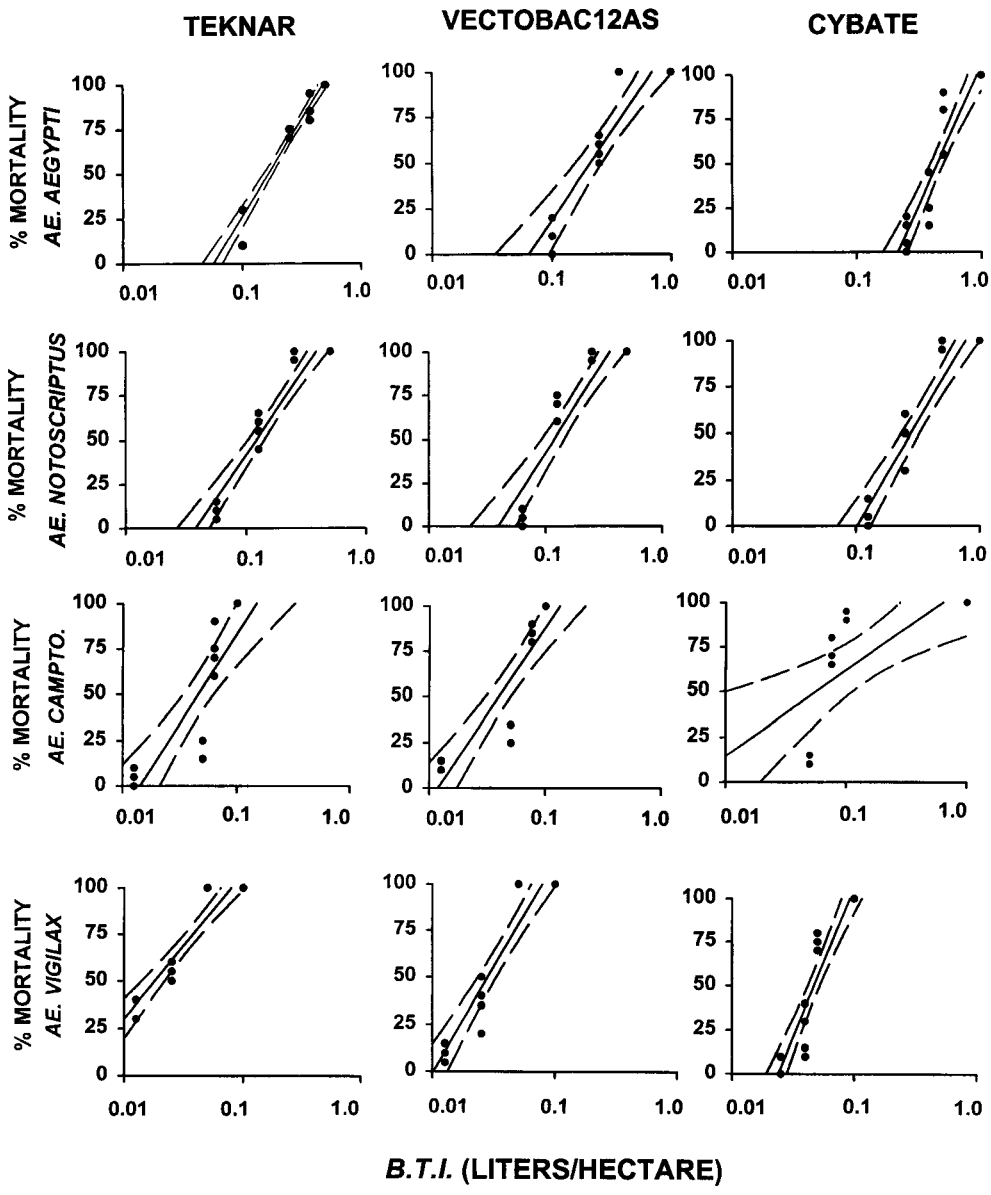

Fig. 1. Observed (circles) and predicted (solid line fitted using probit regression equations; dashed lines indicate 95% CL) mortality of 4 Australian *Aedes* mosquitoes treated with 3 × 1,200 ITU *Bti*/mg liquid formulations.

ingly, we recommend that, where resources permit, laboratory bioassays be compared with the international standard IPS-82.

We have provided baseline data on the variable susceptibility between species to a range of liquid *Bti* products. Accordingly, we recommend that mosquito control agencies evaluate the efficacy of these products against their local target *Aedes*. Products should then be selected using criteria including performance and not just price. Also, the influences on efficacy of various biotic (larval stage, larval density, feeding behavior) and abiotic (water temperature, water quality, and water depth) factors also require definition (Nayar et al. 1999) with regard to *Bti* treatments in Australia. This is important because underdosing our vectors with *Bti* could lead to resistance and loss of valuable products.

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