THE LARVICIDAL ACTIVITY OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* (H-14) AGAINST MOSQUITOES OF THE CENTRAL AMAZON BASIN

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ABSTRACT. A standardized air dried spore and crystal preparation of *Bacillus thuringiensis* var. *israelensis* (IPS-78) produced at the Pasteur Institute, Paris was bioassayed under laboratory conditions against late instars of *Culex quinquefasciatus* and five other mosquitoes found in the vicinity of Manaus, Brazil. The LC_{50} and LC_{95} for *Cx. quinquefasciatus* were .042 and 0.33 ppm, respectively. When an LC_{100} concentration was administered to *Cx. quinquefasciatus* larvae, mortality was noticeable after 2 hrs and was complete within 12 hrs. The primary powder, R-153.78 produced 98.3% mortality at 0.1 ppm compared to 65.0% for the standard at the same conc. Five peridomestic and sylvatic mosquitoes responded variably to 0.1 ppm of the standard. No mortality was produced in *Cx. (Carrollia)* sp.; *Trichoprosopon digitatum* responded with 43.3% mortality and *Cx. mollis* and a mixture of *Limatus durhami* and *L. flavisetosus* responded with 63.3% and 63.6% mortality, respectively.

INTRODUCTION

The insecticidal properties of *Bacillus thuringiensis* Berliner are chiefly derived from 2 toxins: the heat labile δ-endotoxin which is predominantly associated with the crystalline inclusion formed during sporulation and the heat stable β-exotoxin produced during vegetative growth. Disadvantages such as mammalian toxicity, teratogenicity and the possibility of mutagenic effects (Angus 1971, Bond et al. 1971) render the broad spectrum β-exotoxin environmentally undesirable. The larvicidal activity and safety of β-exotoxinfree spore preparations containing the δ-endotoxin used against lepidopterous pests are well documented (Heimpel 1967, Heimpel & Angus 1963, Burgerjon & Martouret 1971).

Only recently, however, have β-exotoxin-free strains of *B. thuringiensis* with high levels of activity against nematocerous Diptera been demonstrated. Several strains which were highly efficacious against Lepidoptera showed fair activity against mosquitoes (Reeves & Garcia 1971, Hall et al. 1977) and black flies (Lacey & Mulla 1977). A new variety, *Bacillus thuringiensis* var. *israelensis* (serovar 14), isolated by Goldberg and Margalit (1977) and serologically characterized by de Barjac (1978b), displays larvicidal activity against mosquitoes and black flies that is comparable to some of the commonly employed chemical insecticides (Goldberg & Margalit 1977, de Barjac, 1978a, Undeen & Nagel 1978, de Barjac & Coz 1979, Garcia & Desrochers 1979, Undeen & Berl 1979). It possesses the additional benefit of being relatively selective for nematocerous Diptera with minimal to no activity against non-target organisms (Garcia et al. 1980 and WHO, Unpublished Document).

Although bioassays of *B. thuringiensis* var. *israelensis* against vector species have been conducted in a number of countries (WHO 1979), very few have been performed in South America. It was the objective of this study to evaluate *B. thuringiensis* var. *israelensis* against *Culex quin-*

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quefasciatus (Say) and other mosquitoes found in and near Manaus, Brazil.

METHODS AND MATERIALS

Field-collected adults of *Cx. quinquefasciatus* were utilized for starting a laboratory colony. Standard techniques (Gerberg 1970) were followed for the rearing of larvae and maintenance of adults. Late 3rd or early 4th instars were utilized for bioassay after the colony had completed at least 3 generations. Fifteen larvae were placed in 200 ml of distilled water (23–24°C) in glass bowls for each replicate and exposed to variable concentrations (conc) of the standardized air dried spore and crystal preparation (IPS-78) formulated and prepared by de Barjac (de Barjac 1978a) at the Pasteur Institute, Paris. Each control and conc was replicated 5 times and observed for 72 hrs at which time cumulative mortality was determined. Larvae which pupated during the first 12 hrs of exposure were subtracted from the original starting numbers. During the course of the bioassay, small amounts of ground lab chow were added to each replicate. Six conc of IPS-78 ranging from 0.025 to 0.8 ppm were utilized for calculation of the $LC_{50\text{-}95}$. The % mortality for each conc was corrected for control mortality with Abbott's formula (Pampana 1969) and graphed on log-probit paper.

At a conc known to produce 100% mortality (0.4 ppm), a mortality curve was generated following the same bioassay procedure except that cumulative mortality was observed at 1, 2, 4, 6, 8, 10, and 12 hrs.

The IPS-78 and the R-153.78 primary powder (produced by Roger Bellon-Biochem group, France) were compared utilizing the aforementioned procedure at a conc of 0.1 ppm against *Cx. quinquefasciatus*. R-153.78 has a reported toxicity of 1350–2400 International Toxicity Units (ITU)/mg against late instars of *Aedes aegypti* (Linnaeus) (Unpublished document, W.H.O.). The IPS-78 was formulated by diluting equal parts of primary powder with clay and powdered diatoms resulting in the arbitrarily assigned toxicity of 1000 ITU/mg (Unpublished document, W.H.O.).

Larvae of 5 other culicine species were field-collected from peridomestic and sylvatic breeding sites and utilized for bioassay. Depending on the number of each species found, 10 to 15 larvae per replicate and 3 to 5 replicates per conc and control were used. When numbers permitted, the IPS-78 was bioassayed at 0.1 and 0.2 ppm. Due to the adverse effects of distilled water on the larvae of *Cx. mollis* Dyar & Knab, it was necessary to use field-collected water for the bioassay procedure. Distilled water was used for all other species. Because of the variable number of larvae per replicate and varying number of replicates utilized for each species, significant differences between the species' mortality responses were determined by non-overlap of 95% confidence intervals.

RESULTS

Table 1 presents the mortality responses of *Cx. quinquefastiatus* to 5 conc of *B. thuringiensis* var. *israelensis* (IPS-78). The LC_{50} and 95 were 0.042 ppm and 0.33 ppm, respectively. The death curve over the first 10 hrs of exposure to a 100% lethal conc is presented in Fig. 1. The primary powder was significantly more efficacious than the diluted standardized formulation. At 0.1 ppm, the primary powder produced 98.3% ± 1.67 mortality, whereas the standard produced 65.0% ± 4.19 mortality (corrected for control mortality).

Table 1. Mortality response of third and fourth instars of *Culex quinquefasciatus* to several conc. of *B. thuringiensis* var. *israelensis* (IPS-78).

Conc.	% Mortality ± s.e.
0	5.45 ± 3.96
0.025	42.35 ± 7.90
0.05	49.73 ± 5.42
0.1	65.0 ± 4.19
0.2	93.33 ± 4.71
0.4	100

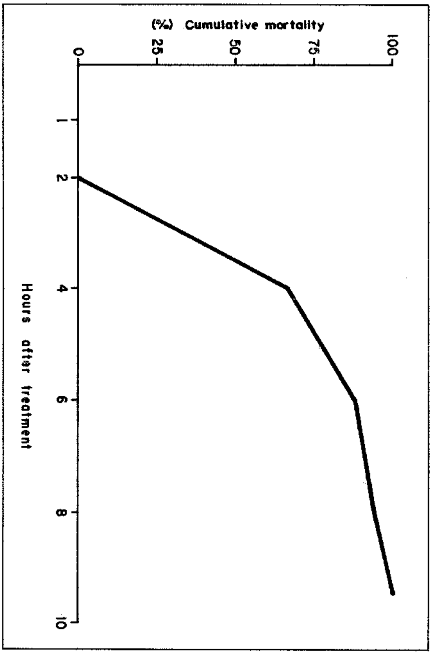

Fig. 1. Curve of mortality response of *Culex quinquefasciatus* zero to 10 hrs after treatment with 0.4 ppm of *B. thuringiensis* var. *israelensis* (IPS-78).

The mortality response of the 5 field-collected species is presented in Table 2. *Limatus durhami* Theobald and *L. flavisetosus* Castro were always found together at the breeding sites and it would have been impossible to separate them without impairing their viability.

Larvae of *Cx. quinquefasciatus*, the most common anthropophilic indoor biting mosquito in Manaus, are both susceptible and accessible enough to consider incorporating *B. thuringiensis* var. *israelensis* into an integrated pest management program. The need to have an effective control strategy against *Cx. quinquefasciatus* is highly warranted. In addition to the possibility of this species vectoring *Wuchereria bancrofti* introduced from endemic foci along the coast of Brazil, it has also been incriminated as a vector of several arboviruses throughout the world (Mattingly et al. 1973). Although DDT is still commonly used against this species in the Central Amazon, J. D. Charlwood (personal communication) found high levels of resistance in both larvae and adults to this insecticide in the Manaus area. In addition to the use of alternative chemical adulticides and cultural methods, *B. thuringiensis* var. *israelensis* could provide an effective larvicide. Its effectiveness against *Cx. quinquefasciatus* under polluted conditions has already been demonstrated by Garcia et al. (1980).

With the exception of *Culex (Carrollia)* sp., the other species were also highly susceptible to *B. thuringiensis* var. *israelensis*. Each of these has a wide distribution in Brazil (Lane 1953), and members of each genus have been implicated in the transmission of various arboviruses in the neotropics (Mattingly et al. 1973). *B. thuringiensis* var. *israelensis* may provide a means of control for peridomestic species such as *Cx. mollis*. Because of their inaccessibility, it would be less feasible to attempt the use of microbial pesticides against *Limatus* spp. which are typically

Table 2. Mortality response of third and fourth instars of 5 mosquito species to two conc. of *B. thuringiensis* var. *israelensis* (IPS-78).

	% Mortality ± s.e.		
Species	0.1 ppm	0.02 ppm	Control
Culex (Culex) mollis	63.62 ± 5.25a	92.0 ± 3.88a	9.33 ± 1.63
Cx. (Carrollia) sp.	0 b	— b	6.66 ± 3.33
Limatus durhami/flavisetosus	63.33 ± 6.67a	92.96 ± 3.53a	3.33 ± 3.33
Trichoprosopon digitatum	43.33 ± 6.67a	63.33 ± 3.44a	2.50 ± 2.89

Means in the same column followed by the same letter are not significantly different. $P<0.05$.

found in secondary forests. There would be even less incentive to control *Trichoprosopon digitatum* (Rondani) due to its use of natural containers as breeding sites in Brazil nut plantations and in both secondary and primary forests.

Control of species such as *Cx. (Carrollia)* sp. may be impossible or would require concentrations which undoubtedly would exceed practical and economic limitations. Further research on the effects of *B. thuringiensis* var. *israelensis* on species in this subgenus may provide a means of defining and elucidating some of the factors which are responsible for decreased susceptibility in mosquitoes to bacterial pathogens. That *Cx. (Carrollia)* sp. did not display the same level of susceptibility to the bacterium as the other *Culex* species is not entirely surprising; Lacey et al. (1978) made similar observations with *B. thuringiensis* (serotype 3a, b) against several *Simulium* spp., and de Barjac and Coz (1979) observed significant differences in mortality between *Anopheles* and *Aedes* mosquitoes exposed to *B. thuringiensis* var. *israelensis*. Since *B. thuringiensis* var. *israelensis* is only active per os and since a large number of mosquito species are highly susceptible to it, the lack of toxicity for *Cx. (Carrolia)* sp. may be explained as a function of feeding habits rather than due to a true physiological resistance.

The results of our evaluation of *B. thuringiensis* var. *israelensis* and that of other investigations indicate that this pathogen offers an alternative means of control of a wide variety of culicine species, especially where cultural methods are not practical and chemical means are undesirable. In addition to the high efficacy of this variety, its other advantages are numerous: it can be grown on artificial media, obviating the need for maintaining host animals, a drawback of most fungal and all viral, microsporidian and mermithid pathogens and parasites of the Diptera; it may be stored for long periods of time in powder form and mammalian toxicity and effects on non-target organisms are non-existent to minimal.

The use of such an effective and selective biological control agent in the Amazon Basin where vector mosquitoes are often combatted with highly residual and frequently ineffective organochlorine insecticides may provide future means of disease and vector control with concomitant environmental protection.

DISCUSSION

The high level of larvicidal activity observed in this study is similar to or less than that reported in earlier investigations (Goldberg & Margalit 1977, de Barjac 1978a, Garcia & Desrochers 1979, de Barjac & Coz 1979). Some difference was also noted in the time that elapsed before onset of death. De Barjac (1978a) obtained 100% mortality in *Ae. aegypti* in 30 to 40 min at high conc and Garcia et al. (1980) observed mortality in as little as 15 min for some *Culex* spp. Mortality was not observed under 2 hrs in our studies at 0.4 ppm of IPS-78. This may be due in part to differences in the age of the preparation and/or the conc utilized. Although mortality was usually complete in less than 12 hrs at higher conc, additional mortality was observed after 12 hrs at lower conc. Hence, 72 hrs was utilized as the period of observation in order to provide a better approximation of the total mortality response. The later slight additional mortality was probably the result of delayed onset of death rather than residual activity. Garcia et al. (1980) found very little residual activity when *B. thuringiensis* var. *israelensis* was applied under a variety of natural conditions and Hembree et al. (1980) made similar observations when high conc were utilized in rice ponds.

As expected, the undiluted primary powder was significantly more efficacious than the IPS-78. Additional research into potency enhancing media and fermentation procedures may yield formulations with even higher larvicidal activity than the 2 preparations evaluated in the present study.

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