OPERATIONAL AND SCIENTIFIC NOTES

THE SUSCEPTIBILITY OF *SIMULIUM VERECUNDUM* (DIPTERA: SIMULIIDAE) TO THREE ISOLATES OF *BACILLUS THURINGIENSIS* SEROTYPE 10 (*DARMSTADIENSIS*)

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In 1979, Ohba et al. identified 35 crystalliferous isolates from silkworm litter of sericulture farms in Ehime Prefecture, Japan, as *Bacillus thuringiensis* serotype 10 (*darmstadiensis*). While most of these isolates produced rhomboidal parasporal inclusions which were toxic to the silkworm, *Bombyx mori*, in 3 of them the inclusions were irregular in shape and these isolates showed no toxicity to *B. mori*. Padua et al. (1980) discovered that 2 of these 3 isolates had a high preferential toxicity for mosquito larvae. In 1981 the 3 isolates designated 73-E-10-2, 73-E-10-16 and 74-E-37-14 were obtained from Japan and the susceptibility of blackfly larvae to them was tested for the first time. The results are reported here.

MATERIALS AND METHODS

Dr. Aizawa, Japan provided the 3 isolates: 73-E-10-2, 73-E-10-16 and 74-E-37-14 on agar slants which were kept at 10°C until used. The bacteria were subsequently grown on nutrient agar, pH. 7.4 at 27–28°C as described by Padua et al. (1980), harvested after 8 days, counted by serial dilution and then stored in distilled water at 10°C for 24–48 hr prior to use in the experiments. The test organism, *Simulium verecundum* (Stone and Jamnback) was reared in the laboratory from field collected eggs in the system described by Colbo and Porter (1979) prior to introduction into the stir-bar system (Colbo and Thompson 1978) in which the susceptibility tests were carried out.

The susceptibility of 3rd, 4th and 5th instars to each of the isolates was tested using the following experimental regime: 30 *S. verecundum* larvae were placed in each of 10 pots and fed with 10 ml/pot Tetra® fish food (1 g/200 ml) 24 hr prior to experimental dosing. The water in each pot was changed and a dose of 10^7, 10^6, 10^5 or 10^4 spores/ml of one of the isolates introduced into each of 2 pots together with food; 2 pots were controls and received food only. After an exposure time of 20 min, the water in each pot was exchanged immediately by siphoning out the water and replacing it with water at the appropriate temperature and again over a 1–2 hr period through a drip system, the larvae were then fed again. Mortality was checked after 48 hr. The entire experiment was replicated 4 times for each instar and each isolate. The temperature ranged from 15–18°C over the experimental period. Concurrently, the susceptibility of 4th instar *Aedes aegypti* (Linn.) (20 larvae in 20 ml water, 5 replicates) to a range of dilutions of each of the isolates was tested. After 48 hr exposure percentage mortality was scored, corrected according to Abbott's formula and analysed using the log probit method of Litchfield and Wilcoxon (1949). The latter experiments were carried out so that the activity of the isolates cultured at RUVP could be directly compared with that of the original isolates used against mosquitoes by Padua et al. (1980).

RESULTS AND DISCUSSIONS

From Table 1 it can be seen that only 2 of the 3 isolates i.e. 73-E-10-16 and 73-E-10-2 but not 74-E-37-14, were effective over the dose range tested against both larval *S. verecundum* and *Ae. aegypti*. These are the same 2 isolates which Padua et al. (1980) had previously shown to have preferential toxicity for mosquitoes. The similarity of activity shown by these 2 compounds towards *S. verecundum* larvae can be clearly seen by reference to LD_{50} and LD_{90} values obtained in Table 1. It is obvious from these data that larval instars do not differ in their susceptibility to either of these compounds.

The compounds 73-E-10-16 and 73-E-10-2 showed greater activity towards *Ae. aegypti* larvae than they did to *S. verecundum*. Also the susceptibility of *Ae. aegypti* to each of these compounds differed, the LD_{50} and LD_{90} for 73-E-10-2 being lower than for 73-E-10-16, Table 1. However, when a comparison is made between the LD_{50} values for *Ae. aegypti* obtained in these experiments and those obtained by Padua et al. (1980) using the same 2 active compounds, a discrepancy arises. The LD_{50} for

Table 1. Susceptibility of *S. verecundum* and *Ae. aegypti* larvae to serotype 10 isolates*

Species and instar		73-E-10-16	95% Confidence interval	73-E-10-2	95% Confidence interval
S. verecundum					
3rd	LD_{50}	1.00×10^5	$9.60 \times 10^4 - 2.60 \times 10^5$	1.10×10^5	$8.60 \times 10^4 - 4.7 \times 10^5$
	LD_{90}	1.57×10^6	$7.96 \times 10^5 - 5.18 \times 10^6$	9.40×10^5	$8.02 \times 10^5 - 1.07 \times 10^6$
4th	LD_{50}	1.73×10^5	$9.27 \times 10^4 - 4.21 \times 10^5$	2.50×10^5	$8.40 \times 10^4 - 6.60 \times 10^5$
	LD_{90}	1.56×10^6	$6.94 \times 10^5 - 6.18 \times 10^6$	1.43×10^6	$8.83 \times 10^5 - 4.03 \times 10^6$
5th	LD_{50}	2.30×10^5	$9.09 \times 10^4 - 3.79 \times 10^5$	1.60×10^5	$9.21 \times 10^4 - 3.99 \times 10^5$
	LD_{90}	1.73×10^6	$9.29 \times 10^5 - 4.17 \times 10^6$	1.23×10^6	$9.53 \times 10^5 - 2.93 \times 10^6$
Ae. aegypti					
4th	LD_{50}	1.00×10^5	$1.00 \times 10^5 - 1.00 \times 10^5$	1.00×10^5	$1.00 \times 10^5 - 1.00 \times 10^5$
	LD_{90}	5.26×10^5	$4.87 \times 10^5 - 5.75 \times 10^5$	5.15×10^5	$5.05 \times 10^5 - 5.25 \times 10^5$

* All values for 74-E-37-14 $> 1.0 \times 10^7$.

73-E-10-2 obtained by Padua et al. (1980) was 3.0×10^5 compared to a value of 1.0×10^5 (Table 1) and for 73-E-10-16 the value was 4.3×10^5 (Padua et al. 1980) compared to 1.0×10^5 (Table 1). Therefore, in these experiments, the activity of both compounds was greater towards *Ae. aegypti* than previously found. This may be attributed to the pre- or post-experimental storage of the bacteria or to differences in the methodology used in the susceptibility tests.

Overall these data indicate that the isolates 73-E-10-16 and 73-E-10-2 exhibit greater activity, over a short exposure time, towards blackfly larvae than the 13 *B. thuringiensis* strains tested by Lacey and Mulla (1977) but are less efficacious as larviciding agents than *B. thuringiensis* var. *israelensis* (serotype 14), the current *B. thuringiensis* isolate of choice for blackfly and mosquito control. Indeed if either of the unformulated compounds 73-E-10-16 or 73-E-10-2 was to be used for control of blackflies, then a dose in the order of 2×10^6 spores/ml would have to be administered and for mosquitoes a dose of 6×10^5 spores/ml. These can be compared to the doses of 1.0×10^5 spores/ml of *B. thuringiensis* var. *israelensis* currently used against blackflies (Undeen and Nagel 1978, Undeen and Berl 1979, Undeen and Colbo 1980) and $1.0 \times 10^4 - 1.0 \times 10^5$ spores/ml used against mosquitoes (de Barjac and Coz 1979, Garcia and Desroches 1979).

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