RESEARCH NOTE

Effects of the Spore-Endotoxin Complex of a Strain of *Bacillus thuringiensis* Serovar *morrisoni* upon *Triatoma vitticeps* (Hemiptera: Reduviidae) under Laboratory Conditions

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Some strains of *Bacillus thuringiensis* (*Bth*) synthetize proteic intracellular crystals (delta-endotoxins), which depending on the serovar, prove to be powerful insecticides against young forms of *Lepidoptera* and larvae of several *Diptera* (Culicidae and Simuliidae) (MT Damage 1982 Acad. Press Inc. London, M Huber-Lulac et al. 1986 *Biochem biophys Commun 126:* 961-965, MEM Habib & CFS Andrade 1986 *Controle Microbiano de Insetos*, Ed. Manole LTDA, part 2, chapter 7: 127-163). The mechanism of action of the delta-toxin, in general, is not yet fully known. However evidence shows that when ingested by susceptible insects, these crystals are dissolved in the intestinal alkaline medium, and hydrolized by proteases, producing active toxins which act upon cells of the medium intestine, lysing them and therefore causing the insect's death (JF Charles & H Barjac 1980 *Bull Soc Pathol Éxot* 91-95, DJ Tyrrel et al. 1981 *J Bacter 145:* 1052-1062, GJP Sing 1985 *Pest Bioch Physiol 24*: 406-414, SS Gill et al. 1987 *Appl Environ Microbiol 53*: 1251-1256).

The present paper aims to study, in the laboratory, the action of the spore-delta-endotoxin complex (SPD-complex) of *Bth* serovar *morrisoni* (*Bthm*) upon *Triatoma vitticeps*. The lethal effects (mortality rates), behavioral (blood ingestion) and physiological (ecdyses) alterations of the remaining insects were observed. Also, recovery of *Bthm* was surveyed in the insects after ingestion of blood containing the SPD-complex.

The tests were carried out in samples of *T. vitticeps* from National and International Reference Laboratory on Taxonomy of Triatomines, at Oswaldo Cruz Institute. In our laboratory, the insects were artificially fed (MM Lima et al. 1989 *Mem Inst Oswaldo Cruz*, Suppl. II *84*: 112).

The SPD-complex from a strain of *Bth* serovar *morrisoni*, LFB-039 - which is toxic for both Lepidoptera *Manduca sexta* and *Spodoptera littoralis* - belonging to the Genus *Bacillus* Culture Collection, of the Department of Bacteriology of Oswaldo Cruz Institute/FIOCRUZ, was grown in soil-extract agar (RE Gordon et al. 1973 *Agriculture Handbook* No. 472), at 33°C, for seven days until sporulation of 99% of the population. A suspension of the material in sterile aqueous solution was prepared (19% glycerol and 21% sorbitol) in order to contain spores, vegetative forms and crystals. This suspension contained 8.8 x 10^7 CFU/ml (CFU=colony forming units). For one of the tests, the suspension of SPD-complex was treated at a temperature of 80° C for 20 min, to eliminate vegetative forms, leaving only thermo resistent spores.

A batch of *T. vitticeps* containing all the evolutive stages (nymphs and winged) homogeneously distributed, was divided into three groups: group I (n=33) received SPD-complex previously submitted to heat treatment, in a suspension of defibrinated sheep blood (1,5 x 10^8 CFU/ml). Of the 33 specimens, 17 were fed until replenishment. Group II (n=33) received SPD complex in the same concentration as the above, but not heated. Of the 33 specimens, 22 reached replenishment state. Group III (n=32), control, received blood added from the suspension carrier, but without the SPD-complex. Twenty five specimens were fed until replenishment.

The three groups stayed in contact with the food for 3 hr and insects that reached replenishment were selected for the experiment. Every 15 days, a new blood-meal was offered to them, with defibrinated sheep blood without SPD-complex or any other suspension. Third, 4th and 5th instar nymphs and the adults were identified individually (JR Mac Cord et al. 1983 *Mem Inst*

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Oswaldo Cruz 78: 473-476), to the group they would belong. First and 2nd instar nymphs were kept in individual test tubes, numbered since the beginning of the tests. All the insects were weighed separately, before and after each meal and kept in climatized chambers (29° C and 60% relative humidity of air).

Insects were observed for death during and up to 4 hr after feeding with SPD-complex and, from there on, 24, 48, 72 and 95 hr. After these intervals, observations were made weekly throughout 13 weeks.

In order to investigate the proliferation or germination of *Bthm*, smears of the gastric content and the hemolymph of the dead and dying insects were made, stained by the Gram method and examined under the optic microscope (2000x). For the statystic analysis of data, the chi-square test was used to compare deaths, length of the evolutive cycle and ecdyses in the three groups, while the Kruskal-Wallis test was used to evaluate the amount of blood ingested (S Siegel 1975 *Estatística Não Paramétrica (Para Ciências do Comportamento)*, 215 pp. Ed. MacGraw-Hill, São Paulo, Brasil).

The results show that group III presented the major number of insects that fed (75%), followed by group II (68%) and group I (53.1%). The amount of ingested blood was also greater in group III, however, the difference of this group from the others was not significant (P>0,05). The 4th instar of group III ingested up to 6.6 times the amount of food in relation to the initial weight. Nevertheless, the 4th instar of group I ingested a maximum of 4.7 times while group II obtained a maximum rate 3.5 times. The 5th instar had the following relations: 6.8 times the maximum weight increase of group II, 4.4 times in group I and 0.6 times in group III. Two weeks after ingestion of blood with SPD-complex, the surviving insects from all three groups began to be fed in regular intervals of 15 days. After 95 days, statystical analysis of data did not present any significant difference between the groups ($H=2.02$; $P>0.05$).

The amount of blood consumed by each group did not present relevant alterations, neither in the first meal (with or without bacterial suspension), nor in subsequent meals. Sometimes, the experimental group overcame the controls in relation to the amount of blood ingested. A similar observation was made by B Subrehmanyan and N Ramakhishman (1981 *J Invert Pathol 36*: 161-168), with *S. litorallis*. When treated with *Baculovirus*, larvae fed more than the control group, throughout the experimental phase.

In relation to mortality, during feeding with the SPD-complex, only two nymphs died: one of 1st and one of 2nd instar, both from group I. In the first three days, a greater number of deaths occurred in group II. However there was not significant difference from group III ($X^2 = 4.29$; $P>0.05$). When comparing the three groups, a significant difference was shown only after the 32th day, remaining untill the end of the observations (Table).

Heating did not change toxicity of the suspensions, that is, the heated preparation (80°C, 20 min) had the same effect upon *T. vitticeps* as those not heated. The rates of mortality observed in the first four days in group II were relatively high in relation to group III. TV Guaycurus et al. (1989 *Mem Inst Oswaldo Cruz* Suppl. *III 84*: 147), working with nine strains of *Bth* and some *Lepidoptera*, obtained better effects than those presented herein. In that report, six of the nine strains of *Bth* led to a 100% mortality in *M. sexta* (250 mg/cm^2 of plant area), four strains were lethal to *Isognatus* spp. and three for *Dione juno juno*.

In relation to the ecdyses, the same number (one) was observed in group II, both in 4th instar, on the 4th and 10th week, respectively. Group III presented three: two on the 3rd and one on the 4th instar. It seems that *Bthm* did not have any influence on the ecdyses of *T. vitticeps*, since the numbers obtained in three groups were very close ($X^2 = 1.03$; $P>0.05$). However these results were relatively low, when compared to those of TCM Gonçalves et al. (1988 *Mem Inst Oswaldo Cruz 83*: 519-523). These authors, under favourable laboratory conditions, observed that 1st and 2nd instars of *T. vitticeps* took less than 30 days to change the skin and 3rd and 4th instars, 90 days at the most.

JE Gomes et al. (1986 *Ciênc Cult* Suppl. *30:* 990), reported that delta-exotoxin of *B. thuringiensis* serovar *thuringiensis* used at concentrations of 0.1, 0.5, and 1.0mg/l had no effect on *R. prolixus*. But concentrations of 10^{-4} and 10^{-3} mg/ml of beta-toxin were enough to completely inhibit the ecdyses of *R. prolixus*, showing that delta-endotoxin would not have any action on the ecdyses of that hemiptera.

In order to investigate the growth of *Bthm* in the digestive trait of *T. vitticeps*, blood and haemolymph smears from 3rd, 4th and 5th instar nymphs of group I were analyzed. The blood smears showed that *Bthm* could only proliferate while in the 3rd instar, even reaching the formation of sporangia. In the 4th and 5th instar nymph smears, development of the bacteria was not observed in the haemolymph slides, *Bthm* could not be detected in any of the samples.

In group II, blood smears from 2nd to 5th instar nymphs and adult insects were examined. Bacterial development was not observed in 2nd and 5th instars. On the 3rd instar and in adult phase, proliferation of *Bthm* occurred. However

TABLE

Mortality of *Triatoma vitticeps* observed until 95 days after oral treatment with *Bacillus thuringiensis* serovar *morrisoni* added to defibrinated sheep blood. Three experimental groups were studied: group I (received the SPD-complex submitted to a thermo-resistance); group II (received the SPD-complex not submitted to a thermo-resistance); group III (control)

Days of observation	Group I		Group II		Group III		Significance
	N	% mortality	N	% mortality	N	% mortality	
0	17(0)	11.7	22(0)	0.0	25(0)	0.0	P>00.5
1	15(0)	11.7	22(1)	4.5	24(1)	4.0	P>00.5
2	15(2)	23.5	21(3)	18.2	23(1)	8.0	P>00.5
3	13(0)	23.5	18(3)	31.8	23(0)	8.0	P>00.5
4	13(1)	29.4	15(0)	31.8	23(0)	8.0	P>00.5
8	12(0)	29.4	15(1)	36.4	18(5)	28.0	P>00.5
15	12(2)	41.1	14(0)	26.4	18(0)	28.0	P>00.5
25	10(0)	52.9	14(1)	40.9	18(0)	28.0	P>00.5
32	8(2)	64.7	13(3)	54.5	18(0)	28.0	P<00.5[a]
38	6(1)	70.6	10(1)	59.1	18(0)	28.0	P<00.5[a]
45	5(0)	70.6	9(0)	59.1	17(1)	32.0	P<00.5[a]
52	5(0)	70.6	9(4)	77.3	17(0)	32.0	P<00.5[a]
59	5(1)	76.5	5(0)	77.3	16(1)	36.0	P<00.5[a]
66	4(0)	76.5	5(0)	77.3	14(2)	44.0	P<00.5[a]
74	4(0)	76.5	5(0)	77.3	14(0)	44.0	P<00.5[a]
81	4(0)	76.5	5(0)	77.3	13(1)	48.0	P<00.5[a]
88	4(0)	76.5	5(0)	77.3	13(0)	48.0	P<00.5[a]
95	4(0)	76.5	5(0)	77.3	13(0)	48.0	P<00.5[a]

[a]: significant difference

on 4th instar, 57% of the analyzed smears pointed out presence of the vegetative form of the bacillus. The haemolymph smears were avaluated on the 3rd, 4th and 5th instars and on the adult phase. On the 3rd and 5th instars there was no growth of *Bthm*. In 4th instar, 66.7% of the slides presented growth of the baccillus, some of them in dying insects, as well as 50% of the slides from adult phase.

It is concluded that the strain of *Bthm* used was able to induce significant mortality in *T. vitticeps*. It is further concluded that spore germination and cell multiplication of *Bthm* occurred in the digestive trait of the triatomine.