Israel Journal of Entomology

Vol. XXIII(1989) pp. 91-93

FEEDING BEHAVIOR OF AEDES AEGYPTI LARVAE AND FATE OF BACILLUS THURINGIENSIS VAR. ISRAELENSIS (B.T.I.) IN B.T.I-KILLED PUPAE

KAMAL KHAWALED, ARIEH ZARITSKY, EITAN BEN-DOV AND ZE'EV BARAK
Department of Biology, Ben-Gurion University of the Negev,
P.O. Box 653, Be'er Sheva, 84105, Israel

ABSTRACT

Two modes of feeding behavior acquired by *Aedes aegypti* larvae were investigated, filtering and scavenging. Spores of *Bacillus thuringiensis* var. *israelensis (B.t.i.)* were used as a tracer for water flow through the larval brushes. Toxicity *of B.t.i.* spores in suspension was compared to that produced during their recycling inside carcasses of B.t.i.-killed larvae and pupae.

The initial rate of spores ingestion by third instar larvae increased with spore concentration, while filtering rate was decreased from 500 µl/min at 10^3 spores/ml to a minimum of 50 µl/min at above 2 x 10^5 spores/ml. These values were used to calculate the lethal dose: six thousand spores ingested within three minutes induced 100% mortality within an hour and a half.

Scavenging activity increased with increasing densities of carcasses of B.t.i.-killed third instar larvae, and with decreasing concentrations of the particulate nutrient Pharmamedia.

For toxicity quantitation, carcasses of the scavengers were introduced singly, intact or homogenized, to different numbers of third instar larvae (secondary scavengers) in 10 ml of sterile water. Mortality of the secondary scavengers was determined twenty-four hours later. This was compared with mortality induced by *B.t.i.*, The latter seemed to be more effective than an equivalent number of spores ingested with a carcass, whether intact or homogenized. A great variability in toxicity of single carcasses was observed and can be explained by a similar variation in the number of spores per carcass at the end of a recycle.

Spores of *B.t.i.* were found to germinate and multiply inside B.t.i.-killed pupae. The latter resulted of treating late fourth instar larvae with a low dose of *B.t.i.* powder. The bacteria completed a growth cycle (i.e. sporulated and produced parasporal crystals of endotoxin) in the pupae, which remained intact for at least 11 days and became toxic to scavenging larvae.

Dipteran larvae are known to ingest small particles from their aquatic environment by filter feeding (Clements, 1963; Dadd, 1971). We have recently described an additional feeding mode of *Aedes aegypti* larvae, in which they gnaw and ingest carcasses of their own and of related species (Zaritsky and Khawaled, 1986; Zaritsky, Khawaled, Chipman and Rabi, 1986).

The time it took a scavenger to effectively approach and scavenge a carcass was seven minutes when the carcass age was 6 hr (Zaritsky et al., 1986). This time decreased with increased carcass concentrations (Khawaled, Barak and Zaritsky, 1988) but increased with increasing concentration of a particulate food source such as Pharmamedia (Fig. 1). In addition, the percent of scavengers was reduced upon raising the food concentration.

This scavenging behavior, which has already been shown to support larval development to adults without an additional food source, is fatal if the carcass is of B.t.i.-killed larva (Zaritsky and Khawaled, 1986). We have previously described ways to quantitate toxicity of carcasses of *B.t.i.*-killed larvae and found that it is caused by a full growth cycle of this bacterium inside the carcass (Khawaled et al., 1988; Khawaled, Barak and Zaritsky, 1986).

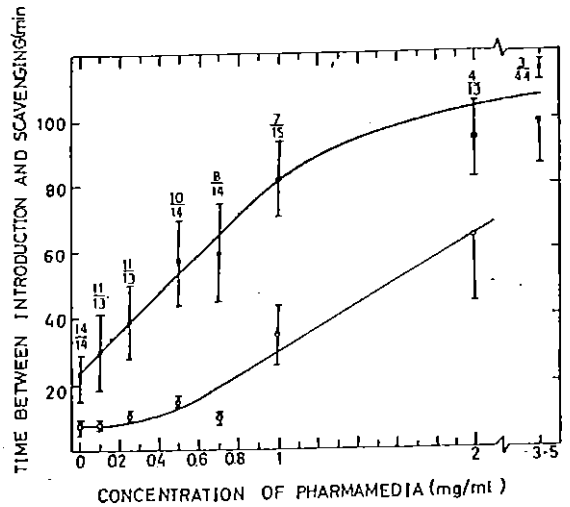

Fig. 1. Scavenging behavior as a function of Pharmamedia concentration. o, average time between introduction and scavenging among the first six scavengers; ● average time between introduction and scavenging among the indicated number of scavengers (numerator) out of the total larvae (denumirator). Bars represent standard errors.

Multiplication of the *B.t.i.* is not restricted to *B.t.i.*-killed larvae only, but is also shown to occur in dead pupae of *Aedes aegypti*. *B.t.i.*-killed pupae were obtained by treating forty-hour-old synchronized fourth instar larvae with a low dose of *B.t.i.* powder (8,000 spores/ml). Such a treatment induced death of twenty percent of the larvae after 24 hr and resulted in thirty and fifty percent dead and living pupae, respectively (Table 1).

It is noteworthy that increased or decreased spore concentration led to a decrease in the number of dead pupae. An increase in the proportion of dead larvae was obtained at the higher concentrations whereas lower concentrations resulted in more living pupae.

The number of spores in the dead pupae was determined at intervals following pupal death (Table 2).

The numbers found in fresh *B.t.i.*-killed pupae were low and remained practically constant up to 20 hrs following pupal death. This number was lower than the lethal dose for fourth instar larvae. It seems therefore that part of the spores were digested or germinated inside the larvae since no difference in spore content was obtained between pupae which died immediately following pupation and those which died later.

Multiplication of *B.t.i.* may not be restricted to carcasses of mosquito larvae. Other organic sources, including carcasses of other organisms, may support its multiplication in natural habitats

TABLE 1
Mortality of *Aedes aegypti* larvae (4th instar) treated with a low dose of *B.t.i.* powder (8,000 spores/ml)

Dead pupae	Living pupae	Dead larvae
30%	50%	20%

Scored after 24 hrs incubation at 30°C. Control mortality under these conditions was zero. Total number of larvae in a three series of experiments was 60.

TABLE 2
Fate of*B.t.i.* in *B.t.i.*-killed *Aedes aegypti* pupae

Time (hr)	Spores per pupal carcass ± S.E.[a]	
0	855 ± 510	(8)[b]
2	674 ± 455	(5)
6	1,200 ± 529	(3)
15	383 ± 283	(3)
20	255 ± 76	(2)
30	2,233 ± 505	(3)
40	1,773 ± 1114	(3)
50	22,000	(1)
60	11,000	(1)
96	600,000	(1)
120	330,000	(1)
168	300,000	(1)

Dead pupae, obtained by treating forty-hour-old fourth instar larvae with 8,000 spores/ml, were homogenized, sonicated and heat shocked (15 min × 70°C) at various times following their death for determination of their spore content.
[a]Standard errors.
[b]Number of carcasses tested.

in certain conditions. Our observations regarding scavenging behavior of mosquito larvae suggest that agents for biocontrol of mosquitoes are not restricted to smaller unicellular organisms ingested by extensive filter feeding. The genes coding for the *B.t.i.* crystal toxins could therefore be engineered into large "delivery organisms" for efficient expression.

ACKNOWLEDGEMENTS

Thanks are due to Dr. J. Margalit for a free supply of *Aedes aegypti* eggs. This study was partially supported by The Fund for Encouragement of Research, Histadrut-The General Federation of Labour in Israel (to K.K.) and by grants from the National Council (Israel) for Research and Development and from US-AID-CDR (both to A.Z. and to Z.B.).

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