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Bioencapsulation and Delivery to Mosquito Larvae of *Bacillus thuringiensis* H14 Toxicity by *Tetrahymena pyriformis*

Using *Bacillus thuringiensis var. israelensis* for controlling mosquitoes and blackflies is limited by low persistence of commercial preparations in the field (C. M. Ignoffo, C. Garcia, M. J. Korha, K. Fukuda, and T. L. Couch, *Mosq. News* **41**, 85, 1981). Much effort is being expended in attempts to produce more persistent preparations.

The approach described here utilizes *Tetrahymena pyriformis* to encapsulate the toxic crystal of *B. thuringiensis* var. *israelensis* and to deliver toxicity to mosquito larvae, which die upon ingesting them. It is based on the following: (1) The toxic crystal is stable at *p*H below 9 (J. P. Insell and P. C. Fitz-James, *Appl. Environ. Microbiol.* **50**, 56, 1985). (2) The *p*H of food vacuoles in *Tetrahymena* is substantially lower than 9 (J. R. Nilsson and E. Weidner, *Insect Sci. Applic.* **7**, 401, 1986). (3) *T. pyriformis* feeds on bacteria (J. R. Nilsson, *C.R. Trav. Lab. Carlsberg* **40**, 259, 1976). (4) *T. pyriformis* shares the natural habitat of mosquito larvae, the upper layers of fresh water (J. O. Corliss, 1973, in "Biology of *Tetrahymena*," A. M. Elliot, Ed., p. 8, Dowden, Hutchinson, & Ross, Inc., Stroudsburg, Pennsylvania). (5) Mosquito larvae prey on microorganisms (L. J. Howland, *J. Ecol.* **18**, 81, 1930).

To demonstrate that *Aedes aegypti* larvae ingest *T. pyriformis,* they were presented with *T. pyriformis* prefed with carmine (Fig. 1A). The cells disintegrated instantly upon entering the larval mouth (Fig. 1B), and the carmine aggregates were pushed into the alimentary canal, staining the larvae a deep red color within 30 min (Fig. 1C).

Active, commercial powder of *B. thuringiensis* var. *israelensis* (R-153-78 of Roger Bellon Laboratories, Belgium) was presented to *T. pyriformis* in a vial submerged in a vessel containing *A. aegypti* larvae (Fig. 2); the latter were unable to enter through the narrow outlet of the vial and reach the toxic powder, whereas *T. pyriformis* could leave it freely. Larvae started to die within 2–24 hr, depending on the experimental conditions employed. Essentially no death was observed in control vessels, either without *T. pyriformis* (Fig. 2B), or without *B. thuringiensis* var. *israelensis* (data not shown). Fig. 2 depicts two typical views of the experimental system. In the presence of *T. pyriformis* (2A), *A. aegypti* larvae dived briefly for short distances, apparently feeding on the cells migrating from the container. In *T. pyriformis*-free devices (2B), they spent longer intervals at the bottom of the outer vessel, apparently searching for food.

This device was tested with varying numbers of *T. pyriformis* and a constant number ($2 \cdot 10^8$) of spores. The results of a typical experiment (repeated three times) are displayed in Fig. 3. A direct relationship between larvicidal activity and the initial number of *T. pyriformis* was found.

The system described here in a preliminary form seems to have a potential to solve a cardinal problem in mosquito biocontrol: effective encapsulation and delivery of the active agent to the target organisms. The *T. pyriformis* cells migrating out of the vial provide a slow release effect for the *B. thuringiensis* var. *israelensis* preparation, which is also protected in the container from various environmental factors. Its real economic value should however be further investigated in the laboratory and tested in nature. Examples of points which must be considered before the method proves its ef-

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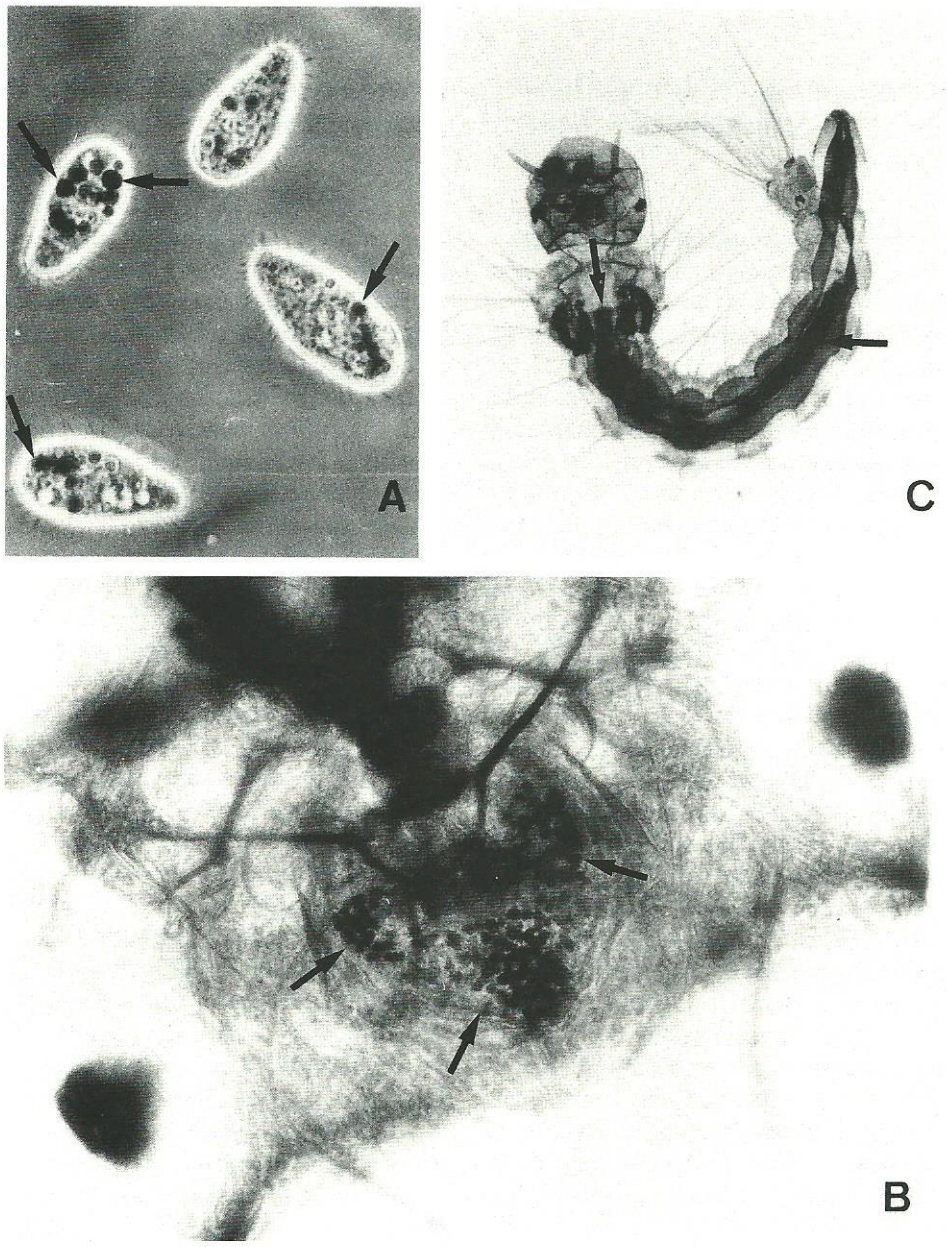

FIG. 1. Ingestion of *Tetrahymena pyriformis* by *Aedes aegypti* larvae. *A. aegypti* larvae were reared as described elsewhere (A. Zaritsky and K. Khawaled, *J. Amer. Mosq. Control Assoc.* **2**, 555–559, 1986). *T. pyriformis* cultures were maintained as described by G. A. Thompson (*Biochemistry* **6**, 2015–2022, 1967), but without glucose. A mid-log phase culture (of $1–2 \cdot 10^5$ cells/ml) was centrifuged, and the cells were washed and resuspended in autoclaved tap water at a density of $6 \cdot 10^4$. (A) Cells of *T. pyriformis*, after 30 min incubation at 27°C with a 1 mg/ml carmine suspension (centrifuged at 1000 rpm for 1 min, to remove aggregates) (magnification, ×400). (B) The head of an *A. aegypti* larva soon after ingestion of a carmine-fed *T. pyriformis* cell (A), which have disintegrated (magnification, ×400). (C) *A. aegypti* larva filled-up with carmine particles, introduced by means of carmine-fed *T. pyriformis* (A) (magnification, ×40). Arrows point at several carmine-filled food vacuoles, in cells (A) and in larval head (B), and at carmine-filled larval gut (C).

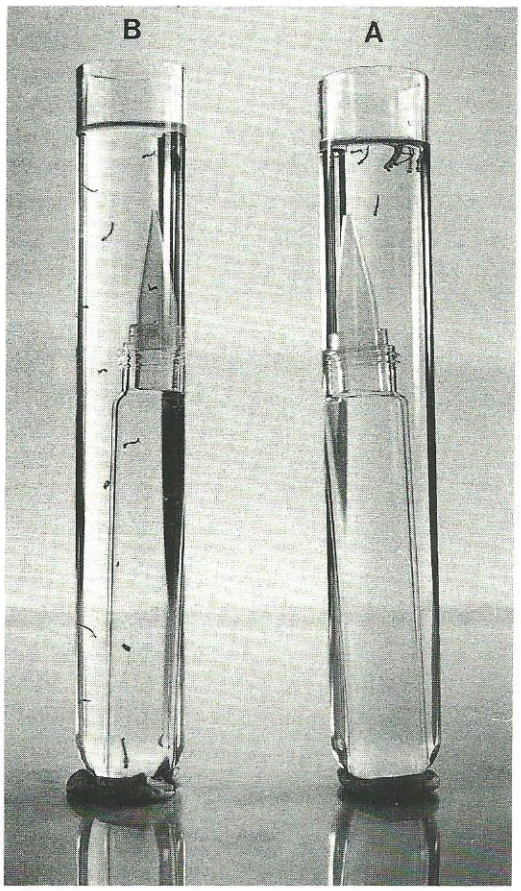

FIG. 2. The experimental arrangement (the device), about half an hour after being established. A glass vessel (15 × 3 cm) with 15 *A. aegypti* larvae and a 15 ml vial, closed by a 1 ml pipetor tip. (A) The test tube containing a mixture of 10^6 *T. pyriformis* and 2 mg of *B. thuringiensis* var. *israelensis* primary powder (with $2 \cdot 10^8$ spores), incubated for 2 hr at 27°C. (B) As in A, but without *T. pyriformis*.

ficacy follow: What is the life expectancy of *T. pyriformis* cells (loaded with the toxic powder)? What is their loading capacity and how long is the toxicity of the ingested powder maintained? What is the competitive-nutritional value of *T. pyriformis* cells for mosquito larvae? What is the LD_{50} of the system? If the answers to these, as well as to many additional questions, confirm the device's potential, its toxicity could perhaps be extended by manipulating its parameters such as the container's dimensions or by adding a food source for the *T. pyriformis* cells.

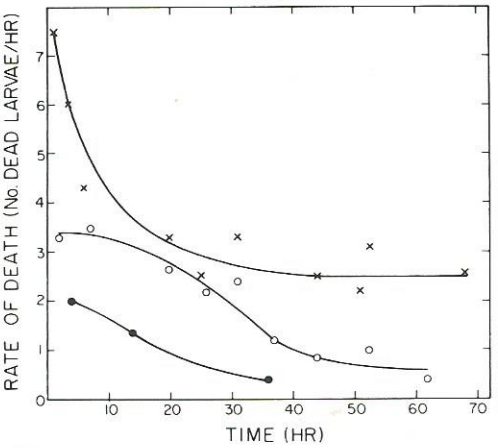

FIG. 3. Change of death rate of *A. aegypti* larvae in the device (Fig. 2) as a function of time. Each test tube contained 2 mg of powder and 700,000 (x), 140,000 (○), or 56,000 (●) *T. pyriformis* cells. Fifteen third instar larvae were introduced to the outer vessel at time 0, and dead larvae scored continuously. Dead larvae were replaced by live ones either when all 15 died or within 18 hr, to avoid secondary mortality due to cannibalism (A. Zaritsky and K. Khawaled, *J. Amer. Mosq. Control Assoc.* **2**, 555–559, 1986). The rate of death is expressed as the number of dead larvae scored divided by the respective time interval (hr).

KEY WORDS: Mosquito biocontrol; *Bacillus thuringiensis* var. *israelensis;* delivery of toxicity; *Tetrahymena pyriformis; Aedes aegypti* larvae; feeding behavior.

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