

# Recycling of *Bacillus sphaericus* in dead larvae of *Culex pipiens* (Diptera, Culicidae)

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## Summary

*Spotulation of Bacillus sphaericus strain 1593-4 was observed in the dead larvae of Culex pipiens. During the digestion (2-24 hours) we observe a decrease in the number of spores in the guts of living larvae accompanied by an increase of the vegetative cells. The viable spore count increased immediately and rapidly in corpses, after 0 time of death, and attained more than that observed in the living larvae. The spore count in corpses was sensitively increased during 3-90 days. The spores in corpses are highly insecticidal to other healthy larvae of Cx. pipiens during this period.*

**Key words :** Recycling — *Bacillus sphaericus* — Larvae — *Culex pipiens*.

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## Résumé

RECYCLAGE DE *BACILLUS SPHAERICUS* CHEZ LES LARVES MORTES DE *CULEX PAPIENS* (DIPTERA, CULICIDAE). *Une sporulation et une augmentation du nombre de spores viables de Bacillus sphaericus souche 1593-4 sont mises en évidence chez les larves mortes de Culex pipiens intoxiquées par cette bactérie. Pendant la digestion (2-24 h.) on constate une chute du nombre de spores ingérées chez les larves vivantes, accompagnée par une augmentation du nombre des cellules végétatives. L'augmentation du nombre de spores commence rapidement et immédiatement après la mort des larves. Le nombre de spores a sensiblement remonté dans les cadavres durant la période du 3<sup>e</sup> au 90<sup>e</sup> jour. De plus, ces spores ont été très toxiques pour les larves saines de Cx. pipiens pendant cette période.*

**Mots-clés :** Recyclage — *Bacillus sphaericus* — Larves — *Culex pipiens*.

## Introduction

*Bacillus sphaericus* strain 1593-4 was originally isolated from diseased *Cx. quinquefasciatus* larvae in Indonesia in June 1974. This strain develops full toxicity during sporulation (Myers *et al.*, 1979). *B. sphaericus* is among those organisms which kill their hosts by means of toxins (Davidson, 1984). The symptoms of intoxication of *B. sphaericus* were detected

after 30 min. (Davidson, 1975, 1981 ; Karch and Coz, 1983 ; Charles *et al.*, 1984). The spores of strain 1593 are not visibly degraded in the larval guts and germinate synchronously at 4-6 hours following ingestion (Davidson, 1981). This study examines the ability of *B. sphaericus* to recycle in corpses and the level of toxicity of the viable spores in these corpses.

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**Materials and method**

3rd and 4th young instars *Cx. pipiens* Linn. (Montpellier strain) were used in all tests and were from colonies maintained in the laboratory at a room temperature of  $25 \pm 2^\circ\text{C}$ . All larvae which were killed 24 hours after exposure to spore suspension (LC 90 = 0.02 mg/l) of lyophilisat powder of *B. sphaericus* strain 1593-4 were harvested and washed with distilled water. These corpses were put in glass jars containing 2 litres of distilled water where they remain during a three month experimental period.

**MICROBIOLOGICAL ANALYSIS**

Three duplicates for each time, five living larvae at 1/2, 4, 6 and 24 hours (or 0 time of death) and five other dead larvae taking at different periods as long as three months. These five living larvae or five dead larvae were crushed with glass rod and diluted in 5 ml of distilled water. Only dilution for the spore counts was pasteurized (12 min.,  $80^\circ\text{C}$ ). One hundred microliters of each dilution was cultivated in a Petri dish using a selective medium M.B.S. (Kalfon *et al.*, 1983) containing 100 mg/l streptomycin. Colonies were counted after a 48 hour incubation at  $35^\circ\text{C}$ .

**BIOASSAYS**

In a parallel direction to the bacteriological analysis, experiments and bioassays were conducted in order to evaluate the larvicidal activity of the viable spores found in corpses. For each bioassay twenty dead larvae intoxicated with *B. sphaericus* strain 1593-4 were crushed and added to 150 ml of distilled water in plastic cup which already includes twenty healthy larvae of *Cx. pipiens*.

**Results and discussion**

The decrease in the viable spore counts (2-24 hours) during the digestion of lyophilisat powder of *B. sphaericus* strain 1593-4 by *Cx. pipiens* larvae was accompanied by the increase of the vegetative cells (fig. 1). This result confirmed that the germination of spores in the guts of larvae occurred during this period. On the other hand, a high increase in the viable spore count started remarkably after death of larvae. Spore counts after the larval death reached a higher level than during the digestion period of living larvae ; from  $1.2 \times 10^2$  spores/larva at 0 hr,

it became  $1.9 \times 10^3$  et  $9.1 \times 10^5$  spores/larva at respectively 24 and 48 hours (fig. 1). Moreover, a small increase in the spore count in corpses was observed during 3-90 days (fig. 2). The data of this figure show that the number of cfu (spores + vegetative cells) is rather similar to the spore count, which means that most of the cfu are spores. It appears that the bacteria are using these corpses not only as a medium for growth but also as a protective shelter.

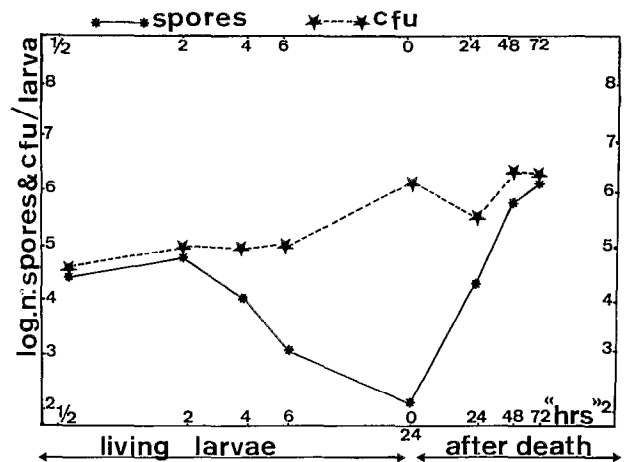


FIG. 1. — Variation in number of spores (average count) and in number of cfu (average count) during the 24 hour period preceding and the 72 hour period following the death of the 3rd and 4th instars of *Culex pipiens* exposed to 0.02 mg/l of *Bacillus sphaericus* strain 1593-4

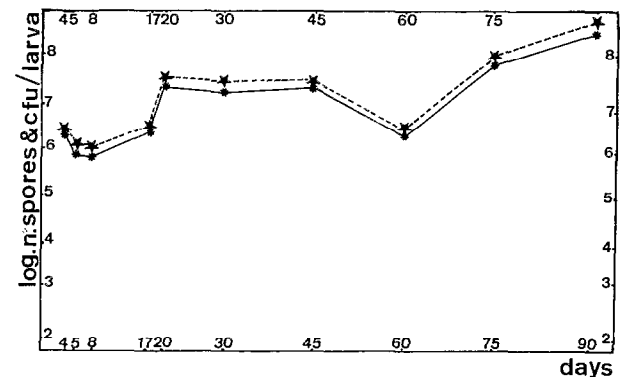


FIG. 2. — Average spore count and cfu count of *Bacillus sphaericus* in dead larvae of *Culex pipiens* 3rd and 4th instars, during three months following the death

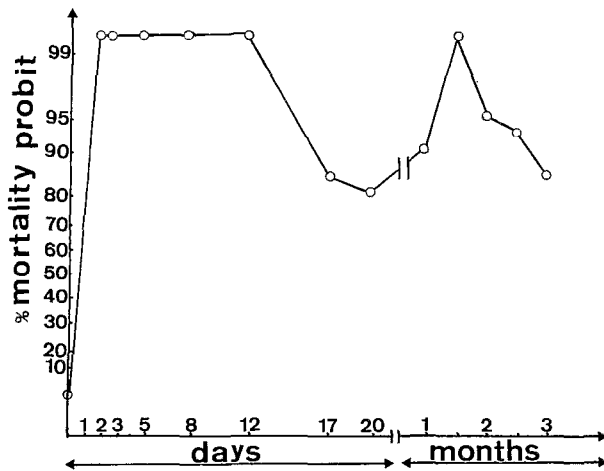


FIG. 3. — Larvicidal activity of the viable spores remaining in corpses of *Culex pipiens* during three months

The levels of toxicity of the viable spores in corpses were bioassayed. The data in figure 3 indi-

cate that the larval activity of the viable spores in corpses caused 100 % mortality between 1-12 days and remained high (82-99 %) during 12-90 days. This result indicates that the spores in corpses remained not only viable but also very toxic for other healthy larvae. Karch and Coz (1984) obtained a high larvicidal activity of spores in corpses which was more active than LC 50 and LC 90 using the powder of the same strain 1593-4.

Davidson (1984) observed that the recycling of *B. sphaericus* can occur in the dead host. This experimental result confirmed that *B. sphaericus* recycles in dead larvae of *Cx. pipiens*. The larvicidal activity of the spores in corpses was related to the number of viable spores remaining in the dead host. These spores were very toxic to other healthy larvae during at least three months.

As far as the mosquito control with *B. sphaericus* is concerned, more field testing should be carried out in order to investigate the possibility of corpse spores to infect and re-infect other larvae after the decomposition of dead larvae.

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