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On the capacity of Bacillus thuringiensis to spread in insect populations

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Abstract

A number of untreated larvae of *Ephestia kuehniella* and *Pieris brassicae* were confined with some larvae of the same species that had recently been infected with *Bacillus thuringiensis*. The majority of the last category died within a few days. Although healthy, diseased and dead specimens were kept together for several days, scarcely any larva of the healthy group became diseased or died by the action of the bacterium. It was concluded that *B. thuringiensis* does not spread easily.

Introduction

Today, numerous laboratories are breeding lepidopterous larvae as test animals for studies with *B. thuringiensis* Berliner but few epidemics due to this bacterium seem to occur. Norris and Burges (1963) reported spontaneous outbreaks in cultures of stored product insects (*Plodia, Ephestia* spp. a.o.). Drastic measures were necessary to check the epidemic which spread through several insectaries of the Pest infestation laboratory at Slough, England. In a mill near Paris, France, Kurstak (1964) found grain infested with *Ephestia kuehniella* Zell. This population contained many diseased larvae the principal agent being *B. thuringiensis*.

The activity of *B. thuringiensis* in the gut does not always induce death. Yamvrias (1962) observed that some larvae of *E. kuehniella* infected with *B. thuringiensis* even recuperated when the supply of contaminated food was discontinued.

As far as *B. thuringiensis* is concerned, stress factors do not seem to accentuate infection. Steinhaus (1958a) showed that crowding was not important in the spread of spore-forming bacteria. However, the susceptibility of *Peridroma*, normally low to *B. thuringiensis*, rose markedly when the insect was kept at 40 °C (Steinhaus, 1958b).

The only clear observation of spreading of a strain of *B. thuringiensis* in the field was reported by Talalaev (1961). In field operations against *Dendrolimus sibiricus* (Tshetv.), the epidemic spread from small initial to extended areas; in addition, dead infected larvae held in the webs served to reinfect the population after hibernation. The application with bacteria was repeated biannually and had to be made just before pupation of the larvae (see also Franz, 1961).

In this paper, results are reported of experiments investigating the capacity of the bacterial disease to spread from infected to untreated larvae in the laboratory.

Methods

The infection of the larvae was performed as follows.
(1) *Ephestia kuehniella.* A tube culture of *B. thuringiensis* was skimmed and 10 ml of distilled water added to the skim. A 0.003 ml droplet of this suspension (approx. 2×10^6 spores) was injected into the anus of 4th instar larvae. Eight out of twelve treated larvae died within 24 h showing the symptoms of the disease. Some of them were externally desinfected with 70% ethanol for 1 min and then placed on a sterile agar plate. After a few days, colonies of *B. thuringiensis* were visible on the plate around the larvae. Control larvae, which were injected with distilled water only, showed no symptoms.
(2) *Pieris brassicae.* 4th instar larvae were starved for 2–3 h and given a 4 mm diameter disk of cabbage leaf, on which a 0.001 ml droplet of a bacterial suspension (approx. 16×10^7 spores) was spotted. This was left to dry for 30 min. Each larva was confined to one disk for 16–18 h at 27 °C and 95% R. H. About 90% of the larvae had eaten the disk during that time. The other 10% were discarded from the experiment. About 80% of the larvae that had eaten the disk died during the following 10 days. Larvae treated in this way were used in the infection experiment. Placing some dying larvae on sterile agar plates gave evidence that death occurred due to *B. thuringiensis.* Infected and healthy larvae were confined together in plastic boxes (19 × 10.5 × 7 cm).

Results

Ephestia kuehniella
Three 4th instar larvae injected as described above, were immediately added to a box containing 10 healthy (3rd instar) larvae. This experiment was done in 6 replicates at 26–28 °C and 60–70% R. H. After 2 days, 10 out of the 18 treated larvae were dead from the bacterium, in each box 1 or 2 dead larvae being found. The dead bodies were left in the boxes. The untreated larvae, 60 in total, were all alive. After 21 days, all these larvae were still alive and most of them had pupated. It is concluded that under the circumstances of this experiment no spread of the pathogen occurred.

Pieris brassicae
The experiments with this species were complicated by the occurrence of a granulosis virus, although the stock originated from the Cambridge strain, which had developed a certain amount of resistance during breeding (David and Gardiner, 1966). If the virus disease appeared just before pupation, the specimens were still evaluated. Four (4th instar) infected larvae were brought together with 20 younger (3rd instar) healthy specimens. In the first experiment, 6 replicates were made but 2 of them were discarded because of early occurrence of the virus disease. Seven days after the beginning of the experiment, 80 untreated specimens were still alive. Of the 80 survivors, 59 pupated and 32 emerged normally. None showed any symptoms of bacterial disease.
In the second experiment, the healthy larvae were much younger (2 days old only) at the time when the infected ones were added. Nine replicates were made. Of the 4 × 9 infected specimens 32 died of bacterial disease within 7 days. In all boxes, 2 to 4 dead caterpillars were found and the dead bodies were left in the boxes. After 9 days, 177 of the untreated larvae (20 × 9) were still alive and 3 had died from the bacterial disease.

The larvae that were still alive (98.3 %) showed no symptoms of bacteriosis, not even during the entire larval development. Granulosis virus, however, started to spread in the cultures 14 days after the onset of the experiments. Forty-eight larvae (28 %) died because of granulosis. Finally 129 larvae pupated and 118 adults emerged. Casualties during pupation were also due to virus disease.

Discussion

B. thuringiensis is today the most promising bacterial pathogen in insect control work. Some laboratories, however, hesitate to work with this organism because it is feared that the insect cultures would be contaminated by this pathogen. Initially, precautions were taken in our laboratory to keep the insect cultures isolated from places where experiments with *B. thuringiensis* were carried out. In the course of the experimental work, it became more and more apparent that *B. thuringiensis* mostly acts as a chemical and very seldom as a biological agent. In our experimental series, treated with lethal or sublethal doses of *B. thuringiensis*, epidemics never occurred. Untreated series of insects kept together with the treated ones never became diseased. The following species of insects are very susceptible to *B. thuringiensis* and are regularly used in our experiments, but in the cultures never symptoms of the bacterial disease developed: *Ephestia cautella*, *E. elutella*, *Galleria mellonella*, *Malacosoma neustria*, *Pieris brassicae* and *Plodia interpunctella*.

From these experiments it is clear that dissemination of *B. thuringiensis* occurred only sporadically, although circumstances were made very favourable to spreading of the disease. With *P. brassicae*, only 3 out of 180 (1.7 %) and with *E. kuehniella* none out of 60 specimens became diseased. No species in which cannibalism occurs were tried. The disease may be spread when dying and dead larvae are consumed by the healthy ones.

Samenvatting

Verspreiding van Bacillus thuringiensis in insektenpopulaties

Onbehandelde rupsen van *Ephestia kuehniella* and *Pieris brassicae* werden samengebracht met een aantal rupsen van dezelfde soort, die kort tevoren anaal, resp. oraal geïnfecteerd waren met een suspensie van *B. thuringiensis*. Van de laatste categorie stierven de meeste na enkele dagen. Ofschoon de gezonde, zieke en dode rupsen daarna nog enige weken werden samengehouden, trad er onder de onbehandelde rupsen praktisch geen sterfte op, die veroorzaakt was door bacteriën.

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