# POSSIBLE REASONS OF RESISTANCE IN LARVAE OF *Spodoptera frugiperda* (ABBOT & SMITH, 1797) INFECTED BY *Bacillus thuringiensis* var. *kurstaki*

M.A. Garcia [1]
M. Simões [1]
M.E.M. Habib [1]

## ABSTRACT

Some extrinsic and intrinsic factors were investigated to determine their effect on the susceptibility of *Spodoptera frugiperda* larvae to *Bacillus thuringiensis* var. *kurstaki*. The effects of hemolymph, mid-gut macerate and regurgitate fluid of *S. frugiperda* larvae, as well as corn leaf extract, on the germination and development of the bacterium were evaluated. Neither the corn leaf extract nor the hemolymph, showed any inhibitory effect. Both mid-gut macerate and regurgitated fluid were considered inhibitory components. The inhibition, in the case of regurgitate, was significantly greater than that caused by the mid-gut macerate. It can be concluded that the gastric juice of the fall armyworm may contain an effective inhibitory factor, possible of chemical nature, which reduces the pathogenicity of *B. thuringiensis*.

## INTRODUCTION

Susceptibility of insects to a given pathogen may

[1] Universidade Estadual de Campinas, SP.

vary substantially within taxonomically similar groups, or even among natural populations of the same insect species. Physiological as well as enrivonmental conditions have been considered by some authors as significant reasons for resistance or suceptibility of insects to *Bacillus thuringiensis* (PRISTAVKO & DOVZHENOK, 1974; GOVINDARAJAN et al., 1975). STEINHAUS (1963) and NARAYANAN et al. (1976a) mentioned that the mid-gut pH regulates the activity of soluble enzimes and toxic substances produced by the bacterium, affecting by this way the germination and multiplication of the pathogen.

NARAYANAN et al. (1976b) demonstrated that, in addition to the role of pH, there are some reducing agents and proteolytic enzimes in the mid-gut which affect the mode of action of *B. thuringiensis* in *Spodoptera litura* and *Papilio demoleus* larvae. On the other hand, the penetration of the bacterium through the gut wall can be facilitated by other entomopathogens which possess a higher invasion capacity, provoking more rapid septicemia. Larvae of *Choristoneura fumiferana*, treated with *B. thuringiensis*, showed higher susceptibility when previously infected by *Nosema fumiferana* (SMIRNOFF, 1973).

Previous work has les us to consider *Spodoptera frugiperda* larvae as non susceptible to *B. thuringiensis* (GARCIA, 1979). The present paper concerns further investigations which attempt to disclose some of the possible reasons for this phenomenon. These investigations comprise analysis of the effect of some intrinsic and extrinsic factors (in relation to *S. frugiperda*) on germination, development and multiplication of *B. thuringiensis*, one of the most common microbial control agents of insect pests.

## MATERIAL AND METHODS

Thuricide-HP, produced by Sandoz LTD, Agro Division, USA, with $30 \times 10^6$ to $52 \times 10^6$ viable spores / mg, equivalent to 16,000 IU/mg was utilized as source of *B. thuringiensis* var. *kurstaki*.

Corn leaf extract, hemolymph, mid-gut macerate and regurgitate fluid of *S. frugiperda* larvae were tested as possible inhibitory factors to the pathogen. All these components were filtrated through Millipore membrane (025 µ) before evaluating their effect. Each of these substances was tested in five Petri dishes (2 repetitions each) with Nutrient Agar inoculated with 15 - 25x$10^7$ viable spores of the pathogen per Petri dish. This concentration corresponds to 75-125 spores per $cm^2$ of medium.

Four sterilized glass rings (1.5 cm diameter; 8 mm high) (Fig. 1) were partialy embedded in the medium of each Petri dish. In each ring a quantity of 6 µl of the tested substance was dropped. One of these rings, in each dish, was treated as control, receiving an equal volume of sterilized distilled water.

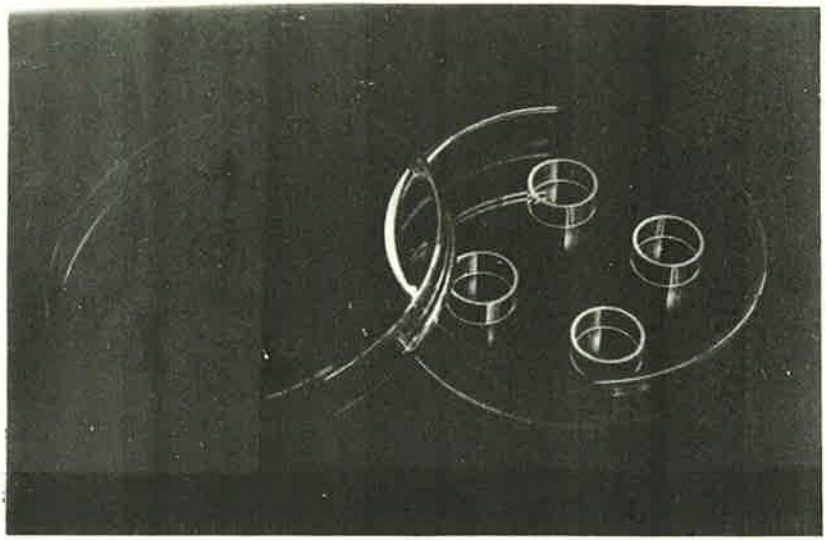

FIGURE 1 - Utilization of glass rings in Petri dishes for tests of inhibition.

The Petri dishes were incubated at 30ºC for 24 hours. Subsequently, bacterial germination and development were evaluated by comparison with the other treatments as well as control and untreated areas.

RESULTS AND DISCUSSION

The present study showed no inhibitory effect of the corn leaf extract on *B. thuringiensis*, in spite of its acidity level (pH = 5). This result indicates the absence of bacteriostatic as well as bactericidal substances in the natural host plant of *S. frugiperda* larvae. Similar results were obtained by GOVINDARAJAN **et al.** (1976) when they used extract of *Ricinus communis*, a host plant of *S. litura*, against the same pathogen. On the other hand, MORRIS (1969) mentioned that the high acidity of certain leaf extracts may reduce the intestine alcalinity, difficulting, in this case, the dissolution of the parasporal body of *B. thuringiensis*. Moreover, even when dissolution occurs, such condition may prevent bacterial and disease development.

The resistance of *S. frugiperda* larvae to *B. thuringiensis*, observed previously by GARCIA (1979) and HABIB & GARCIA (1981), led us in the present work to test three intrinsic factors. Figure 2 shows that the regurgitate fluid (R) inhibited almost completely the germination of the pathogen, while the hemolymph (H), as well as the distiled water (C) did not show any significant effect. The mid-gut macerate (I) provoked an inhibition slightly less intensive than the regurgitate.

These observations indicate that corn leaves, as well as hemolymph, are not involved in the non-susceptibility of *S. frugiperda* larvae to *B. thuringiensis*. Moreover, responsible factor, in this case, may exist in the alimentary canal.

Some physiological characteristics of the digestive system in many insects may influence the germination and development of insect pathogens. These characteristics include pH level, presence of reducing agents or substances acting as inhibitors.

HABIB & GARCIA (1981), studying pathology of *S. frugiperda*, showed that the pH in two levels did not have

any inhibitory effect on the germination and development of *B. thuringiensis*. These two pH levels tested correspond to those of hemolymph (6, 8) and of mid-gut (8,6). Therefore, when the pathogen was treated with mid-gut macerate or regurgitate fluid of *S. frugiperda* larvae, in the present study, the inhibition is not due to pH in itself. Moreover, since the regurgitate fluid provoked inhibition more intensive than the gut macerate, indicating more dilution of the inhibition factor in the later, it can be concluded that the factor responsible for inhibition occurs, more evidently, in the digestive juice itself, and not in the gut tissue.

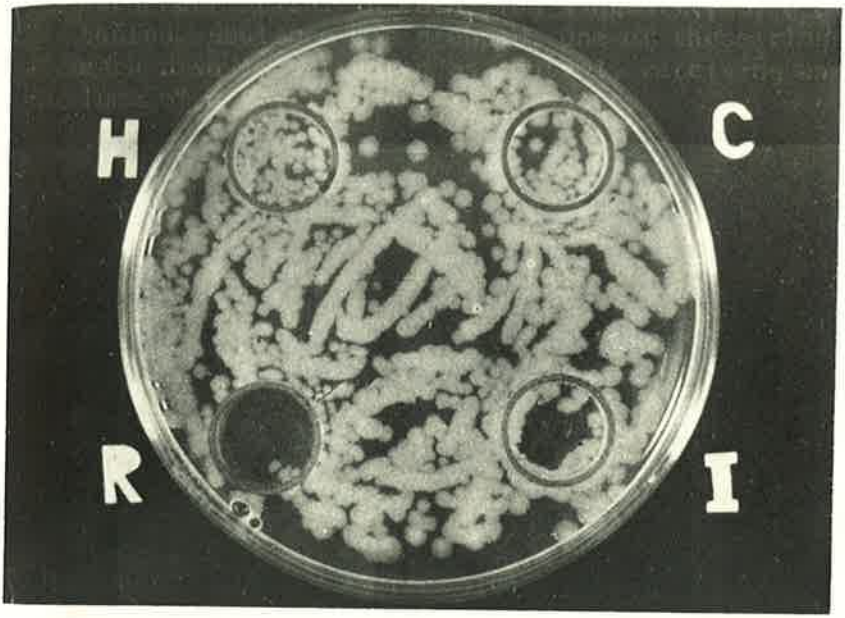


FIGURE 2 - Effect of hemolymph (H), regurgitate fluid (R), mid-gut macerate (I) and water (C) on germination of *B. thuringiensis*.

This conclusion is in agreement with GOVINDARAJAN et **al.** (1975) and NARAYANAN et **al.** (1976b). Both of these two groups suggested that the presence of chemical compo

nents, enzymes and reducing agents in the mid-gut lumen may be responsible for the non-susceptibility of *S. litura* larvae to *B. thuringiensis*.

Determination of the real nature of the inhibiting factor of *B. thringiensis* in *S. frugiperda* larvae would merit more detailed studies comprising isolation, purification and identification of the components of the gastric juice that would have bacteriostatic or bactericidal effect. Meanwhile, the results suggest that this factor is of a chemical nature.

RESUMO

Fatores extrínsecos e intrínsecos a *Spodoptera frugiperda* foram investigados como possíveis causas da não susceptibilidade deste noctuídeo a *Bacillus thuringiensis*.

Os efeitos da hemolinfa, conteúdo do intestino médio e regurgitado de larvas de *S. frugiperda* e extrato de folha de milho sobre a germinação e desenvolvimento de *B. thuringiensis* foram analisados.

O extrato de folha de milho, assim como a hemolinfa não afetam o patógeno, enquanto que o conteúdo do intestino médio e, em maior grau, o regurgitado atuam como anti-bacterianos.

Assim, sugere-se que o suco gástrico deve conter um fator inibidor de *B. thuringiensis* e que este fator, seja de natureza química.

LITERATURE CITED

GARCIA, M.A., 1979. **Potencialidade de alguns fatores bióticos e abióticos na regulação populacional de** *Spodoptera frugiperda* **(Abbot & Smith, 1797)** (Lepidoptera, Noctuidae), M.Sc. Thesis, UNICAMP.

factors affecting the pathogenicity of *Bacillus thuringiensis* var. *thuringiensis* to the European corn borer and fall armyworm. **J. Invert. Pathol.** 8: 365-375.

SMIRNOFF, W.A., 1973. The possible use of *Bacillus thuringiensis* plus chitinase formulation for the control of spruce budworm outbreaks. **J.N.Y. Ent. Soc.** 81: 196-200.

STEINHAUS, E.A., 1963. **Insect Pathology, an advanced treatise**, vol. I (661pp) and II (689 pp), Academic Press, N.Y. & London.