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## Comparison Between Preparations of *Bacillus thuringiensis* Berliner and Insecticides, Against *Leptinotarsa decemlineata* (Say) (Col. Chrysomelidae) in Potato Field.<sup>(+)</sup> (\*)

### INTRODUCTION

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera Chrysomelidae), is the key potato pest in Italy. In both the adult and larva stage it also damages eggplant and sometimes pepper and tomato by destroying leaves, sprouting flowers and young fruits. The farmer thus has to intervene with specific pest control techniques, or face losing all the crop.

In Emilia-Romagna, there are 2-3 generations per year: on the plain of the Campania region the CPB completes 3 generations (Priore and Tremola, 1971).

The CPB overwinters as an adult in diapause at a depth of 20-45 cm. It generally starts its activity in the following spring when the ground temperature is around 14 °C. In Italy the CPB control is compulsory by law. However rapid induction of resistance prevents chemical control (Forgash, 1981; Casagrande, 1987). In eggplant greenhouses, chemical control against this key pest renders even more problematic the biological control strategy against other pests using beneficial arthropods.

Some years ago in Italy the possibility of introducing the *Perillus bioculatus* F. (Heteroptera, Pentatomidae) was investigated. In the United States however, where this predator is naturally present, promising results were obtained (Hough-Goldstein and Keil, 1991; Biever and Chauvin, 1992), but this is not the case in Italy (Tremblay and Zouliamis, 1968). Recent studies of the possible applications of seasonal inoculative releases of the neotropical egg parasitoid *Edovum puttleri* Grissell on eggplant greenhouses and on potatoes together with microbial insecticides are on-going (Pucci and Dominici, 1988; Maini *et al.*, 1990; Pucci, 1992; Colazza and Bin, 1992).

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(<sup>+</sup>) Research supported by the Regional Project of IPM (Emilia-Romagna, Italy).

(\*) Accepted for publication September 9, 1993.

Microbial control using the entomopathogenic fungus *Beauveria bassiana* (Balsamo) is also being investigated in several countries (Roberts *et al.*, 1981).

Large scale prospective application of the *Bacillus thuringiensis* Berliner (*B.t.* subsp. *kurstaki* = *B.t.k.* and *B.t.* subsp. *tenebrionis* = *B.t.t.*) selective for Coleoptera is the most promising of all CPB control methods.

Among several species and strains of *B.t.*, the *B.t.t.* was tested against various species of Coleoptera (Burgio *et al.*, 1992; Ferrari and Maini, 1992; Tommasini *et al.*, 1992) and *B.t.t.* preparations are already on the market in many countries.

The efficacy of *B.t.t.* against CPB has been demonstrated in numerous field tests. Experiments have been conducted in Germany, Canada, USA, Switzerland and Italy. They have been seen to be efficient against young larvae, also in populations resistant to chemical insecticides, whether in open field trials on potato crops, or in the greenhouse on eggplant crops. So the efficacy of commercial preparations of *B.t.k.* and *B.t.t.* on potato crops, in Po Valley conditions, was compared with the efficacy of widely used chemical insecticide (Langenbruch *et al.*, 1985; Bassand, 1989; Guarda and Tassoni, 1990; Maini *et al.*, 1991).

#### MATERIALS AND METHODS

The trials were carried out in 1990 and 1992. In both cases the experiment consisted in a randomized design, with five replications per treatment. The plots were 22 m<sup>2</sup> by 6 rows of potato plants. The Monalisa variety of potato was used in 1990, and the Primura variety in 1992. 700 l/ha of water was used in each treatment, using a knapsack sprayer MOD. KWH S/40 Martignani.

In the first year were compared: - a commercial preparation of *B.thuringiensis* subsp. *tenebrionis*, (H8a8b, strain BI 256-82 according to Huger and Krieg, 1989), that belongs to *morrisoni* in conformity with the classification of deBarjac and Frachon (1990) strain "tenebrionis" (Novo strain NB 176: Novodor® FC), 15,000 BTTU/g, 3% active protein (7% of coleopteran active toxin CryIII A), at 5,000 cc/ha; - a commercial preparation of *B.thuringiensis* subsp. *kurstaki* (H3a3b3c), strain EG2424 (strain constructed by conjugal plasmid transfer), containing 2.25% of coleopteran active toxin CryIII A and 5.25% of lepidopteran active toxin CryIA(c), 5,300 IU on *T. ni* - 26,000 IU CPB/mg, at 14,000 cc/ha, provided by Siapa (Italy) as Foil® OF; - Deltamethrin (Decis®) at 30 ml/hl.<sup>(1)</sup>

The first spray was made at the beginning of the appearance of young larvae (May 23); after a week it was repeated with just microbiological preparations.

The second generation larvae was sprayed once (July 7) in the same way for each treatment. This was necessary because of a high level of larvae population in a short period of time.

In the second year, Novodor® FC at 5,000 cc/ha; the same formulation with an added protective substance, pinolene (1-p-menthene, Vapor Gard®), at 70 g/hl, produced by Miller Chemical and Fertilize Corp., Hanover, Pennsylvania, USA and distributed by Intrachem, Italy; and a Teflubenzuron (Escort®) insecticide,

<sup>(1)</sup> Until now, none of these *B.t.* have taxonomic standing according to the approved list of bacteriological names (*International Journal of Systematic Bacteriology*, up to 1992). The International Units are no longer be the accepted method of quantification of *B.t.* commercial formulations in USA, but we indicate as stated on the label of the *B.t.* products tested.

distributed by Rhône Poulenc Agro, at 150 ml/ha, were compared.

In 1992 the first treatment was carried out at the appearance of first generation larvae (May, 26) and repeated, with the microbiological formulation, after a week. No treatment was carried out on the second generation larvae since its outbreak coincided with the harvest.

In both the tests, the results were intended to quantify the number of larvae present on leaves and stems of the sampled plants. At each sampling were randomly chosen 4 plants/plot, and collected in the four central rows. In 1990, 4 weekly samplings were taken from the first generation, and 2 from the second generation, whereas only 3 were taken from the first generation in 1992. The sampled bushes were initially put in plastic bags and subsequently examined in the laboratory, one by one.

In 1992, moreover, Damage Index (DI) was calculated on the leaves and stems [DI =  $\sum(n \cdot cd)/N$ , where n = number of plants in a class of damage, cd = class of damage (1: 0-5 % , 2: 5-50%, 3: 50-100 % leaves and/or stems eaten) , N = total of sampled plants].

Data of the experiments were subjected to analysis of variance (one-way ANOVA), followed by orthogonal comparisons when significant ( $P < 0.05$ ) statistical difference occurred between treatments. The data were transformed into  $\log(x+1)$  for the elaborations; the homogeneity of variance was checked by Levene's test (Snedecor and Cochran, 1980).

#### RESULTS AND DISCUSSION

In 1990 significant differences were observed between the treatments and the control in the first and second generations, even if Deltamethrin manifested a modest insecticide activity against the second generation larvae (Figg. I-III; Tab. 1). The 2 microbiological formulations provided positive and significantly better results in respect of Deltamethrin.

Tab. 1 - Orthogonal comparison of the average number of CPB larvae (1990).

Comparison	Ist generation		IInd generation	
	F	P	F	P
Control vs. treatments	45.23	< 0.001	36.64	< 0.002
Deltamethrin vs. <i>B.t.</i>	5.41	< 0.05	40.75	< 0.001
<i>B.t.t.</i> vs. <i>B.t.k.</i>	0.06	> 0.05	0.002	> 0.05

Since *B.t.t.* was as effective as *B.t.k.* against CPB, and in Italy no other target insects, such as Lepidoptera larvae are damaging potato plants, the wider insecticide spectrum of this *B.t.k.* preparation, in our opinion is not completely necessary for practical application in such conditions. Recently in Italy was registered a *B.t.k.* preparation for CPB control: Foil, BFC that contains 9.75% of the coleopteran active toxin and no activity against Lepidoptera. The product, registered and distributed by Intrachem, Italy, is named: Jack Pot, BFC, and is registered and distributed under the name Ecotech Extra® by Roussel Hoechst Agrovet.

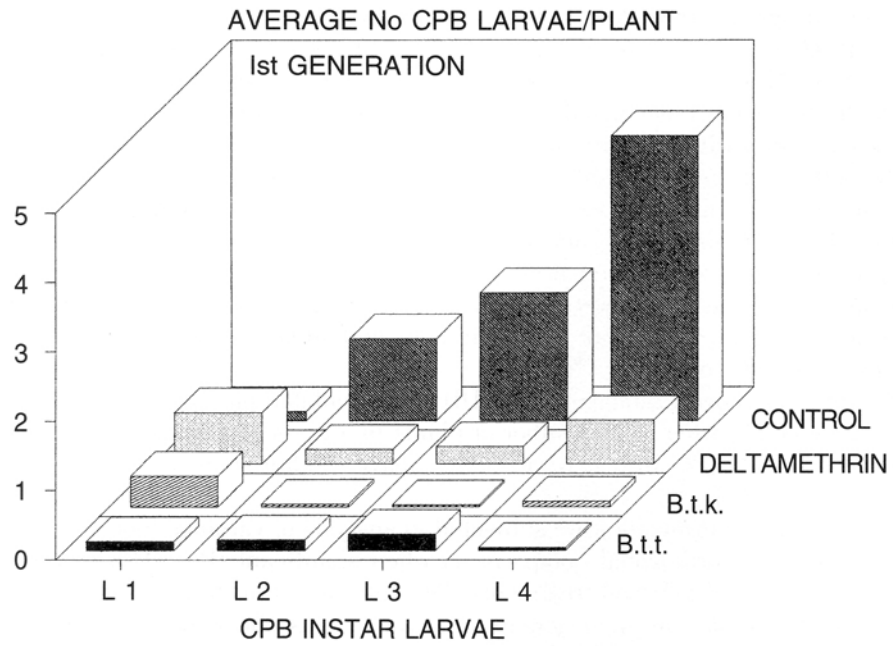


Fig. I - Average number of CPB larvae/plant (1st gen.) after treatments (1990).

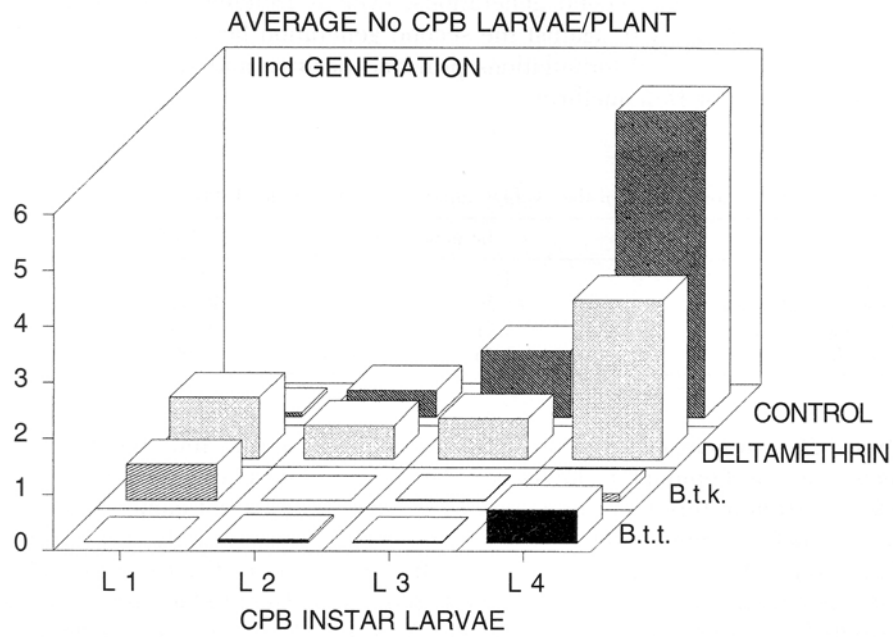


Fig. II - Average number of CPB larvae/plant (IIInd gen.) after treatments (1990).

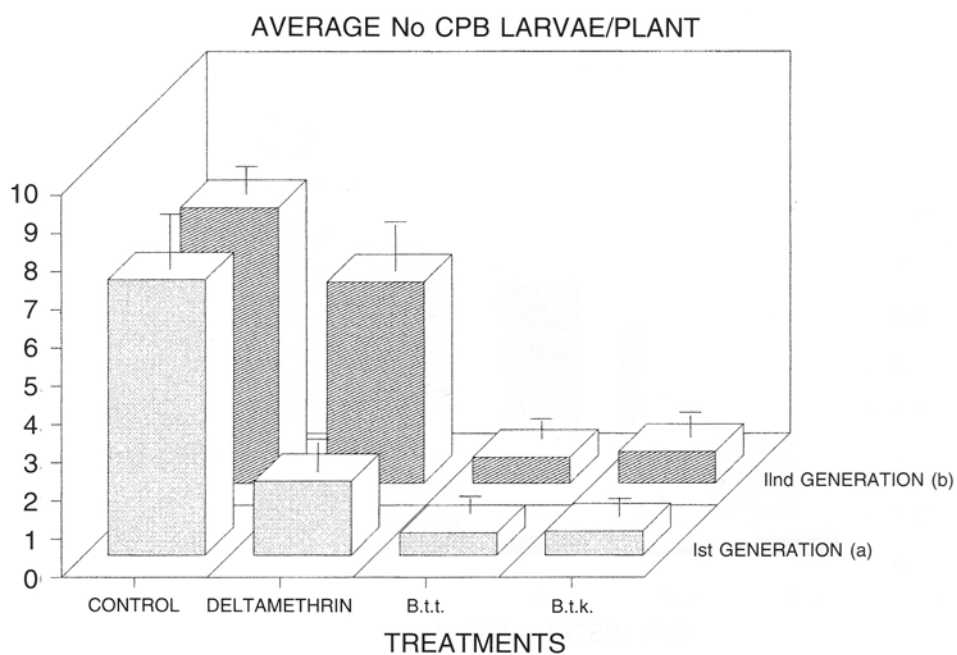


Fig. III - Average number of CPB larvae/plant in Ist and IIInd generation (1990);  
a:  $F = 16.9$ ; d.f. = 3,16;  $P < 0.001$  ; b:  $F = 25.79$ ; d.f. = 3,16;  $P < 0.001$  (ANOVA).

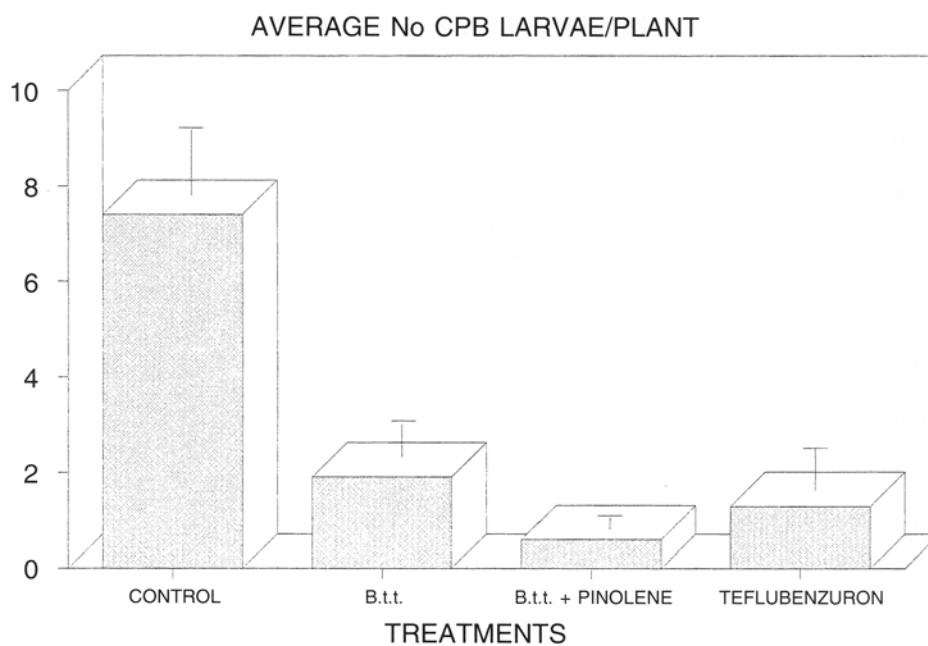


Fig. IV - Average number of CPB larvae/plant in Ist gen. (1992);  
 $F = 4.34$ ; d.f. = 3,16;  $P < 0.05$  (ANOVA).

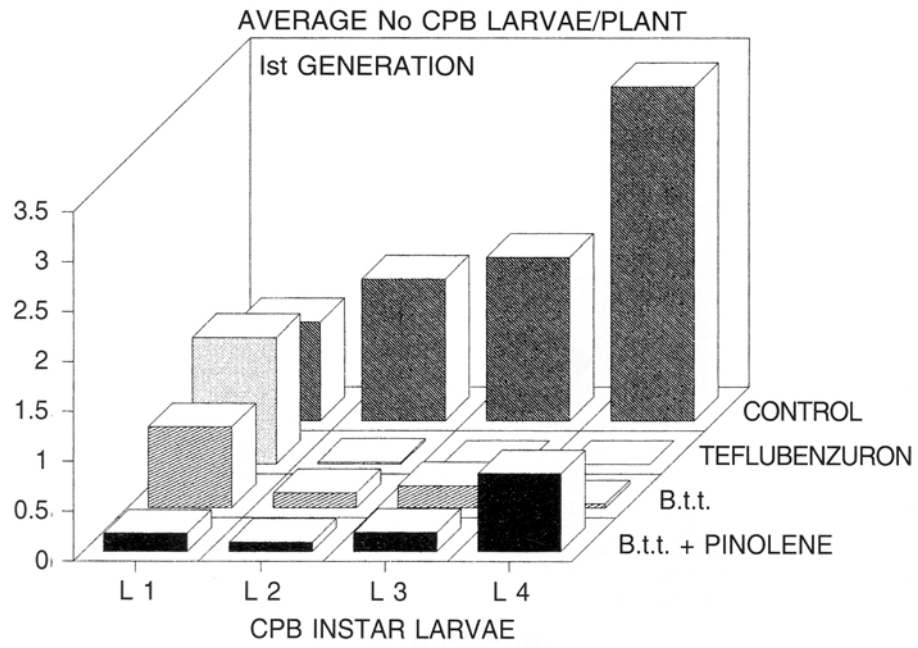


Fig. V - Average number of CPB instar larvae/plant (1st gen.) after treatments (1992).

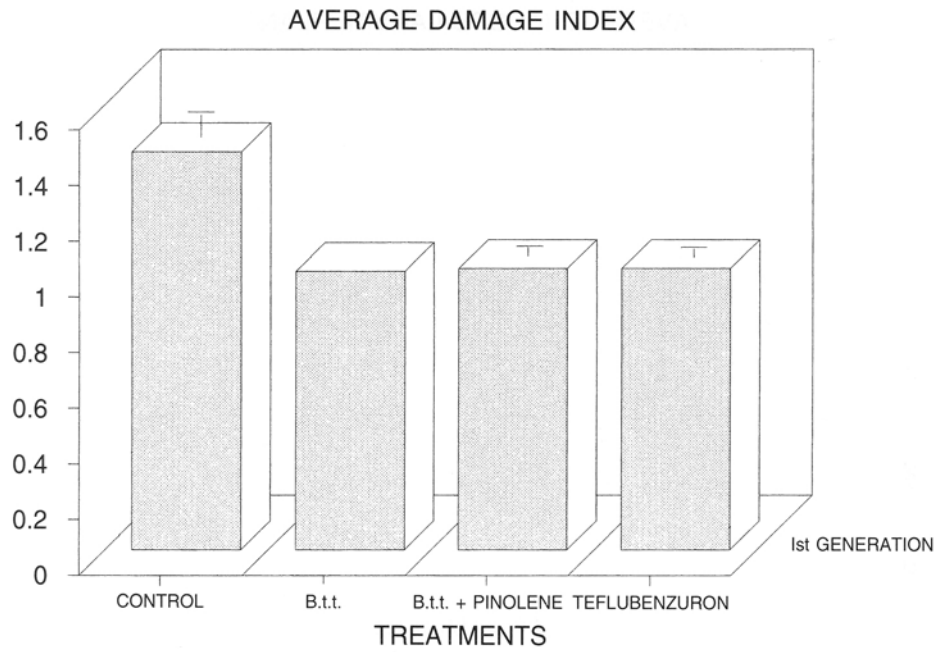


Fig. VI - Damage Index (DI) of CPB 1st gen. larvae (1992).  
 $F = 10.58$ ; d.f. = 3,16;  $P < 0.01$  (ANOVA).

Tab. 2 - Orthogonal comparison of the average number of CPB larvae ( $\bar{x}$  larvae) and Damage Index (DI) (1992).

Comparison	$\bar{x}$ larvae		DI	
	F	P	F	P
Control vs. treatments	12.68	< 0.01	31.74	< 0.001
Teflubenzuron vs. <i>B.t.t.</i>	0.24	> 0.05	0.005	> 0.05
<i>B.t.t.</i> vs. <i>B.t.t.</i> + pinolene	0.1	> 0.05	0.01	> 0.05

In 1992 significant differences were observed between the treatments and the control, while no significant differences were noted between *B.t.t.* and Teflubenzuron, which demonstrated a notable insecticide activity against CPB larvae (Fig. IV-VI; Tab. 2). The addition of 1-p-menthene to the microbiological formulation, did not have any particular influence on its efficacy (Fig. IV; Tab. 2). The *B.t.t.* treatments, even without the addition of a protective substance, conserved their validity. Ferro and Lyon (1991), in laboratory experiments showed the effects of temperatures on *B.t.* subsp. *san diego* activity against CPB larvae. During our field trials, the range of temperatures, did not apparently affect the level of control.

Guarda and Tassoni (1992) observed good results with frequent spraying (every 3 days) with *B.t.t.*; here however, we demonstrate how weekly applications are sufficient to guarantee a constant control of larval infestation, in accordance with Pucci (1992) and Jelušić *et al.* (1992).

In Italy, field condition and temperature after *B.t.* application are, therefore, favourable to control CPB larval population below economically damaging levels.

#### SUMMARY

Preparations of *Bacillus thuringiensis* Berliner subsp. *tenebrionis* (*B.t.t.*) (Novodor® FC), *B.thuringiensis* subsp. *kurstaki* (*B.t.k.*) (Foil®OF) and the insecticides Deltamethrin (Decis®) and Teflubenzuron (Escort®) against Colorado Potato Beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera, Chrysomelidae), were tested. In 1990 field trials, no differences in effectiveness between the two microbiological preparations were found against the larvae, but both of them, were found more effective than the Deltamethrin. In 1992 field trials, no differences in effectiveness between *B.t.t.* and Teflubenzuron, were found.

#### Confronto tra preparati a base di *Bacillus thuringiensis* Berliner e insetticidi contro la *Leptinotarsa decemlineata* (Say)(Col. Chrysomelidae).

#### RIASSUNTO

Sono stati sagggiati contro la Dorifora della patata, *Leptinotarsa decemlineata* (Say) (Coleoptera Chrysomelidae), dei preparati a base di *Bacillus thuringiensis* Berliner : *B. thuringiensis* ssp. *tenebrionis* (*B.t.t.*) (Novodor® FC), *B. thuringiensis* ssp. *kurstaki* (*B.t.k.*)(Foil® OF) e gli insetticidi Deltamethrin (Decis®) e Teflubenzuron (Escort®).

Nella prova di campo del 1990 non furono evidenziate differenze di efficacia nei riguardi delle larve del fitofago tra i due preparati microbiologici, ma questi manifestarono migliore attività insetticida rispetto a Deltamethrin.

Nel 1992, non si riscontrarono differenze di efficacia tra *B.t.t.* e Teflubenzuron.

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