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Susceptibility of *Leptinotarsa decemlineata* (Say) (Col. Chrysomelidae) Larvae and Adults to *Bacillus thuringiensis* Berliner subsp. *tenebrionis* Preparations. (*)

Introduction

Among the Coleoptera Chrysomelidae, *Leptinotarsa decemlineata* (Say), the Colorado potato beetle (CPB) plays a very important role as a target for *Bacillus thuringiensis* Berliner subsp. *tenebrionis* (*B.t.t.*). Because CPB, both in Europe and North America, is resistant to traditional insecticides, the use of microbial formulations appears to be a very promising alternative control tool that lacks harmful side effects (Casagrande, 1987; Ferrari and Maini, 1992). The susceptibility of CPB larvae to *B.t.t.* has been reported in several instances, but the effects of *B.t.t.* on CPB adults has not been clearly demonstrated (Hough-Goldstein *et al.* 1991; Keller and Langenbruch, 1993).

We carried out laboratory experiments on CPB larvae and adults to evaluate the efficacy of standard field dosages of *B.t.t.* preparations to complete the data on our wild strain of CPB (Ferrari *et al.* 1993). We tested the susceptibility of CPB adults because the effects of *B.t.t.* on adults of other Chrysomelidae species have been clearly shown, like reduced life span (Tommasini *et al.* 1992; Burgio *et al.* 1992; Keller and Langenbruch, 1993).

Materials and methods

The preparations tested were: - Novodor® FC (Novo Nordisk) a commercial preparation of *B. thuringiensis* subsp. *tenebrionis*, (H8a8b, strain BI 256-82, Novo strain 176, according to Huger and Krieg, 1989; 15,000 BTTU/g - 3 % active protein, 7% of coleopteran active toxin CryIIIA) at the dose of 5 l/1000 l of water (1.056 g/200 ml distilled water) and San 418 I WG 64 (Sandoz) (7400 BTTU/mg) at the dose of 1250 g/1000 l of water (0.25 g/200 ml distilled water).

The control was treated only with distilled water.

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The CPB strain tested was collected from potato and eggplant fields in the Emilia-Romagna Region and then reared for several generations on potato in a greenhouse at the Institute of Entomology "G. Grandi", Bologna University.

Test units were 1/4 litre glass jars closed on top with a perforated lid and tulle screen and with a piece of absorbent paper inside on the bottom. Ten individuals per replicate for 4 replicates were assayed.

Experiment 1: Larval susceptibility. All larval instars (L1, L2, L3, L4) were tested. Potato leaves were dipped in the *B.t.t.* solutions and dried at room temperature and then offered to larvae inside the jars. One day later, untreated potato leaves were added to feed the surviving larvae. Three potato leaves a day were given to L3-L4 CPB and one to L1-L2 CPB larvae. Larvae were observed daily until death or moulting occurred, for a maximum of 10 days.

Experiment 2: Adult susceptibility. Newly emerged adults (0-24 h old) were tested as in the experiment described above. Five potato leaves were put inside the jars. We tested two food regimes (FR): a) continuous supply of *B.t.t.* treated leaves (FR1), b) CPB adults supplied for the first 24 h with *B.t.t.* treated leaves and then with untreated leaves only (FR2). The mortality rate was observed up to the 10th day.

Statistical analyses: Percent mortality data were transformed by arcsin $\sqrt{x}$ and analysed by factorial ANOVA for repeated measures. The comparison of the control mean to each other group mean was detected by Dunnet procedure.

To analyse experiment 1 data, we considered the factors: larval instars (S) (levels: L1, L2, L3, L4), preparation (P) (levels: Novodor, San 418), post-treatment time (T) (levels: 1-till moult or 1-10 days).

To analyse experiment 2 data, we considered the factors: food regime (FR) (levels: *B.t.t.* treated leaves only once, *B.t.t.* treated leaves continuously), preparation (P) (levels: Novodor, San 418), time (T) (levels: 1-10 days).

The relationship between percent mortality and time was analysed by curvilinear regression $Y = A/(1+B\ \rho^x)$ (Snedecor and Cochran, 1980).

Results and Discussion

Experiment 1: in table 1 we report the mortality rates of the different CPB instar larvae, related to post-treatment time and preparations. First and second instar larvae are the most susceptible, followed by third instar and the least affected are the last instar larvae. In table 2, the 3-way ANOVA of mortality related to the factor studied is shown. Significant effects were detected for larval instars, with higher susceptibility of L1-L2 than for L3-L4. The increase of mortality over time and the interaction between Stage*Time were significant. No difference in insecticide efficacy was shown between preparations. The trends of mortality of the two preparations relative to different instars over post-treatment time, are shown in fig. I. Statistical parameters of the calculated curvilinear relations are presented in tables 3, 4. The mortality rate of the control larvae was less than 5% and regarding only the L4 CPB larvae at 8-10 days post-treatment. For this reason the mortality of control was not considered.

Tab. 1 - Mortality (%) (mean ± se) of CPB larvae, in the different instars and over time by two *B. thuringiensis* subsp. *tenebrionis* preparations; C = control, Nov = Novodor, San = San 418.

Days post-treatment	C	L 1 Nov	L 1 San	C	L 2 Nov	L 2 San	C	L 3 Nov	L 3 San	C	L 4 Nov	L 4 San
2	0	22.50 ±4.78	50.00 ±28.86	0	10.00 ±7.07	35.00 ±12.58	0	0	0	0	0	0
3	0	97.50 ±2.50	100	0	25.00 ±13.22	72.50 ±20.96	0	0	0	0	0	
4	0	100	100	0	47.50 ±14.93	82.50 ±11.08	0	5.00 ±2.88	0	0	2.50 ±2.50	2.50 ±2.50
5	/	100	100	0	57.50 ±11.08	82.50 ±11.08	0	10.00 ±4.08	5.00 ±5.00	2.50 ±2.50	7.50 ±4.78	5.00 ±2.88
6	/	100	100	0	75.00 ±8.66	82.50 ±11.08	2.50 ±2.50	25.00 ±6.45	15.00 ±6.45	2.50 ±2.50	20.00 ±10.80	22.50 ±4.78
7	/	100	100	0	75.00 ±8.66	85.00 ±8.66	7.50 ±2.50	57.50 ±12.50	72.50 ±7.50	7.50 ±2.50	27.50 ±7.50	40.00 ±7.70
8	/	100	100	0	75.00 ±8.66	85.00 ±8.66	7.50 ±2.50	75.00 ±10.40	80.00 ±7.07	7.50 ±2.50	37.50 ±6.29	47.50 ±8.53
9	/	100	100	0	75.00 ±8.66	85.00 ±8.66	10.00 ±0.0	77.50 ±8.53	80.00 ±7.07	7.50 ±2.50	50.00 ±4.08	60.00 ±4.08
10	/	100	100	0	75.00 ±8.66	85.00 ±8.66	10.00 ±0.0	80.00 ±7.07	85.00 ±2.88	10.00 ±0.0	57.50 ±2.50	65.00 ±2.88

Tab. 2 - ANOVA of mortality for CPB larvae. S = larval instars (L1, L2, L3, L4); P = preparation (Novodor, San 418); T = post-treatment time (1-till moult or 1-10 days).

Treatments	F value	P level
S	62.4	< 0.001
P	1.7	> 0.05
T	215.3	< 0.001
S*P	1.64	> 0.05
S*T	22.2	< 0.001
P*T	1.2	> 0.05
S*F*T	1.1	> 0.05

Tab. 3 - Parameter estimates of the curvilinear regressions related to larval mortality (Novodor). Model: $Y = A/(1+B\rho^x)$.

Larval instar	A	B	ρ	r
L1	100	61958	0.007	0.99
L2	75.72	67.93	0.31	0.99
L3	80.37	19238	0.21	0.99
L4	64.41	307.52	0.46	0.99

Tab. 4 - Parameter estimates of the curvilinear regressions related to larval mortality (San 418). Model: $Y = A/(1+B\rho^x)$.

Larval instar	A	B	ρ	r
L1	100.06	28663.35	0.005	0.99
L2	83.87	221.81	0.08	0.99
L3	82.09	$170293 \cdot 10^4$	0.03	0.98
L4	$898256 \cdot 10^2$	$185282 \cdot 10^2$	0.76	0.90

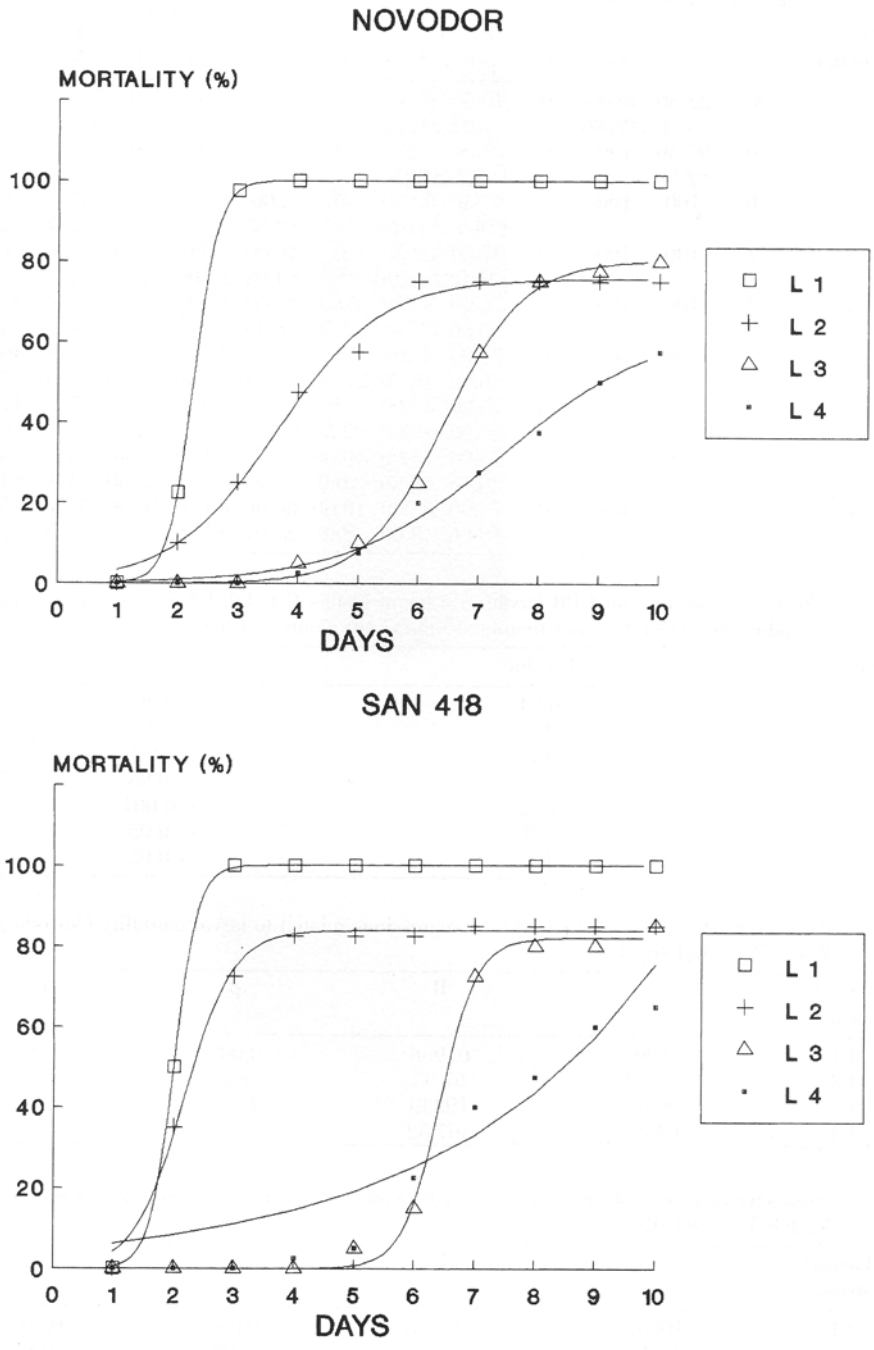

Fig. I - Trends of cumulative mortality for the four instars of *Leptinotarsa decemlineata* larvae.

Experiment 2: in table 5 are reported the results of CPB adults fed with *B.t.t.* treated potato leaves and for the two different food regimes. After 5 days for the group continuosly fed *B.t.t.* treated leaves and after 6 days for the group fed *B.t.t.* treated leaves only once, a significant effect of both preparations *vs.* control was detected (Dunnett test, $P < 0.05$). Higher mortality occurred when CPB consumed only *B.t.t.* potato treated leaves (Table 6). As with CPB larvae, no difference was detected between the activity of *B.t.t.* preparations on the adults. The interaction between Food Regime*Time was significant as shown in fig. II and therefore was analysed separately for the two preparations.

These effects of *B.t.t* ingestion on CPB adults have not been clearly documented in experiment carried out by other researchers so far. Possible reasons may be different susceptibility of CPB strains (Keller and Langenbruch, 1993), or because previous bioassays were done by observing adults mortality only throughout

Tab. 5 - Mortality (%) of CPB adults, in the different food regimes over time, by two *B. thuringiensis* subsp. *tenebrionis* preparations; C = control, Nov = Novodor, San = San 418.

Days	FR 2			FR 1		
	C	Nov	San	C	Nov	San
2	0	0	0	0	2.50 ±2.50	5.00 ±2.88
3	0	2.50 ±2.50	2.50 ±2.50	0	7.50 ±4.78	17.50 ±8.53
4	0	12.50 ±2.50	5.00 ±2.88	0	12.50 ±6.29	22.50 ±6.29
5	7.50 ±4.78	25.00 ±9.57	12.50 ±4.78	7.50 ±4.78	22.50 ±4.78	32.50 ±7.50
6	12.50 ±2.50	35.00 ±8.66	25.00 ±8.66	12.50 ±2.50	47.50 ±19.31	47.50 ±9.46
7	12.50 ±2.50	40.00 ±10.00	32.50 ±10.30	12.50 ±2.50	62.50 ±13.14	75.00 18.92
8	15.00 ±2.88	40.00 ±10.00	42.50 ±8.53	15.00 ±2.88	75.00 ±13.22	77.50 ±16.52
9	17.50 ±2.50	45.00 ±8.66	45.00 ±9.57	17.50 ±2.50	90.00 ±4.08	85.00 ±11.90
10	17.50 ±2.50	52.50 ±4.78	47.50 ±7.50	17.50 ±2.50	92.50 ±2.50	92.50 ±4.78

Tab. 6 - ANOVA of mortality for CPB adults.
FR = food regimes (continuos supply of *B.t.t.* treated leaves); P = preparation (Novodor, San 418); T = time (1-10 days).

Treatments	F value	P level
FR	11.9	< 0.01
P	0.04	> 0.05
T	83.4	< 0.001
FR*P	0.2	> 0.05
FR*T	5.6	< 0.001
FR*P*T	0.93	> 0.05

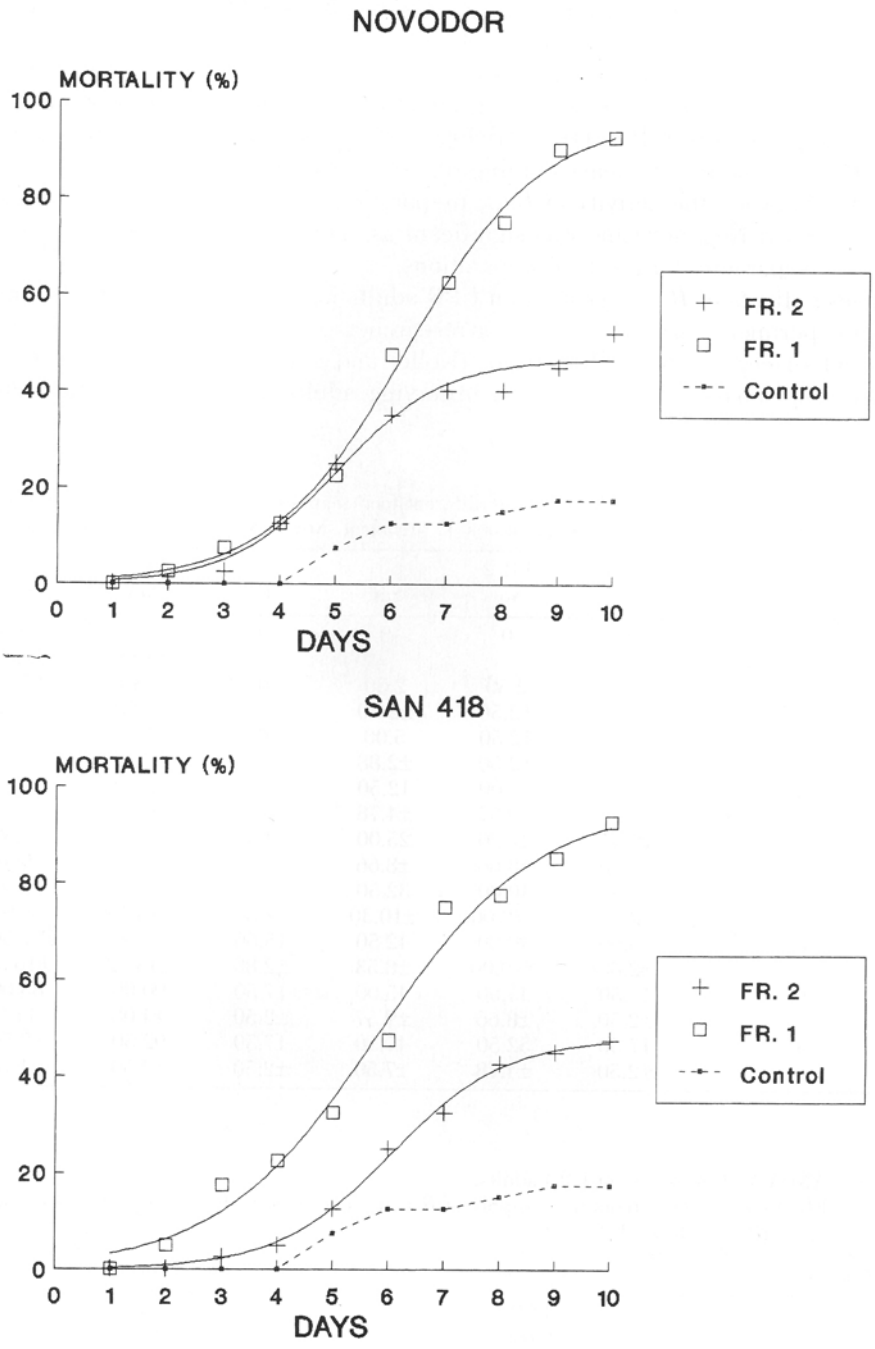

Fig. II - Trends of cumulative mortality for *Leptinotarsa decemlineata* adults (FR 1= continuous supply of *B.t.t.* treated potato leaves; FR 2= supply of *B.t.t.* treated potato leaves for the first 24 h and then with untreated leaves only).

Tab. 7 - Parameter estimates of the curvilinear regressions related to adult mortality (Novodor). Model: $Y = A/(1+B\rho^x)$.

Food Regimes	A	B	ρ	r
Feed once (FR 2)	46.82	185.48	0.35	0.98
Feed continuosly (FR 1)	96.77	174.78	0.43	0.99

Tab. 8 - Parameter estimates of the curvilinear regressions related to adult mortality (San 418). Model: $Y = A/(1+B\rho^x)$.

Food Regimes	A	B	ρ	r
Feed once (FR 2)	48.1	361.17	0.37	0.99
Feed continuosly (FR 1)	96.21	57.51	0.49	0.99

the first three days post-treatment. Moreover the efficacy of *B.t.t.* preparations on adults of different Chrysomelidae species is well documented (Tommasini *et al.*, 1992; Burgio *et al.*, 1992; Keller and Langenbruch, 1993), so, we can assume that, at least related to the amount of *B.t.t.* crystals ingested, the effects on CPB adults are not simply feeding inhibition but an action that causes significant mortality after 5 days. Similar results were observed by Hough-Goldstein *et al.* (1991), where in experiments to demonstrate the effect of the consumption of foliage treated with the M-One formulation of *B.t.t.* (*B.t.* var. *san diego*), all CPB adults died within 8 days of the continuous ingestion of *B.t.t.* treated potato leaves. In the case of the consumption of M-One treated leaves only once and then of untreated ones, the CPB adults showed some recovery in consumption but still adult life span was less than for the control. In our opinion the fact that consumption of *B.t.t.* treated foliage reduces the life span and egg laying of CPB indicates that the use of *B.t.t* is an effective control method for CPB.

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SUMMARY

Laboratory experiments on CPB larvae and adults were carried out to evaluate the efficacy of two *Bacillus thuringiensis* Berliner subsp. *tenebrionis* preparations (Novodor® FC and San 418 I WG 64). First and second instar larvae were the most susceptible, followed by the third and fourth instar. The trends of mortality of the two preparations over post-treatments time are shown and discussed. Mortality of CPB adults was significantly greater for preparations compared to control. The higher mor-

tality when adults consumed continuosly treated potato leaves compared to when they consumed treated leaves once was shown. No difference in insecticide efficacy was observed between preparations for either larvae or adults.

Attività di due preparati a base di *Bacillus thuringiensis* Berliner ssp. *tenebrionis* nei confronti di larve ed adulti di *Leptinotarsa decemlineata* (Say) (Col. Chrysomelidae).

RIASSUNTO

Sono state condotte prove di laboratorio per valutare l'efficacia di due preparati (Novodor® FC e San 418 I WG 64) nei confronti delle larve e gli adulti di *Leptinotarsa decemlineata* (Say). Le larve di prima e seconda età si sono mostrate più sensibili rispetto alla larve di terza e quarta età; quest'ultime registrarono una mortalità significativa rispetto al testimone solo dopo 5-6 giorni dal trattamento. I due formulati hanno evidenziato inoltre un'attività insetticida verso gli adulti, con un effetto significativo verso il testimone, dopo 5-6 giorni dal trattamento; una mortalità maggiore fu osservata per il gruppo di adulti alimentati continuamente con foglie trattate. Non è stata rilevata differenza di efficacia fra i due formulati, sia nell'attività larvicida che adulticida.

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