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THE PATHOGENICITY OF *BACILLUS THURINGIENSIS* STRAINS HD-263 AND HD-251 TO THE LARVAE OF *BOARMIA SELENARIA* (LEPIDOPTERA : GE OMETRIDAE)

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ABSTRACT

Several isolates of *Bacillus thuringiensis* Berliner *(B.t.)* belonging to serovar 3a3b, *kurstaki* were incorporated into an artificial diet and tested for their larvicidal activity against the giant looper, *Boarmia selenaria* Schiffermuller (Geometridae), which is a major pest of avocado in Israel. The mortality rate and retardation in development of second, third, fourth and fifth-instar larvae were determined, as well as the pupation rate of fifth instars. Isolate HD-263 had a higher insecticidal activity against all tested larvae than Dipel^{R} WP or the standard HD-1-S-1980 (both serovar 3a3b, *kurstaki).* The potencies found ranged from 1.7 to 22.8 times the potency of the reference. HD-251 was in general slightly more effective than Dipel and the standard, with potencies ranging from 0.8 to 13.8 times the reference potency; the high potencies were obtained against fifth instars. All products had an increasing effectivity with decreasing larval size and with increasing concentration. For HD-263 the LC_{50} after 5 days ranged from 2.2 *B.t./ml* diet for second instars to 19.6 *µg B.t./ml* diet for fifth instars. For HD-251, these figures were 3.0 and 52.1, respectively. Based on the results of this research, the HD-263 strain is now produced commercially in Israel and used for the control of *B. selenaria* in avocado orchards.

KEY WORDS: *Bacillus thuringiensis* var. *kurstaki;* HD-263 and HD-251 strains, pathogenicity of; *Boarmia selenaria;* bioassay.

INTRODUCTION

Bacillus thuringiensis Berliner *(B.t.)* is used to control *Boarmia selenaria* Schiffermiiller in avocado groves in Israel. Only young larvae (first and second instars) of this pest are sensitive to the commercial preparations of *B.t.,* and then at relatively high concentrations (Swirski et al., 1988; Wysoki and Izhar, 1986). Attempts have been made to improve the control by searching for isolates with greater activity against more developed larvae (Cohen et al., 1983; Wysoki and Jarvinen, 1986; Wysoki and De Haan, 1988; Wysoki and Scheepens, 1988). Therefore, four isolates of the serovar 3a3b, *kurstaki, viz.,* HD-251A, HD-251B, HD-263A and HD-263B, were bioassayed and compared with the standard HD-1-S-1980 and the commercial product Dipel^{R} WP of the same serovar.

MATERIALS AND METHODS

Boarmia selenaria was reared at 27 ± 1°C and an RH of 45% on a semi-artificial diet (Shorey and Hale, 1965). Modifications were made in the diet used in the tests: a formaldehyde solution replaced

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synthomycine and a B-vitamin complex mixture, and choline chloride was used instead of yeast. The dry powders HD–251A, HD–251B (2×10^9 spores/g), and HD–263A, HD–263B (9×10^8 spores/g) were tested and compared with the standard HD–1–S–1980 (containing 16,000 IU/mg) or the commercially available product Dipel^R WP (all of serovar 3a3b, *kurstaki*); A, B are different batches of the product. Each bioassay included a control with Tween only. Transparent plastic cups (65 mm diam., 40 mm depth) were used, with five larvae per cup and three of the fifth-instar larvae. Experiments were carried out with second ($n = 35$), third ($n = 50$ or 100), fourth ($n = 50$) and fifth ($n = 30$) instars on diet samples which contained 1.0, 2.1, 4.2, 8.3, 16.5, 25.0 and 33.3 μg bacterial powder per ml food. Larvae were kept for 5 days at 27 ± 1°C, 70% RH and at 16L, 8D photoperiod and weighed before and after the test. Mortality was recorded on days 2, 3, 4 and 5 following initiation of the test, corrected by Abbott's formula (1925) and LC_{50} values were calculated from dosage mortality regressions using log probit analysis (SAS, 1985). The potency of the *B.t.* isolates was calculated according to Dulmage et al. (1971). Data were evaluated statistically according to the logit model by the CATMOD procedure of SAS (1985).

RESULTS AND DISCUSSION

Isolate HD–251A was less effective against second, third and fourth instars, and more effective against fifth instars than the standard or Dipel after 5 days. Isolate HD–251B was more effective than the standard or Dipel against all sizes (Tables 1, 2, 3 and 4). At all observations, after 3, 4 or 5 days of feeding with *B.t.*, the isolate HD–263B caused the highest mortality, followed by HD–263A (Tables 1, 2, 3 and 4). After 2 days of exposure of third and fourth instars, mortality was less than 35% (third instar) or 12% (fourth instar) in all treatments and concentrations. After 2 days, HD–263A caused a significantly ($P = 0.05$) higher mortality at a concentration of 33.3 μg/ml than did Dipel against third-instar larvae. In all other cases, after a 2-day period, no differences were found to be significant according to logit analysis. After 3, 4 or 5 days, however, HD–263A and HD–263B were much more effective than the other isolates (Tables 2 and 3). Generally, the susceptibility of all isolates decreased with an increase in larval size and with a decrease in concentration.

TABLE 1

LC_{50} (μg *B.t.*/ml diet), 95% fiducial limits (μg *B.t.*/ml diet), slope and potency (IU/mg) of *Bacillus thuringiensis* isolates on second-instar *Boarmia selenaria* larvae after 3 and 5 days of exposure

Bacterial preparation	LC_{50}	Fiducial limits	Slope ± SE	Potency	Exposure period (days)
HD–1–S–1980	33.5 a	b	0.463 ± 0.251	16,000	3
HD–251A	8.8	7.1–11.1	1.188 ± 0.142	60,909	3
HD–251B	7.4 a	b	0.658 ± 0.213	72,432	3
HD–263A	3.6 a	0.2–16.4	0.677 ± 0.185	148,889	3
HD–263B	1.5 a	b	0.258 ± 0.225	357,333	3
HD–1–S–1980	4.2	3.4–5.3	1.098 ± 0.130	16,000	5
HD–251A	5.6 a	1.7–22.6	1.138 ± 0.282	12,000	5
HD–251B	3.0 a	b	1.111 ± 0.423	22,400	5
HD–263A	2.2 a	b	1.188 ± 0.549	30,545	5
HD–263B	c	b	–383.096	b	5

[a]Values followed by the same letter differ significantly at $P < 0.01$, according to the chi-square test.
[b]The value could not be calculated.
[c]The LC_{50} could not be calculated, but mortalities at different concentrations were highly significantly greater than those of the standard, according to logit analysis.

TABLE 2

LC_{50} (μg *B.t.*/ml diet), 95% fiducial limits (μg *B.t.*/ml diet), slope and potency (IU/mg) of *Bacillus thuringiensis* isolates on third-instar *Boarmia selenaria* larvae after 3 and 5 days of exposure

Bacterial preparation	LC_{50}	Fiducial limits	Slope ± SE	Potency	Exposure period (days)
Dipel	19.8	17.3–23.1	0.974 ± 0.097	16,000 c	3
HD–251A	20.2	16.6–25.8	0.659 ± 0.100	15,683	3
HD–251B	17.7	14.9–21.4	0.760 ± 0.102	17,898	3
HD–263A	4.7 a	d	0.598 ± 0.272	67,404	3
HD–263B	3.8 a	d	0.488 ± 0.296	83,368	3
Dipel	6.5 a	4.4–8.8	1.318 ± 0.191	16,000 c	5
HD–251A	7.2 ab	–16.1	1.080 ± 0.374	14,444	5
HD–251B	5.9	4.9–6.7	1.441 ± 0.155	17,627	5
HD–263A	2.1 a	d	0.792 ± 0.345	49,524	5
HD–263B	1.7 a	d	0.700 ± 0.353	61,176	5

[a]Values followed by the same letter differ significantly at $P < 0.01$, according to the chi-square test.
[c]Potency according to the manufacturer.
[d]The value could not be calculated.

TABLE 3

LC_{50} (μg *B.t.*/ml diet), 95% fiducial limits (μg *B.t.*/ml diet), slope and potency (IU/mg) of *Bacillus thuringiensis* isolates on fourth-instar *Boarmia selenaria* larvae after 4 and 5 days of exposure

Bacterial preparation	LC_{50}	Fiducial limits	Slope ± SE	Potency	Exposure period (days)
HD–1–S–1980	30.1 a	13.6– b	0.818 ± 0.228	16,000	4
HD–251A	29.5	23.6–40.2	0.973 ± 0.137	16,325	4
HD–251B	25.3 a	8.4– b	0.863 ± 0.284	19,036	4
HD–263A	16.9 a	5.8– b	0.822 ± 0.232	28,497	4
HD–263B	11.6	9.8–13.6	1.297 ± 0.126	41,517	4
HD–1–S–1980	15.2	12.7–18.4	1.092 ± 0.116	16,000	5
HD–251A	17.2	14.5–20.7	1.178 ± 0.129	14,140	5
HD–251B	14.3	8.3–26.6	1.202 ± 0.216	17,007	5
HD–263A	8.8 a	0.5–61.5	0.990 ± 0.289	27,636	5
HD–263B	7.3	4.1–12.8	1.443 ± 0.269	33,315	5

[a]Values followed by the same letter differ significantly at $P < 0.01$, according to the chi-square test.
[b]The value could not be calculated.

The larvae that survived feeding with *B.t.* (Tables 5, 6; Fig. 1) were significantly affected, as demonstrated by slower development and reduced consumption and excretion. Even the low *B.t.* concentrations that caused low mortality affected larval weight. The pupation rate of fifth instars decreased with increasing *B.t.* concentration (Fig. 3). Except at a concentration of 4.2 μg/ml, approximately 30% of the larvae developed into live prepupa/pupa, compared with 60% of the control. Based on the results of this research, the HD–263 strain is now produced commercially in Israel (by Becker Microbial Products) and used for the control of *B. selenaria* in avocado orchards.

Commercial products of *B.t.* are effective only against young stages of *B. selenaria* in avocado groves in Israel (Swirski et al., 1988). Therefore, selection of new strains of *B.t.* that may be more

TABLE 4

LC_{50} (μg *B.t.*/ml diet), 95% fiducial limits (μg *B.t.*/ml diet), slope and potency (IU/mg) of *Bacillus thuringiensis* isolates on fifth instar *Boarmia selenaria* larvae after 4 and 5 days of exposure

Bacterial preparation	LC_{50}	Fiducial limits	Slope ± SE	Potency	Exposure period (days)
HD-1-S-1980	550.2	79.4- b	0.397 ± 0.239	16,000	4
HD-251A	40.0	31.6-76.0	1.499 ± 0.411	220,080	4
HD-251B	45.7	32.0-111.8	0.950 ± 0.243	193,053	4
HD-263A	26.8 a	b	1.415 ± 0.554	328,478	4
HD-263B	26.3	20.4-39.4	0.915 ± 0.183	334,722	4
HD-1-S-1980	98.3	41.6- b	0.467 ± 0.179	16,000	5
HD-251A	52.1	33.2-183.8	0.750 ± 0.202	30,188	5
HD-251B	30.8	22.1-60.2	0.726 ± 0.171	51,065	5
HD-263A	19.6	15.1-27.5	0.827 ± 0.159	80,245	5
HD-263B	15.3	b	0.544 ± 0.213	102,797	5

[a]Values followed by the same letter differ significantly at $P < 0.01$ according to the chi-square test.
[b]The value could not be calculated.

TABLE 5

Mean weights (± SE) of surviving second-instar *Boarmia selenaria* larvae after 5 days of exposure to different *Bacillus thuringiensis* isolates. Initial larval weight ± SE = 0.60 ± 0.98 mg ($n = 175$)

Treatment	Mean weight ± SE (mg)	Number of larvae
Control	5.88 ± 1.10 a	139
HD-1-S-1980	2.47 ± 1.58 ab	67
HD-251A	3.26 ± 1.49 ab	75
HD-251B	2.04 ± 1.73 ab	56
HD-263A	1.78 ± 1.91 ab	46
HD-263B	2.07 ± 2.64 ab	24

[a]Values followed by the same letter do not differ from each other, but do differ significantly from initial weight at $P = 0.05$, according to weighted analysis of variance and Duncan's Multiple Range Test.
[b]Values followed by the same letter do not differ from initial weight at $P = 0.05$, according to weighted analysis of variance and Duncan's Multiple Range Test.

TABLE 6

Mean weights (± SE) of surviving fifth-instar *Boarmia selenaria* larvae after 5 days of exposure to different *Bacillus thuringiensis* isolates. Initial larval weight ± SE = 231.1 ± 15.2 mg ($n = 150$)

Treatment	Mean weight ± SE (mg)*	Number of larvae
Control	358.8 ± 16.3 a	131
HD-1-S-1980	298.9 ± 18.1 b	106
HD-251A	300.7 ± 18.1 b	106
HD-251B	280.8 ± 19.5 bc	91
HD-263A	257.2 ± 20.9 bc	79
HD-263B	244.4 ± 22.4 bc	69

*Values followed by a common letter do not differ at $P = 0.05$, according to weighted analysis of variance and Duncan's Multiple Range Test.
a and b differ from initial weight; c do not.

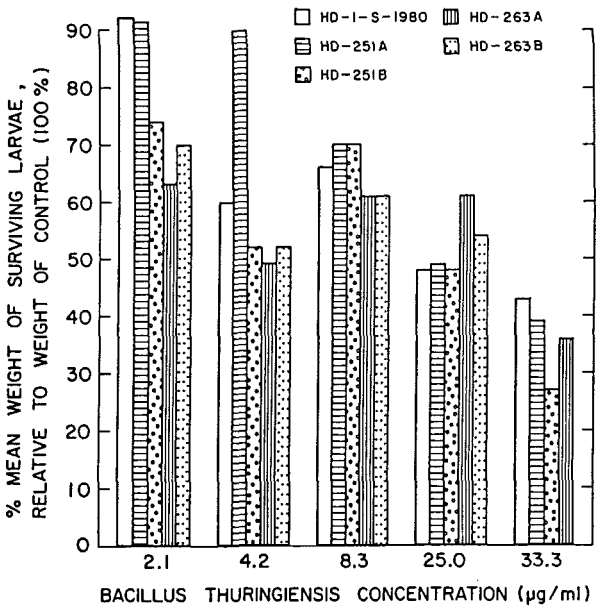

Fig. 1. Mean weight of surviving fourth-instar *Boarmia selenaria* larvae, as percentage of mean final weight of control ($x \pm SE = 292.2 \pm 10.0$ mg), after 5 days of exposure to different *Bacillus thuringiensis* isolates at various concentrations. Mean initial weight was $x \pm SE = 141.3 \pm 9.4$ mg.

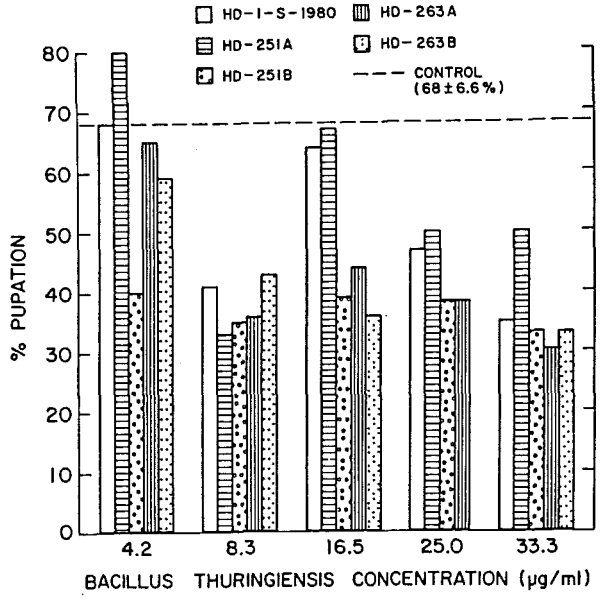

Fig. 2. Percentage of fifth-instar *Boarmia selenaria* larvae which developed into live prepupae or pupae after 5 days of exposure to different *Bacillus thuringiensis* isolates at various concentrations ($n = 30$).

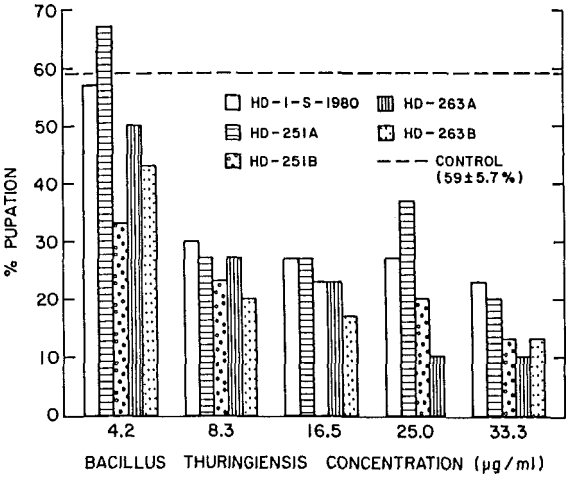

Fig. 3. Fifth-instar *Boarmia selenaria* larvae which developed into live prepupae or pupae, as percentage of surviving larvae after 5 days of exposure to different *Bacillus thuringiensis* isolates at various concentrations.

lethal to advanced stages may improve control of this pest. Previous screening failed to discover isolates more effective against *B. selenaria* than Dipel. *Boarmia selenaria* seems to be most susceptible to the isolates *kurstaki* and *thuringiensis* and less so to the isolates *entomocidus, aizawai, kenyae* and *ostriniae* (Cohen et al., 1983; Navon et al., 1983; Wysoki and Jarvinen, 1986; Wysoki and Scheepens, 1988), with the exception of HD–251, which was effective against fourth instars (Wysoki and De Haan, 1988).

Isolates HD–263 and HD–251 were on the average more effective than Dipel and the standard. The susceptibility of *B. selenaria* larvae to the isolates decreased with increasing larval size as observed before on *B. selenaria* (Cohen et al., 1983) and other Lepidoptera (Ignoffo et al., 1977; Izhar et al., 1979; Sneh et al., 1981). However, HD–263 caused high mortality even on fourth- and fifth-instars of *B. selenaria.*

The mean final weight of *B. selenaria* larvae which survived *B.t.* treatment was lower than that of the control. Sneh et al. (1981) found in *Spodoptera littoralis* Boisduval that larval weight relative to the controls decreased with an increase in *B.t.* spore concentration on food. Increased duration of development might be of economic value, as the probability that such larvae survive in the field is low. Prolongated development in different Lepidoptera fed on a diet containing *B.t.* was also reported by Ignoffo et al. (1968), Matter and Zohdy (1981), McGaughey (1978), Salama et al. (1981), Sutter and Raun (1966), and Yamvrias and Angus (1970). The giant looper larvae affected by *B.t.* do not cause much damage and will not develop into adults or will develop very slowly, as demonstrated also by the lower pupation rate, a phenomenon that Salama et al. (1981) found in several other Lepidoptera. In determining the effectiveness of *B.t.* preparations, it is important to consider the wide differences in susceptibility among populations of the same species (Kinsinger and McGaughey, 1979; Kinsinger et al., 1980).

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