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FEEDING RATE AND SURVIVAL OF *PLUTELLA XYLOSTELLA* (L.) LARVAE (LEPIDOPTERIA: PLUTELLIDAE) AFTER INTOXICATION BY *BACILLUS THURINGIENSIS* BERLINER VAR. KURSTAKI AND VAR. *AIZAWAI*

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Laboratory tests were conducted to determine the effect of two varieties of *Bacillus thuringiensis* (Bt) on food consumption and survival of diamondback larvae, *Plutella xylostella* L. Third instar larvae were allowed to feed for 48 h on cabbage disks treated with a series of concentrations (0.006 to 100 µg AI/ml water) of the formulations Dipel 2X (6.4% AI; Bt var *kurstaki*) and Xentari (10.3% AI; Bt var *aizawai*). Surviving larvae were transferred to untreated leaves to complete their life cycle until pupation or death. After two days of feeding on treated leaves, Bt subsp. *aizawai* with LC50 (0.82 µg/ml) was less efficacious than Bt subsp. *kurstaki* with LC50 (0.45 µg/ml). Moreover, mortality of larvae on treated leaves increased, whereas food consumption was reduced with increasing concentrations of delta-endoxin.

Consumption of untreated leaves by surviving larvae and duration of larval stage were reduced significantly in both cases (expected 0.006 µg AI/ml) in comparison to control. Reduction in duration of larval stage by Bt var. *kurstaki* was more effective compared to Bt var. *aizawai*.

KEY WORDS: *Bacillus thuringiensis* Berliner subspecies, *Plutella xylostella*, survival, food consumption

INTRODUCTION

The use of microbial pesticides in crop insect control is well established und has attained an increasing importance in the field of the Integrated Pest Management. *Bacillus thuringiensis* (Bt) is one of the most potent microbial pesticides, which is most frequently employed in management of lepidopterous pests on many crops. With increasing interest in the use of Bt for pest control, more effective strains have been found and the potency of Bt formulations has been increased (Carlton, 1988).

The Bt varieties *kurstaki* and *aizawai* are the Bt strains, which produce a paraspo-ral protein crystal (delta-endotoxin). The action of delta-endotoxin is involved in a rapid midgut paralysis and selective permeability of midgut epithelium in susceptible

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lepidopteran larvae (Fast and Angus, 1965). Some studies indicate that larval feeding is inhibited by Bt or Bt-toxins (Herbert and Harper, 1987; Gould *et al.*, 1991) and food consumption and survival time of intoxicated larvae by Bt were reduced on untreated leaves (Gharib and Wyman, 1991). Feeding inhibition could have a negative effect on the efficacy of Bt formulations under field conditions. Because the endotoxin is a rapid-acting toxin in comparison for example with beta-exotoxin (Gharib and Wyman, 1991), there is no significant nagative impact on its efficacy on susceptible pest insects.

The present study deals with the intoxication of *Plutella xylostella* larvae after exposure to Bt var. *kurstaki* and var. *aizawai* and the delayed effects of both Bt varieties on the food consumption and survival time of the diamondback larvae on untreated leaves.

MATERIALS AND METHODS

All bioassays were done on third-instar larvae of *Plutella xylostella* (average weight $\pm$SE, 1.11 $\pm$ 0.08 mg each) at 23 $\pm$ 2°C, 60 % r.h. and a photoperiod of 14:10 (L:D). The formulations of *Bacillus thuringiensis* were Dipel 2X (6.4% AI; 32,000 IU per mg, Abbott Laboratories, North Chicago, IL) for var. *kurstaki* and Xentari (10,3% AI; 15,000 IU per mg, Abbott Laboratories, North Chicago, IL) for var. *aizawai*.

Stock solutions of Bt were prepared in water and diluted with 0,02% Tween to a series of concentrations ranging from 0.006 to 100 µg AI/ml water. Cabbage leaf disks (24 cm^2) were treated by dipping for 10 s in the application solution. Each disk was then placed in a petri dish on filter paper, and after 3 h of drying at room temperature larvae were placed on it. Ten larvae were tested per concentration in each of six replicates. Larvae were allowed to feed daily on fresh treated leaves disks for 48 h.

Mortality was recorded and surviving larvae from all treatments were transferred to untreated disks (24 cm^2), which were maintained on moistened filter paper in a 9 cm petri dish until the larvae had died or reached pupation. Leaf squares were replaced daily. For statistical analysis concentration mortality responses for larvae at 48 h after intoxication were subjected to the probit analysis procedure (Finney, 1971) to obtain estimates of slope and LC_{50} values. Leaf area was measured with an area meter, Model LI-3000, Lambda Instruments, Lincoln Nebr. Leaf consumption by larvae was determined by totaling the differences between leaf areas before and after each 24-h feeding period until pupation or death of larvae. Total leaf consumption was summed up for each replicate of each treatment and average leaf consumption per larva and per concentration was calculated. Mean consumption per larva and average duration of larval stage on untreated leaves were compared among analyses of variance with the computer program Instat2.

RESULTS AND DISCUSSION

The intoxication of the Diamondback larvae, *Plutella xylostella* by two strains of *Bacillus thuringiensis* has been tested under laboratory conditions. LC_{50} values after 48 h feeding on treated leaves are presented in Table 1.

Table 1 Efficacy (LC_{50}) of Bt var. *kurstaki* and var. *aizawai* on 3 rd instars *P. xylostella* larvae in leaf dip-test after 48 h

Bt var.	*n*	LC_{50}			
		Slope ± *SE*	(*µg AI/ml*)	*95% FL*[1]	*EF*[2]
kurstaki	60	1.26 ± 0.1	0.45	0.25–0.83	1.8
aizawai	60	1.22 ± 0.1	0.82	0.44–1.55	–

[1] 95% fiducial limits; [2] Efficacy factor = LC_{50} of var. *aizawai*/LC_{50} of var. *kurstaki*.

The results show that Bt subsp. *aizawai* had a longer LC_{50} (0.82 µg AI/ml water) in comparison with Bt subsp. *kurstaki* (0.45 µg AI/ml water). Although mortality of larvae from Dipel 2X and Xentari did not differ significantly. As these mortality levels are high, the use of both Bt varieties may have a considerable importance in the control of Diamondback. Differences in the effect of both Bt varieties are always reported. In the experiments of Perez *et al.* (1995), Bt subsp. *kurstaki* caused higher larval mortality of *P. xylostella* than *aizawai* at early head formation, but the effects of both depended of the application technology. In contrast, Leibee *et al.* (1995) indicate that Bt var. *kurstaki* was less efficacious than Bt *aizawai* in the control of *P. xylostella* in head cabbage.

Distinct differences were detected in the inhibitory effects of both subspecies on the food consumption of larvae on treated leaves. Figures 1 and 2 show that the consumption of endotoxin-treated leaves within 48 h caused mortality, but mortality increased and leaf squares consumption decreased with increasing endotoxin concentration. The reduction in leaf consumption by Dipel 2X (Bt subsp. *kurstaki*) was more effective compared with Xen Tari (Bt subsp. *aizawai*).

Gould *et al.* (1991) reported that young larvae of *Heliothis virescens* avoided Bt-endotoxin-containing diets. According to Hoy and Hall (1993) third-instar *P. xylostella* consumed greater proportions of untreated leaf disks than droplets containing Bt-delta-endotoxin. In the present study, extremely but not total reduction in food consumption by larvae on treated leaves was absorbed only at the Bt-concentrations of 25 and 100 µg AI/ml water. Moreover the lower consumption of treated leaves was enough for a rapid midgut paralysis and high rate of mortality after two days.

The relatively better effect of Dipel 2X on third instar larvae of *P. xylostella* may be a result of the specificity of the two Bt-strains.

To determine the delayed toxicity of delta-endotoxin, surviving larvae were transferred on untreated cabbage leaves after 48 h feeding on treated leaves. Survival of treated larvae with the concentrations 100, 25 and 6,25 µg AI/ml could not be measured, because high mortality as a result of high toxicity was recorded at these concentrations.

Mortality and the reduction of leaf consumption continued to increase. Mortality rate of surviving larvae was at 42 to 100% by var. *kurstaki* and 47 to 78% by var. *aizawai* (Table 2). Consumption of untreated leaves per surviving larva until death or pupation after intoxication was reduced significantly by both in comparison with control larvae, but the difference between both varieties was not significant. Moreover larval duration was significantly shorter after delta-endotoxin intoxication than for the control (Table 2).

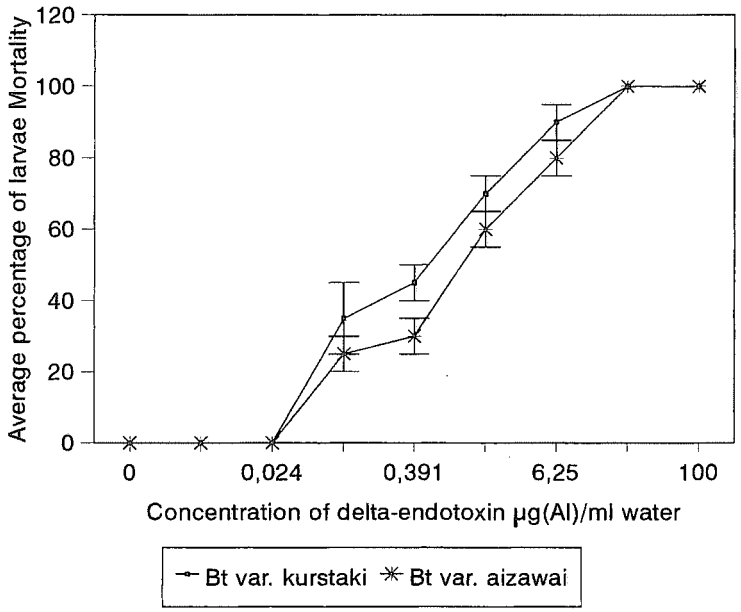

Fig. 1 Mortality rate of *Plutella xylostella* on cabbage leaves treated with the formulations Dipel 2X (*Bacillus thuringiensis* var. *kurstaki*) or Xen Tari (*Bacillus thuringiensis* var. *aizawai*).

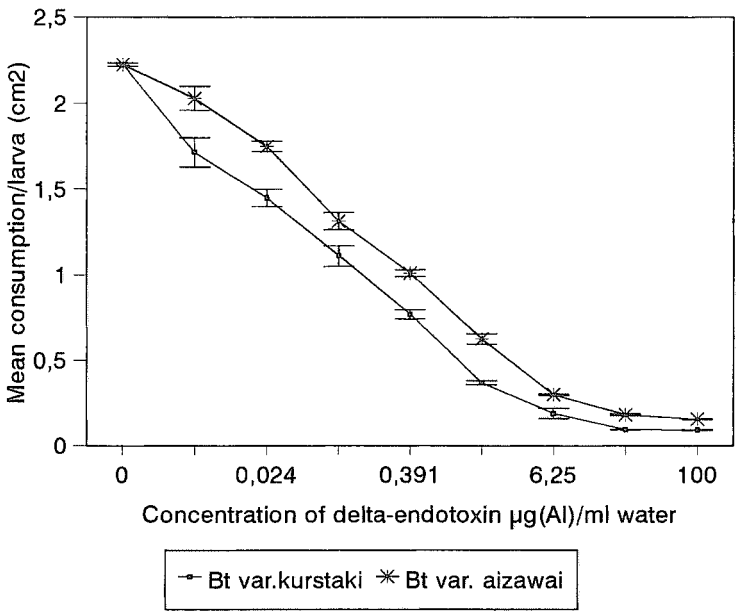

Fig. 2 Food consumption (cm^2) of *Plutella xylostella* on cabbage leaves treated with the formulations Dipel 2X (*Bacillus thuringiensis* var. *kurstaki*) or XenTari (*Bacillus thuringiensis* var. *aizawai*).

Table 2 Survival and consumption of *P. xylostella* on untreated leaves after feeding for 48 h on leaves treated with *B. thuringiensis* var. *kurstaki* or var. *aizawai*

Bt-var.	*No Larvae surviving intoxication*	*Died before pupation*		*Consumption of untreated leaves cm²*		*Duration of larval stage (days)*[1]
		Total	*%*	*Total*	*per larva*	
Untreated control	60	0	0	211.80	3.03 ± 1.18	2.25 ± 0.37
Bt var. *kurstaki* (µg AI/ml water)						
0.006	60	25	42	129.00	1.65 ± 0.17 ns	2.01 ± 0.13 ns
0.024	60	35	58	79.20	0.82 ± 0.18*	0.90 ± 0.22**
0.098	39	25	64	36.27	0.43 ± 0.03**	0.72 ± 0.33**
0.391	33	30	91	14.19	0.08 ± 0.08***	0.51 ± 0.16***
1.562	18	18	100	0.00	0.00 ± 0.00***	0.11 ± 0.05***
Bt var. *aizawai* (µg AI/ml water)						
0.006	60	28	47	132.60	1.71 ± 0.25 ns	2.15 ± 0.11 ns
0.024	60	30	50	73.80	0.73 ± 0.12*	1.20 ± 0.21**
0.098	45	23	51	46.08	0.52 ± 0.17**	1.02 ± 0.12**
0.391	42	30	71	16.38	0.10 ± 0.05***	0.98 ± 0.07**
1.562	14	11	78	0.98	0.07 ± 0.03***	0.23 ± 0.08***

[1] Duration on untreated leaves until death or pupation after intoxication for 2 days. Significance in consumption of untreated leaves (cm^2) per larva and duration of larval stage (days) was calculated for each Bt variety in comparison to untreated control by Tukey-Kramer multiple comparisons test: ns = no significant to untreated control; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

The results suggest that the increasing mortality and the reduction in total leaf consumption reflect both latent toxicity and feeding inhibition on untreated leaves. Gharib and Wyman (1991) reported a similar effect in larvae of *Trichoplusia ni* following intoxication by Bt var. *kurstaki*. Moreover latent effects of *Bacillus thuringiensis* in lepidopterous larvae were mentioned by Herbert and Harpert (1987) as well as by Abo El-Ghar *et al.* (1995).

P. xylostella responded also less susceptibly to the potential of feeding inhibition of both Bt-varieties. The consumption of delta-endotoxin conducted to an antifeedant effect, but this effect was probably the result of rapid midgut paralysis.

Pest control by *B. thuringiensis* as unconventional insecticide plays an important role in Integrated Pest Management. More effort should be invested to discover Bt strains with high control efficacy and low production costs. Moreover financial support and additional investigations are necessary for realizing this request.

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