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NOTE

Effect of a Formulation of *Bacillus thuringiensis* Berliner var. *kurstaki* on *Podisus nigrispinus* Dallas (Heteroptera: Pentatomidae: Asopinae)

The impact of biocontrol agents on beneficial organisms has been increasingly considered and evaluated worldwide for the growing interest on biodiversity conservation. *Bacillus thuringiensis* var. *kurstaki* (Btk) is a biopesticide widely used in Brazil for the control of lepidopteran pests in reforestation areas. *Podisus nigrispinus* Dallas is commonly found in such environments and commonly produced in laboratories for the control of lepidopteran pests.

Tests were conducted in a room at 25 ± 2°C, 70 ± 10% RH, and 12 h photophase to determine the effects of a formulation of Btk, strain HD-1 (Dipel, concentrated suspension), commercialized by Abbot Laboratory Brasil Ltd., on the biology of *P. nigrispinus*. The formulation had 17,600 IU/mg; about 2.6×10^9 viable spores/ml were detected at the product by pour plate counting in nutrient agar (Thompson and Stevenson, 1984). For the experiment Dipel was diluted 5 g/100 ml.

Predators used in the experiments were collected from a stock colony initiated with individuals from Mogi Guaçu-SP, Brazil. Five couples were held individually in plastic cages (15 × 10 × 10 cm) for oviposition. The bottom of each cage was covered with a piece of filter paper, on top of which there was a 1-cm^3 cup containing a piece of cotton soaked in distilled water. Two second- or third-instar larvae of *B. mori* were added daily to each cage as prey. Twenty eggs of *P. nigrispinus* were randomly taken from each cage as soon as they became available to initiate life table studies. Eggs and the emerged first-stage nymphs of each couple were maintained together in a Petri dish (9.0 cm diameter) whose bottom was covered with a filter paper on top of which there was a 1-cm^3 cup containing a piece of cotton soaked with 20% honey solution. After reaching the second nymphal stage, predators were isolated until adulthood in plastic cups (10 cm diameter × 9 cm height) topped with screen to allow air circulation. To reduce variability, 10 second-stage nymphs of each parental couple were assigned to each treatment. F1 couples were formed with individuals originating from the same treatment, and both individuals were maintained together until death. From the second nymphal stage on, predators were fed daily a second or third instar larva of *B. mori* reared on clean mulberry leaves (t1) or on mulberry leaves that had been submerged for 30 s in a Btk suspension (t2). In the latter case, larvae were allowed to feed at will 14–16 min; 1 h after stopping to feed, they were offered to the predators. For both treatments, prey were replaced daily.

Eggs used in the second generation were obtained from the corresponding treatments in the first generation. Fertility life tables were constructed (Southwood, 1978), starting with 50 eggs per treatment. Calculated biological parameters were compared using *t* tests, after using the "jackknife" method (Meyer, 1986) to estimate variances. Daily oviposition rates were statistically compared by *F* tests.

For microbiological observations of midguts, 20 recently emerged adults of *P. nigrispinus* were randomly taken from the stock colony and put individually in plastic cups (10 cm diameter × 9 cm height) similar to those previously described. They were fed infected larvae of *B. mori,* as previously described for treatment t2. After 4 weeks, they were submerged in a solution of 10% sodium hypocrite and rinsed 4 times in distilled water. Predators were then immobilized to remove their legs at the level of the coxae. A sample of the exuding hemolymph was taken from each insect, homogenized, diluted, and inoculated onto nutrient agar plates for total and spore counts of Bt (Thompson and Stevenson, 1984). Predators were then dissected to extract their midguts, which were individually homogenized (30 s) in sterilized water, conveniently diluted, and inoculated into nutrient agar plates in order to obtain total counts and spore counts after overnight incubation at 30°C. In the latter case, the homogenized midgut was heated at 80°C for 10 min before inoculation into agar (Thompson, 1984). Part of the suspension was submitted to direct microscope observation of characteristic Bt vegetative cells and spores or crystals (Smirnoff, 1962). The presence of Bt was checked in feces of adult predators deposited onto filter paper in

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TABLE 1

Associated Life Table Parameter Estimates of *P. nigrispinus* in Two Consecutive Generations, When Exposed to Healthy *Bombyx mori* Larvae or Larvae of the Same Prey Intoxication by *Bacillus thuringiensis* var. *kurstaki*

	Generation 1					Generation 2				
	Control		Treated			Control		Treated		
	Estimate	STD	Estimate	STD	*P* value	Estimate	STD	Estimate	STD	*P* value
Ro	243.90	21.75	94.65	12.77	<0.0001[a]	223.50	17.02	53.32	8.526	<0.0001[a]
r_m	0.17	0.003	0.12	0.005	<0.0001[a]	0.13	0.005	0.11	0.004	0.013[a]
λ	1.17	0.004	1.12	0.005	<0.0001[a]	1.14	0.006	1.12	0.005	0.013[a]
MGT	32.30	0.982	35.68	0.910	0.05596[b]	42.49	1.686	35.80	0.924	0.999[b]
DT	4.00	0.083	5.90	0.021	0.9999[b]	5.44	0.228	6.21	0.237	0.001[b]

Note. (STD) standard error of estimate.
[a] Uppertailed *t* test.
[b] Lowertailed *t* test.

the plastic cups where they were reared for the microbiological observation of the midgut. Pieces of the filter paper with feces were cut off and put in distilled water. The material was homogenized, diluted, and inoculated onto nutrient agar plates for Bt total and spores counts.

In both generations, the intrinsic rate of increase (r_m), the net reproductive rate (R_o), and the finite rate of increase (λ) were significantly lower, and the doubling time (Dt) was significantly higher for *P. nigrispinus* fed *B. mori* treated with Btk (Table 1). The first three parameters were slightly lower and the latter parameter was slightly higher in the second generation. The mean generation time (MGT) was significantly longer for *P. nigrispinus* fed treated *B. mori* in the first but not in the second generation. In the first generation, the mean number of eggs laid per female was 579.40 (±51.68) for the control and 318.43 (±43.00) for treated insects; in the second generation, it was 532.00 (±40.15) for the control and 300.00 (±48.00) for treated insects. Fecundity was slightly lower in the second generation for both treatments. In both generations fecundity was significantly higher (*F* test, $P < 0.005$) in the control.

In both generations oviposition started in the first week when *P. nigrispinus* was fed healthy *B. mori* and in the second week when it was fed treated *B. mori.* Oviposition rate always peaked a week after starting. When the predator was fed with healthy *B. mori,* the oviposition period was significantly longer (*t* test, $P < 0.05$). The oviposition period was also significantly longer in the second generation, especially for predators fed healthy prey.

Neither spores nor vegetative cells were detected in the hemolymph of the predator. They were only observed in the midgut (0.8×10^2–1.0×10^4 CFU/midgut) and feces (where it was not quantified).

Differences in behavior between generations indicate changes in the inherent characteristics of *P. nigrispinus* when maintained even for a short time under artificial conditions in the laboratory, perhaps because of unintentional selection.

The results indicated adverse effects of the biopesticide on the biology of *P. nigrispinus.* The absence of the pathogen in the hemolymph of the predator indicates that the effects were not related to actual pathogenicity. The cause for such effects could not be demonstrated, but could be related to activated insecticidal crystal toxins produced by Btk (Dulmage *et al.,* 1981). Alternatively, it could be related to components of the formulation, other than the bacterium or its products (Haverty, 1982). The observed adverse effect could still be related to deterioration of the food available to the predator because of eventual colonization of the moribund prey by different microorganisms. This effect could have occurred despite the daily replacement of the prey. To elucidate those different possibilities, future studies should include treatment corresponding to *B. mori* fed mulberry leaves treated with inactivated Btk.

The work reported here corresponds to Phase I of the evaluation of the impact of a biopesticide on nontarget organisms, in accordance with protocols utilized for the purpose of registration of such products for commercial use (Anonymous, 1989). In this phase, nontarget organisms are exposed to high dosages of biopesticides, under conditions most favorable for deleterious effects to be shown. Thus, the results of this study may not reflect the actual impact of Btk on *P. nigrispinus* populations in the field, where *P. nigrispinus* would have an array of alternative food items available, which could allow it to escape the effects of a full dependence on prey infected by Btk.

Key Words: Bacillus thuringiensis; Podisus nigrispinus; biological control; pathogen; risk assessment.

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M. L. Nascimento*
D. F. Capalbo†
G. J. Moraes‡
E. A. De Nardo†
A. H. N. Maia†
R. C. A. L. Oliveira*

**Fellowship Capes and PADCT*

‡Department of Zoology, ESALQ-USP
13418-900 Piracicaba-SP, Brazil

†EMBRAPA, CNPMA
13820-000, Jaguariúna-SP, Brazil

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Deise Maria Fontana Capalbo
CNPMA
EMBRAPA
C P 69
Jaguariúna, 13820-000
BRAZIL

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