Enhanced Activity of *Anticarsia gemmatalis* Hüb. (Lepidoptera: Noctuidae) Nuclear Polyhedrosis Vírus by Boric Acid in the Laboratory

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An. Soc. Entomol. Brasil 26(1): 115-120 (1997)

Aumento da Atividade do Vírus de Poliedrose Nuclear de *Anticarsia gemmatalis* Hüb. (Lepidoptera: Noctuidae) pelo Ácido Bórico, em Laboratório

RESUMO - Avaliou-se em laboratório o efeito de diferentes concentrações de ácido bórico sobre a atividade do vírus de poliedrose nuclear (VPN) da lagarta-da-soja, *Anticarsia gemmatalis* Hub., através da sua incorporação na dieta do inseto. O ácido bórico foi testado nas concentrações de 0,020,0,030,0,045,0,067 e 0,101 gramas/100 ml de dieta. A concentração letal média (CL_{50}) do VPN isoladamente, calculada no sétimo dia após a infecção, foi de 1,52 x 10^5 corpos poliédricos de inclusão (CPI)/ml de dieta, enquanto que, para a suspensão adicionada de 0,045 g de ácido bórico, foi de 7,95 x 10^2 CPI/ml de dieta. Em datas subsequentes de avaliação (9, 11 e 14 dias da infecção) os valores da CL da mistura foram cerca de quatro vezes inferiores aos obtidos com o VPN isoladamente. O tempo letal médio (TL_{50}) do VPN isoladamente, na dose de 250 CPI/ml de dieta, foi de 13,6 dias, enquanto que para doses crescentes do ácido bórico, em mistura com o VPN, o TL_{50} variou de 13,7 dias (0,02g de ácido bórico/100 ml de dieta) a 7,4 dias (O,l0l g de ácido bórico/100 ml de dieta). Portanto, a adição de ácido bórico ao VPN aumentou a virulência do patógeno para A *gemmatalis e* diminuiu seu tempo de atuação sobre o inseto.

PALAVRAS-CHAVE: Insecta, lagarta-da-soja, controle biológico, baculovirus.

ABSTRACT - Boric acid concentrations (0.02,0.03,0.045,0.067 and 0.101 g/l00 ml of diet) were evaluated in combination with the *Anticarsia gemmatalis* Hub. nuclear polyhedrosis virus (AgNPV) for enhanced virali activity against the insect. Seven days after inoculation, the median lethal concentration (LC_{50}) was 1.52 x 10^5 for the AgNPV alone and 7.95 x 10^2 for the NPV mixed with 0.045g of boric acid/100 ml of diet. At subsequent evaluation dates (9,11 and 14 days after inoculation) LC_{50}'s for NPV+boric acid were ca. 4x lower than those observed for the NPV alone. The median lethal time (LT_{50}) was 13.6 days when the NPV was used alone, while, when in mixture with increasing concentrations of boric acid, LT_{50} values ranged from 13.7 days (boric acid at 0.02g/100 ml of diet) to 7.4 days (boric acid at 0.101 g/ml of diet). Therefore, boric acid added to the AgNPV significantly increased *A. gemmatalis* larval mortality and shortened mortality time by the pathogen.

KEYWORDS: Insecta, velvetbean caterpiliar, biological control, baculovirus.

A nuclear polyhedrosis vírus (NP V) of the velvetbean caterpiliar, *Anticarsia gemmatalis* Hub. (Lepidoptera: Noctuidae), has been yearly applied as a microbial insecticide m Brazil in approximately 1,000,000 ha of soybean (Moscardi 1989, Moscardi & Sosa-Gómez 1992). It has provided successful control of the insect. However, intrinsic characteristics of the VPN such as time to kill the host and susceptibility to deactivation by U V light, as well as extrinsic factors such as formulation type, climatic conditions, pest population dynamics, and quality of application, pose limitations to a wider use of the virus. Moreover, farmers tend to mix the N P V with reduced dosages of chemical insecticides, mainly because of the NPV slow speed of kill. Several substances have been tested to increase virulence of entomopathogens (Doane & Wallis 1964, Shapiro & Bell 1982,Young&Yearian 1986,Bijjur *et al*. 1991, Webb *et al.* 1994). One of them, the boric acid, has been shown to potentialize the activity of the NPVs of *Lymantria dispar* (L.) (Shapiro & Bell 1982), *Heliothis armigera* (Hüb.) (Bijjur *et al.* 1991) and *Spodoptera litura* Fabr. (Chaudhari 1992) at boric acid concentrations of 0.5% or lower. The objective of this work was to evaluate the effect of boric acid on the activity and speed of kill of the *A. gemmatalis* NPV.

Material and Methods

The *A. gemmatalis* larvae utilized in the bioassays were obtained from an insect colony established at Embrapa, Londrina, and reared according to Hof fmann-Campo *et al.* (1985) on an artificial diet modified from Greene *et al*. (1976). NPV and boric acid concentrations, either isolated or in combinations, were obtained by their incorporation in the insect diet at 50 °C, at the ratio of 20 ml of treatment suspension/180ml of diet,homogenized in a becker by an electrical hand mixer (Morales & Moscardi 1993). Treated diet (ca. 10 ml) was placed in plastic cups (50 ml) and offered to two 2nd-instar larvae/cup, maintained at 25 ± 2 °C, 70 ± 10% RH, and 14L: 10D photoperiod. In a 1st trial, four NPV dosages were tested individually or in combinations with a single concentration of boric acid (0.045 g/l 00 ml of diet). In a 2nd trial, five concentrations of boric acid (0.02,0.03,0.045,0.067, and 0.101 g/ 100 ml of diet) were tested individually or in combinations with a single dosage of the NPV (250 polyhedron inclusion bodies - PIB/ml of diet). Around 40 larvae were used/treatment/ trial, with each trial being replicated 3 x. Total numbers of larvae for respective treatment dosages are shown in Tables 1 and 2. Insects were observed daily up to pupation, with mortality being corrected according to Abbott (1925). Data were submitted to probit analysis (Finney 1971) for determination of median lethal concentration (LC_{50}), median lethal time (LT_{50}), and respective fiducial limits (95%).

Results and Discussion

The mixture of boric acid to *A. gemmatalis* NPV increased larval mortality up to 5x and significantly reduced the LT_{50} of the pathogen, when compared to the effect of the NPV alone (Table 1). At the higher NPV concentration (1,215 PIB/ml of diet), larval mortality 7-d after treatment (DAT) was 12%, compared to 63% when this NPV dosage was mixed with boric acid at 0.045g/100 ml of diet. At this dosage, boric acid alone caused only 0.8% larval mortality. The same trend was observed at 405 PIB/ml of diet, but the effect of boric acid was not significant at the two lowest NPV dosages. At 11 DAT, larval mortality did not differ between NPV alone and NPV+boric acid for the lowest virus concentration; however, mortality was ca. 2x higher for the mixtures in relation to NPV alone at 405 and 1,215 PIB/ ml of diet. Similarly, Shapiro & Bell (1982) reported a 11 x increase in *L. dispar* mortality when a NPV of this species was mixed with boric acid at 1.0%. However, they did not observe increases in *L. dispar* larval mortality at boric acid concentrations of 0.10 and 0.25%. These authors also reported that boric acid alone did not cause larval mortality at concentrations up to 1.0%, but that at 2.5% resulted in 25% larval mortality. Chundurwar *et*

Table 1. Effect of boric acid mixed with different concentrations of the nuclear polyhedrosis virus (NPV) of *Anticarsia gemmatalis*, on larval mortality by NPV and on the medial lethal time (LT_{50}) of the pathogen.

NPV Concentration (PIB/ml of diet)	N[1]	Mortality (%) 7 DAT	Mortality (%) 11 DAT	LT_{50} (days)	FL(95%)[2]
Control	120	0	0.8	-	-
Boric Acid (BA)[3]	123	0.8	1.6	-	-
45 (NPV)	118	2.5	13.6	19.7	17.4-23.9
45 (NPV)+BA	122	5.7	12.3	17.1	16.1-18.5
135 (NPV)	120	5.0	20.0	16.4	15.1-18.2
135 (NPV)+BA	122	9.0	30.3	13.2	12.4-14.1
405 (NPV)	119	5.9	29.4	14.1	13.2-15.4
405 (NPV)+BA	122	32.8	59.0	10.0	9.3-10.8
1215 (NPV)	117	12.0	51.3	11.3	10.4-12.5
1215 (NPV)+BA	122	63.0	82.8	7.2	6.7-8.3

[1]Number of tested insects.
[2]Fiducial limits (95%) for the LT_{50}.
[1]Concentration of boric acid = 0.045 g/100 ml of diet.

al. (1990) observed an increase of up to 4x in *H. armigera* mortality when boric acid (0.5%) was added to the insect NPV. In field experiments, Bijjur *et al. (*1991) reported significant reductions of *H. armigera* larval populations in sunflower treated with NPV + boric acid, in comparison with the NPV alone.

When a single NPV concentration of 250 PIB/ml of diet was used, observed larval mortality was 9.8 and 32.5%, respectively at 7 and 9 DAT. However, as it was mixed with increasing dosages of boric acid, mortality varied from 11.7 to 56.2% at 7 DAT, and from 32.5 to 92.4 at 9 DAT (Table 2), confirming that boric acid does potentialize the activity of *A. gemmatalis* NPV at certain concentrations. Also in this trial, the lower dosage of boric acid did not result in an increase in larval mortality by the NPV The different boric acid concentrations, when used alone, resulted in larval mortality between zero and 5.0 %, showing that this chemical was not toxic to the insect. Median lethal concentrations (LC_{50}) of the NPV, either used alone or in combination with boric acid, estimated at 7,9,11, and 14 DAT (Table 3), also showed the impact of boric acid in potentializing virus activity on *A. gemmatalis* larvae. At 7 DAT, LC_{50} for the virus alone was 1.52×10^5 PIB/ml of diet compared to$7.95 \times I0^2$ PIB/ml of diet for the NPV+boric acid (0,045 g/100 ml of diet). For subsequent dates of evaluation, differences were also significative, based on non overlap of 95% fiducial limits, and tended to stabilize at 4x between the mix-

Table 2. Effect of boric acid (BA) concentrations, in grams/100 ml of diet, on the activity of the nuclear polyhedrosis virus (NPV) of *Anticarsia gemmatalis*, expressed by larval mortality by NPV, at 7 and 11 days after treatment (DAT) and by the median lethal time (LT_{50}) of the pathogen.

Treatment	N[1]	Mortality (%)		LT_{50} (days)	FL (95%)[2]
		7 DAT	11 DAT		
Control	120	0	0.8	-	-
NPV[3]	122	9.8	32.5	13.6	12.5-15.2
BA 0.020	121	0	0.8	-	-
BA 0.020 + NPV	120	11.7	35.5	13.7	12.5-15.6
BA 0.030	120	1.7	2.5	-	-
BA 0.030 + NPV	118	23.7	48.3	11.6	10.9-12.5
BA 0.045	120	0	1.7	-	-
BA 0.045 + NPV	118	38.1	70.3	9.1	8.5-10.0
BA 0.067	119	1.7	2.5	-	-
BA 0.067 + NPV	120	52.5	80.0	8.2	7.6-9.0
BA 0.101	118	2.5	5.0	-	-
BA 0.101 + NPV	121	56.2	94.2	7.4	7.0-7.9

[1]Number of tested insects.
[2]Fiducial limits (95%) for the LT_{50}.
[3]PV concentration = 250 PIB/ml of diet.

ture and the virus alone. Non significant Chis-quare values (χ^2) indicated that the data used to estimate LC_{50}'s fitted the model (probits) utilized in their calculation.

The mixture of boric acid to the NPV of *A. gemmatalis* caused increased mortality of larvae, but also prommoted differences of up to four days in the mean time to cause 50% larval mortality (LT_{50}). At the higher NPV concentration (1,215 PIB/ml of diet) the LT_{50} was reduced from 11.3 to 7.2 days, when boric acid was added at 0.045 g/l 00 ml of diet (Table l). As increasing dosages of boric acid were added to a low NPV concentration (250 PIB/ml of diet), the LT was substantially reduced with the increase in boric acid concentration, varying from 13.6 (NPV used alone) to 7.4 (VPN+boric acid at 0.101 g/100 ml of diet) (Table 2). Similarly, Shapiro & Bell (1982) reported a LT_{50} of 20.5 days for the *L. dispar* NPV alone, compared to 16.2 days for the NPV+boric acid. Aiso, Chundurwar *et al.* (1990) reported a LT_{50} of 194.5 hours for the NPV of *H. armigera* compared to 136.8 hours for the NPV+boric acid (0.5%). Consequently, results obtained by these authors and those

Table 3. Median lethal concentrations (LC_{50}) of the *Anticarsia gemmatalis* NP V, when used alone or in combination with boric acid (BA), at different days after treatment (DAT).

Treatment	LC_{50}[2]	Fiducial Limit (95%)	χ^2
NPV (7 DAT)	1.52 x 10^5	1.73x10^4 - 8.9x10^6	0.69 NS
NPV + BA[1] (7 DAT)	795.8	643.3 - 1,038.9	4.41 NS
NPV (9 DAT)	2,340.6	1,418.1 - 5,200.8	3.58 NS
NPV + BA (9 DAT)	441.7	369.1 - 538.8	1.83 NS
NPV (11 DAT)	1,370.4	882.1 - 2,574.1	1.90 NS
NPV + BA (11 DAT)	300.4	250.7 - 364.6	0.28 NS
NPV (14 DAT)	567.1	405.3 - 874.3	1.40 NS
NPV + BA (14 DAT)	131.9	101.7 - 165.8	0.54 NS

[1]Dosage of boric acid = 0.045 g/100 ml of diet.
[2]PIB/ml of diet.

presented in the present work show that boric acid, at very low concentrations, may be used in viral formulations to increase virulence and speed of kill by entomopathogenic viruses. The mechanisms leading to the enhancement of viral activity in the respective hosts by boric acid are not well understood, and should be further investigated. Boric acid is a plant nutrient and is of low cost. If proven effective at field levei, this product may be usefull in integrated pest management programs.

The results of this work show that boric acid has a great potential to potentialize the activity and to speed up death provoked by NPV on *A. gemmatalis.* However, its practical utilization ought to be evaluated at field conditions to verify its efficiency and cost/benefit at specified concentrations in mixture with the NPV of *A. gemmatalis.*

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Received 11/1/96. Accepted 12/11/97.