

## EFFECT OF A NUCLEAR POLYHEDROSIS VIRUS ON *Spodoptera frugiperda* (SMITH) (LEPIDOPTERA: NOCTUIDAE) LARVAE, ITS DAMAGE AND YIELD OF MAIZE CROP

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**ABSTRACT** - The efficiency of *Baculovirus* in a wettable powder formulation to control the fall armyworm *Spodoptera frugiperda* (Smith) was evaluated using variations of the recommended dose of 50 grams per hectare ( $2.5 \times 10^{11}$  polyhedron inclusion bodies - PIB ha<sup>-1</sup>): 50 or 100 g ha<sup>-1</sup> applied once, and variations of two applications at seven-day intervals, of 25+25, 25 + 50, 50+25 and 50+50 g ha<sup>-1</sup>, compared to untreated plots. The treatments were arranged in a randomized complete block design with six replications. Each plant at 6-8 or 8-10-leaf growth stages, was artificially infested with 10 newly hatched larvae. The virus suspension (300 L ha<sup>-1</sup>) was applied 24 hours after larvae infestation using a back-pack-manual sprayer at 40 PSI (2.8 kg cm<sup>-2</sup>) and a regular flat fan nozzle. Larval mortality rate obtained from plots with the application of 50 g of *Baculovirus* wettable formulation ha<sup>-1</sup>, at once (96.2%), was significantly higher than that obtained with the application of 25+25 g ha<sup>-1</sup> (85.3%) or 25 + 50 g ha<sup>-1</sup> (87.8%). However, it was lower than that obtained from the other virus treatments, which had no significant difference among them (mean of 99.2%). The damage caused by *S. frugiperda* on the leaves of plants of untreated plots (average of 4.5 in a visual scale from 1 to 5) was significantly different from that observed in plots sprayed with virus (1.75 to 2.33). Plants protected with the virus produced significantly higher yield (mean of 7,577 kg ha<sup>-1</sup>) than plants unprotected (6,140 kg ha<sup>-1</sup>), that is, 23.4% higher.

**Key words:** Insecta, fall armyworm, biological control, *Baculovirus*, *Zea maiz*.

## EFEITO DO VÍRUS DE POLIEDROSE NUCLEAR SOBRE LARVAS DE *Spodoptera frugiperda* (SMITH) (LEPIDOPTERA: NOCTUIDAE), SEU DANO E RENDIMENTO DE GRÃOS DA CULTURA DE MILHO

**RESUMO** – Foi avaliada a eficiência de uma formulação pó molhável de *Baculovirus* para o controle de lagartas de *Spodoptera frugiperda* (Smith), utilizando variações de uma dose padrão de 50 gramas da formulação (concentração de  $2,5 \times 10^{11}$  corpos de inclusões poliédricas – CIP ha<sup>-1</sup>): 50 ou 100 g ha<sup>-1</sup>, aplicadas de uma única vez, e variações de duas aplicações a intervalos de sete dias, de 25+25, 25+50, 50+25 e 50+50 g ha<sup>-1</sup>, comparado com uma testemunha sem aplicação de vírus. Os tratamentos foram arranjados em blocos ao acaso, com seis repetições. Cada planta nos estádios de 6 a 8 ou 8 a 10 folhas, foi artificialmente infestada com 10 lagartas recém-nascidas. A suspensão de vírus (300 litros ha<sup>-1</sup>) foi aplicada 24 horas após a infestação, utilizando um pulverizador manual costal, com bico leque e na pressão de 40 PSI (2,8 kg ha<sup>-1</sup>). A taxa de mortalidade obtida de parcelas com uma única aplicação de

50 g da formulação de *Baculovirus* (96,2%) foi significativamente maior que aquela obtida com a aplicação de 25+25 g ha<sup>-1</sup> (85,3%) ou 25+50 g ha<sup>-1</sup> (87,7%). Entretanto, ela foi menor do que aquela obtida nas outras parcelas tratadas com o vírus nas doses de 50+50 e 100 g ha<sup>-1</sup>. Nessas duas parcelas, não se obteve diferença significativa entre as médias de mortalidade, que foi de 99,2%. O dano causado pela *S. frugiperda* nas folhas de plantas nas parcelas testemunhas (média de 4,5 numa escala visual de 1 a 5) foi significativamente diferente daquele observado em parcelas pulverizadas com o vírus (1,75 a 2,33). Plantas que receberam aplicações do vírus tiveram maior rendimento (média de 7.577 kg ha<sup>-1</sup>) que plantas sem proteção (6.140 kg ha<sup>-1</sup>), ou seja, 23,4% a mais.

**Palavras-chave:** Insecta, lagarta-do-cartucho, controle biológico, *Baculovirus*, *Zea maiz*.

The fall armyworm, *Spodoptera frugiperda* (Smith) is a serious pest of corn in the Americas. It feeds on all growth stages of corn but most frequently in the whorl of young plants. First instar larvae usually eat the green tissue from one side of the leaf leaving the membranous epidermis in the other side intact. Older instars make holes in the leaf and may completely destroy small plants and strip large ones (Cruz, 1995ab). In Brazil, the fall armyworm, *S. frugiperda* is one of the key pests of maize (*Zea maiz* L.) and other row and vegetable crops (Cruz, 1992, 1995b). Ruppel *et al.* (1957) stated that the fall armyworm is the most severe pest of corn in Colombia. This is also true in Venezuela (Horovitz, 1960), Peru (Campos, 1968; Pena, 1974), Chile (Etcheverry, 1957), Mexico (Sifuentes, 1967) and the United States of America (Barfield, *et al.*, 1978). According to Lopez-Edwards *et al.* (1999) the fall armyworm, is a tropical insect species, endemic to the Western Hemisphere, where it ranges from Brazil northward, throughout Central and North America.

Yield reductions due to fall armyworm larvae vary from 17.7 to 57.6 according to the development stage and maize genotypes (Cruz & Turpin, 1982, 1983; Williams & Davis, 1990; Willink *et al.*, 1991; Cruz *et al.*, 1996, 1999). Control of *S. frugiperda* is based on chemical pesticides, employed when defoliation is noticed in the crop. However, because of the problems associated with the use of pesticides to control this pest, especially

the possibility of increasing insecticide resistance level on pest population and reduction of beneficial insects, emphasis has been shifted to biological control (Cruz 1995a; Cruz *et al.*, 1997). Among biological control agents, *S. frugiperda* nuclear polyhedrosis virus (SFNPV) is believed to have great potential to control this pest (Gardner *et al.* 1984; Moscardi & Kastelic 1985; Valicente *et al.*, 1989; Valicente & Cruz, 1991; Hamm *et al.*, 1994, 1997; Valicente & Costa, 1995; Cruz *et al.*, 1997, Cruz, 2000).

The objective of this study was to evaluate a wettable powder formulation of SFNPV, applied on two different growth stages of maize plants to control *S. frugiperda* larvae.

### Material and Methods

The experiment was conducted at the Embrapa Maize and Sorghum Research Center, Sete Lagoas, MG, Brazil, during summer, in a dystrophic red latosol soil, under savanna vegetation, using the maize hybrid Pioneer 3041. The treatments were arranged in a randomized complete block design with six replications. The experimental unit consisted of six rows of 8 m (240 plants). Plants at 6-8 or 8-10-leaf growth stages were artificially infested with 10 newly hatched larvae obtained from laboratory culture. A basic wettable power was produced according to Cruz (2000) and applied as an aqueous suspension using a back-pack-manual sprayer at 40 PSI (2.8 kg cm<sup>-2</sup>) and a regular flat fan nozzle (Cruz

& Santos, 1984). Spraying ( $300 \text{ l ha}^{-1}$ ) was carried out in the morning, between 08:00 and 10:00 h. The virus was applied 24 hours after larvae infestation, using variation of the standard commercial dose of  $50 \text{ g}$  ( $2.5 \times 10^{11}$  polyhedron inclusion bodies – PIB  $\text{ha}^{-1}$ ):  $50$  or  $100 \text{ g}$ , applied once, and variations of two applications (at seven-day interval):  $25 + 25$ ;  $25 + 50$ ;  $50 + 25$  and  $50 + 50 \text{ g ha}^{-1}$ , compared to a check plot (untreated control).

Measurements were based on virus efficacy, pest damage and yield. Evaluations on virus efficacy were made in each plot at seven and 10 days after the first virus application by randomly selecting 30 plants  $\text{plot}^{-1}$  and counting dead and alive larvae. Alive larvae were taken to the laboratory and placed individually in plastic cup with artificial diet. Insects were maintained at  $25 \pm 2^\circ\text{C}$ , 70% RH and photophase of 12 h, and observed daily, and mortality recorded. Damage evaluations were based on a visual scale (Cruz, 1980) from 1 (no visible damage) to 5 (plant with the whorl destroyed). Plants in each experimental unit in the four central rows were visually rated for leaf damage, 20 days after infestation. At harvest, ears were placed in cloth bags and taken to the laboratory where the damage and the grain weight were taken for each plot. Data on larval mortality, leaf and ear damage and yield were submitted to the analysis of variance and the means were compared using Duncan's multiple range test ( $p \leq 0.05$ ). Before running analysis of variance, tests were conducted to determine if data set met the necessary assumptions. The Burr-Foster Q-test was used to test equality of variance. For testing the normality of the data, the W test developed by Shapiro and Wilk was used. Descriptions of both tests are found in Anderson & McLean (1974). Transformations, when applied, were used according to the criteria suggested by Ostle & Mensing (1975).

## Results and Discussion

### *Larval mortality*

In the first evaluation made at the seventh day after the virus application, there was no significant difference in field mortality rate among treatment with virus applied at the 6-8-leaf stage. However, when applied at the 8-10-leaf stage, significantly lower mortality was observed from plots where the virus was applied at  $25 \text{ g ha}^{-1}$  (Table 1). In spite of these differences field mortality was high in all plots with the virus application (57.6 to 71.2%). The virus lethal period usually is between 6-8 days (Valicente *et al.* 1989). Therefore, at the seventh day after the application, some larvae were still alive. However, considering the total mortality, that is, adding the field to the laboratory data, the mortality rate increased in all virus treatments. There was no significant difference among treatments where the virus was applied at a minimum dose of  $50 \text{ g ha}^{-1}$ . The average mortality rate was 96.2 and 92.3%, at the 6-8 and 8-10-leaf stages, respectively. Lower mortality was obtained from plots where virus was applied at  $25 \text{ g ha}^{-1}$ .

In the second evaluation, made three days after the second virus application, field mortality varied from 77.6 to 80.7% at the 6-8-leaf stage, and from 76.6 to 84.3% at the 8-10 leaf stage. As in the first evaluation, there was no significant difference among mortality rates from plots with virus application in the 6-8-leaf stage; at the 8-10-leaf stage, higher mortality was obtained from plots where the virus was initially applied at the dose of  $50 \text{ g}$ . Finally, considering the total mortality, all the virus treatments, provided at least, 84.1% mortality (Table 2). One application of  $50 \text{ g ha}^{-1}$  24 hours after infestation, provided a mortality rate of 96.1 and 88.2%, when applied at 6-8 or 8-10-leaf stage, respectively. Cruz *et al.* (1997) reported a mortality rate of 90.5% in evaluation five days after the application of  $50 \text{ g ha}^{-1}$ . They found a significant gain

in efficiency when the virus was applied at a 5-fold concentration. Significant gain was obtained from plots where the virus was applied at the dose of 100 g ha<sup>-1</sup> (2-fold), at once, or applied at seven-day interval, at the doses of 50+25 (1.5-fold) or 50+50 (2-fold), especially at the 8-10-leaf stage (Table 2).

In both growth stages, significantly lower rate of mortality was obtained from plots where the initial application was 25 g ha<sup>-1</sup>, even with a second application of 50 g, seven days later. Apparently, the initial application of 25 g ha<sup>-1</sup> was not enough to provide control rate compared to the other treatments.

**TABLE 1.** Effect of dose and interval application of SFNPV on the larval mortality of *Spodoptera frugiperda* (sampled 7 days after first virus application).

Dose (g ha <sup>-1</sup> )	Field mortality (%) <sup>1</sup>			Total mortality (%) <sup>1</sup>		
	Leaf stage			Leaf stage		
	6-8	8-10	Average	6-8	8-10	Average
50	66.9 A	69.8 A	68.4 A	95.5 A	93.6 A	94.6 A
25 + 25	63.3 A	57.6 B	60.5 B	81.1 B	76.4 B	78.8 B
25 + 50	60.5 A	60.9 B	60.7 B	79.2 B	76.5 B	77.8 B
50 + 25	67.6 A	66.5 A	67.1 AB	95.5 A	91.4 A	93.5 A
50 + 50	71.2 A	65.2 A	68.2 A	97.2 A	90.9 A	94.1 A
100	67.0 A	70.1 A	68.5 A	96.5 A	93.4 A	95.0 A
Untreated	1.2 B	1.6 C	1.4 C	1.2 C	3.2 C	2.2 C
Average	56.8 a	56.0 a		78.0 a	75.1 a	
CV (%)	17.4	12.4	15.0	4.95	5.21	6.2

<sup>1</sup>Means followed by the same capital letter within each column or small letter across each line are not significantly different according to Duncan's multiple range test ( $p \leq 0.05$ ).

**TABLE 2.** Effect of dose and interval application of SFNPV on the larval mortality of *Spodoptera frugiperda* (sampled 3 days after second virus application).

Dose (g ha <sup>-1</sup> )	Field mortality (%) <sup>1</sup>			Total mortality (%) <sup>1</sup>		
	Leaf stage			Leaf stage		
	6-8	8-10	Average	6-8	8-10	Average
50	79.4 A	83.7 A	81.6 AB	96.1 A	88.2 B	92.2 B
25 + 25	79.2 A	76.6 B	77.9 B	86.6 B	84.1 B	85.3 C
25 + 50	78.8 A	80.7 AB	79.8 AB	88.5 B	87.0 B	87.7 C
50 + 25	77.6 A	84.3 A	80.9 AB	100.0 A	99.3 A	99.6 A
50 + 50	80.7 A	83.5 A	82.1 A	100.0 A	98.2 A	99.1 A
100	80.1 A	82.7 A	81.4 AB	99.4 A	98.5 A	99.0 A
Untreated	0.0 B	1.0 C	0.5 C	0.0 C	1.0 C	0.5 D
Average	68.0 a	70.3 a		81.5 a	79.5 b	
CV (%)	6.71	4.86	6.69	5.24	3.71	4.40

<sup>1</sup>Means followed by the same capital letter within each column or small letter across each line are not significantly different according to Duncan's multiple range test ( $p \leq 0.05$ ).

According to the combining analysis considering the two growth stages, there was no significant difference in the total mortality rate obtained from the virus application in the first evaluation (average of 76,5%). Significant difference existed between growth stages, in the second evaluation. However, the difference was very low (81.5% at the 6-8 and 79.5% at the 8-10-leaf stage) (Table 2). A greater difference was obtained in terms of dose of virus. The total mortality rate obtained from plots with the application of 50 g ha<sup>-1</sup>, at once (96.2%), was significantly higher than that obtained with the application of 25+25 g ha<sup>-1</sup> (85.3%) or 25 + 50 g ha<sup>-1</sup> (87.8%). However, it was lower than that obtained from the other virus treatments, which had no significant difference among them (average of 99.2%).

**Leaf damage**

The damage caused by *S. frugiperda* larvae was severe on the leaves of untreated plots. An average of 4.92 and 4.17 was obtained for infestation at the 6-8 and 8-10-leaf stages, respectively (Table 3). The damage was observed inside the plant whorl, with many plants getting the rate 5 (plants with the whorl destroyed). The damage caused to plants in

plots sprayed with virus varied from 1.75 to 2.33 at the 6-8-leaf stage and from 1.83 to 2.33 at the 8-10-leaf stage. In both cases, lowest damage was verified on plots with one application of 50 g ha<sup>-1</sup>, at least. Applications of 25+25 or 25+50 g ha<sup>-1</sup>, at seven-day interval, did not provide the same protection to the plants. However, the leaf damage was significantly lower than that observed from untreated plots. The leaf damage caused by fall armyworm larvae was very close in both growth stage, and there is no interaction between doses of virus and growth stage. However, significant differences existed among doses (Table 3).

**Ear damage and grain yield**

The damage on ear was very low and confined to the silk and ear tip. Therefore it does not interfere in the results. Significant differences existed among treatments in relation to grain yield (Table 3). Plants protected with the virus produced significantly higher yield than plants unprotected. There is no significant difference among the yield obtained from plots where the virus was applied at the initial dose of 50 g ha<sup>-1</sup>, in both growth stages (average of 8,056.5 kg ha<sup>-1</sup> at the 6-8-leaf and 7,643.5 kg ha<sup>-1</sup> at the 8-10-leaf stage). These means are, respectively,

**TABLE 3.** Effect of dose and interval application of SFNPV on the leaf damage caused by *Spodoptera frugiperda* and on grain yields of maize.

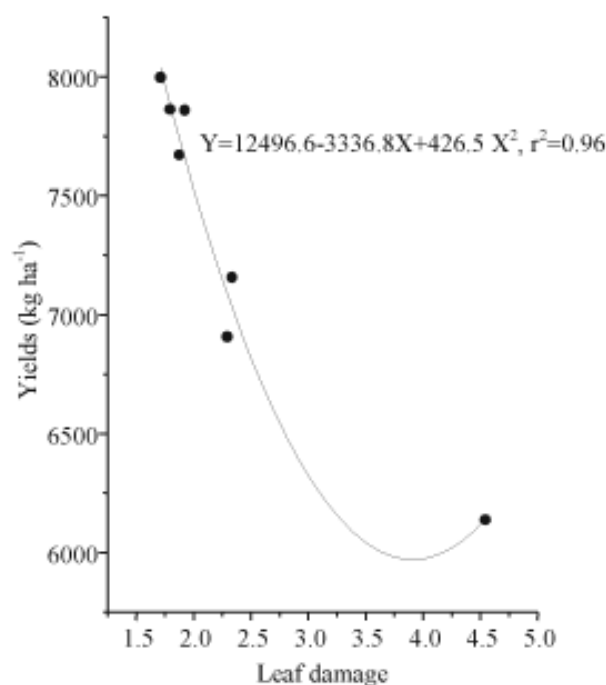
Dose (g ha <sup>-1</sup> )	Leaf Damage <sup>1</sup>			Yield (kg ha <sup>-1</sup> ) <sup>1</sup>		
	Leaf stage			Leaf stage		
	6-8	8-10	Average	6-8	8-10	Average
50	1.83 C	1.92 BC	1.87 C	7.705 AB	7.642 AB	7.673 A
25 + 25	2.25 B	2.33 B	2.29 B	6.887 C	6.931 C	6.909 B
25 + 50	2.33 B	2.33 B	2.33 B	7.215 BC	7.104 BC	7.159 B
50 + 25	2.00 BC	1.83 BC	1.92 C	8.145 A	7.579 AB	7.862 A
50 + 50	1.75 C	1.83 BC	1.79 C	8.204 A	7.527 AB	7.865 A
100	1.75 C	1.67 C	1.71 C	8.172 A	7.826 A	7.999 A
Untreated	4.92 A	4.17 A	4.54 A	6.166 D	6.115 D	6.140 C
Average	2.4 a	2.3 a		7.499 a	7.246 b	
CV (%)	13.8	20.3	17.23	4.1	5.0	6.57

<sup>1</sup>Means followed by the same capital letter within each column or small letter across each line are not significantly different according to Duncan's multiple range test (p ≤ 0.05).

1,890.5 and 1,528.5 kg ha<sup>-1</sup> higher than the averages obtained from untreated plots. In other words, they represent differences of 30.7 and 25.0% in yield. Applications of 25+25 or 25+50 g ha<sup>-1</sup> resulted in similar yields (average of 7,051 and 7,286.5 kg ha<sup>-1</sup>, at 6-8 and 8-10-leaf stage, respectively). These yields are 14.3 and 19.1% higher than those obtained from respective untreated plots.

As in the other parameters, there was interaction between doses and growth stages. However, the average yield was higher in the 6-8-leaf stage (difference of 253 kg ha<sup>-1</sup>), probably due to higher larval mortality obtained in that stage.

Considering the data from the two growth stages (Table 3), the grain yield from untreated plots was 6,140 compared with an average of 7,577 kg ha<sup>-1</sup>, from the treated plots (difference of 23.4%). According to Cruz & Turpin (1983) maize yield is closely related to fall armyworm leaf damage. Plotting the average leaf damage against yields the functional relationship was quadratic (Figure 1).



**FIGURE 1.** Relationship between maize yield and fall armyworm leaf damage from plots protected with different treatment with virus application.

*Baculovirus spodoptera* formulated as a wettable powder can be used in maize crop for fall armyworm (*S. frugiperda*) larvae control at the dose of 50 g ha<sup>-1</sup> ( $2.5 \times 10^{11}$  PIB ha<sup>-1</sup>). The use of 50 or 100 g ha<sup>-1</sup>, applied 24 hours after infestation, or application at seven-day interval of 50+25 or 50+50 g ha<sup>-1</sup>, in maize fields, may produce up to 27.8% higher yield in relation to untreated fields.

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