INTERACTION BETWEEN *Baculovirus spodoptera* AND NATURAL ENEMIES ON THE SUPPRESSION OF *Spodoptera frugiperda* (J. E. SMITH) (LEPIDOPTERA: NOCTUIDAE) IN MAIZE

MARIA DE LOURDES CORRÊA FIGUEIREDO[1], IVAN CRUZ[1], ANGÉLICA MARIA PENTEADO-DIAS[2] and RAFAEL BRAGA DA SILVA[2]

[1]*Embrapa Milho e Sorgo, Caixa Postal 151, CEP 35.701-970, Sete Lagoas, MG, Brasil, E-mail: figueiredomlc@yahoo.com.br, ivancruz@cnpms.embrapa.br*
[2]*Universidade Federal de São Carlos (UFSCar), Caixa Postal 676, CEP 13.565-905, São Carlos, SP, Brasil, E-mail: angelica@ufscar.br, rafaelentomologia@yahoo.com.br*

ABSTRACT - The impact of the application of the *Baculovirus spodoptera* (2.5 x 10^{11} polyhedra/ha), a nuclear polyhedrosis virus, on maize crop and the possible additional contribution of natural control agents to the management of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) were evaluated. The experimental design consisted of randomized blocks with seven *B. spodoptera* treatments (at two days application intervals, beginning four days after artificial infestation with fall armyworm egg masses and finishing after 16 days), and five replications. The main natural enemies found in the experimental area were *Chelonus insularis* (Cresson) (Hymenoptera: Braconidae) and *Eiphosoma laphygmae* Costa Lima (Hymenoptera: Ichneumonidae), egg/larval and larval parasitoid, respectively, and *Doru luteipes* Scudder (Dermaptera: Forficulidae), predator of eggs and larvae. Along with NPV, the natural enemies provided a good control of the target insect reducing the damage caused by *S. frugiperda* larvae in maize plants.

Key words: biological control, nuclear polyhedrosis virus, parasitoids, predators, fall armyworm.

INTERAÇÃO ENTRE *Baculovirus spodoptera* E INIMIGOS NATURAIS NA SUPRESSÃO DE S*podoptera frugiperda* (J. E. SMITH) (LEPIDOPTERA: NOCTUIDAE) NA CULTURA DO MILHO

RESUMO - Foi avaliado o impacto da aplicação do *Baculovirus spodoptera* (2,5 x 10^{11} poliedros/ha) sobre lagartas de *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), e a possível contribuição adicional de seus inimigos naturais na cultura do milho. O delineamento foi em blocos ao acaso com sete tratamentos com *B. spodoptera* (época de aplicação do inseticida a intervalos de dois dias, iniciando-se quatro dias após a infestação artificial, com posturas de *S. frugiperda* e finalizando 16 dias após), em cinco repetições. *Chelonus insularis* (Cresson) (Hymenoptera: Braconidae) e *Eiphosoma laphygmae* Costa Lima (Hymenoptera: Ichneumonidae), respectivamente, parasitóides de ovo/larva e de larva e o predador de ovos e lagartas, *Doru luteipes* Scudder (Dermaptera: Forficulidae), foram os principais agentes de controle biológico natural na área. Juntamente com o vírus, os inimigos naturais propiciaram um bom controle do inseto alvo, reduzindo o dano foliar causado pela lagarta nas plantas de milho.

Palavras-chave: controle biológico, vírus da poliedrose nuclear, parasitóides, predadores, lagarta-do-cartucho.

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is a major maize pest in Brazil, and may infests the crop from plant emergence to harvesting stage. Depending on injury, the pest may cause losses in grain yield up to 55% (Figueiredo et al., 2006a).

The pest control has been achieved by chemicals, used often in an abusive manner, causing a prompt elimination of the complex of natural enemies in the area and favoring the build-up of resistant populations to these products besides other negative effects on the environment (Cruz, 2002). For these reasons, researchers have been looking for alternative management, such as biological control focusing in mass releasing of natural enemies and/ or protection of the predators and parasitoids through the use of selective insecticides (Lucchini & Almeida, 1980; Dutcher, 1993; Simões et al., 1998; Figueiredo et al., 1999; Cruz et al., 2002; Cruz, 2002). The importance of predators and parasitoids in association with *S. frugiperda* has been highlighted not only in

Brazil (Figueiredo et al., 2006b), but also in other countries (Notz, 1972; Silva et al., 1997; Molina-Ochoa et al., 2004; Murúa et al., 2006, 2009). Entomopathogenic microorganisms, such as the natural occurrence in Brazil of the nuclear polyhedrosis virus (NPV), *Baculovirus spodoptera* are also considered important in suppressing the pest (Murray et al., 1995; Escribano et al., 1999, 2000; Cruz et al., 2002). The efficiency of a wettable powder formulation in field applications on maize was demonstrated by Cruz et al. (2002). Synergistic effect of the virus and natural biological control agents was reported by Cruz et al. (2002) and Figueiredo et al. (1999).

The objective of this study was to evaluate the application time of the biopesticide *B. spodoptera* on the suppression of *S. frugiperda* in maize and its interaction with the natural enemies of the class Insecta.

Material and Methods

The study was conducted in Sete Lagoas, Minas Gerais state, Brazil (19° 28' 00'' S and 44° 15' 00'' W), in a savanna region, in the National Maize and Sorghum Research Center (Embrapa / CNPMS), using the maize hybrid BRS 3123.

The effect of a wettable powder formulation of *B. spodoptera* (Nuclear Polyhedrosis Virus - NPV) on maize plant to control the fall armyworm. The virus was applied at a single dose of 2.5×10^{11} polyhedra/ha (50g/ha) (Cruz et al., 1997a) was evaluated

using a randomized complete block design with five replications and the treatments were times of virus application at 2-days intervals, since virus application four days after artificial infestation with *S. frugiperda* egg masses (Treatment 1) until virus application 16 days after artificial infestation (Treatment 7). Each plot had six 4 m long rows (area of 24 m²), with 20 plants per row. Plots were kept four meters apart from each other to avoid contamination. Artificial infestation was made fifteen days after plant emergence. Each plot was infested with one egg mass of *S. frugiperda* on every fifth plant (Cruz & Turpin, 1983). Egg masses containing about 100 eggs and embryonic development up to 24 hours were selected from a laboratory colony. The virus was applied using a CO_2 pressurized backpack sprayer with a flat fan nozzle 8003, maintaining the pressure to 40 PSI and providing a spray volume equivalent to 300 liters per hectare (Cruz et al., 2002). Of the six rows of each experimental plot three clusters were left to assess the damage caused by pest and to obtain the yield. The other rows were used to quantify the presence of caterpillars and their natural enemies.

Before each insecticide application, 20 plants in a row were picked at random. The same procedure was performed 72 hours after the product application. Plants were cut close to the ground individually placed in plastic bags and taken to the laboratory. Egg masses and/or larvae of *S. frugiperda* were collected

and counted. Then, egg masses and live larvae were individually transferred to PVC cups (50 mL) containing artificial diet (Figueiredo et al., 2006b) which were sealed with acrylic cover. Evaluations were made daily to detect the evolution of pest development or the presence of dead insects, and in this case, recording the mortality factor. In each plant it was also evaluated the presence of *Doru luteipes* Scudder (Dermaptera: Forficulidae), an important predator of eggs and small larvae of *S. frugiperda*, once it is very common in the whorl of maize plants (Cruz & Oliveira, 1997; Figueiredo et al., 2006ab). In the field, 19 days after infestation, assessments of damage caused by *S. frugiperda* larvae were made in all plots, using the following visual rating scale: 0 - Plants without damaged leaves; 1 - Plants with scraped leaves; 2 - Plants bearing holes in the leaves; 3 - Plants showing leaf damage and some damage to the whorl; 4 - Plants bearing whorl destroyed; 5 - Dead plants (Cruz & Turpin, 1983). The damage scale was applied to the central leaves of the plant, considering all plants in the plot. At harvest, plant yield was obtained for each plot.

Data of the experiments were analyzed by one-way Analysis of Variance (ANOVA) through the computer program SISVAR (Ferreira, 2000) and treatment means were compared with the Scott-Knott test ($P = 0.05$) (Scott & Knott, 1974). Before running the ANOVA, tests were conducted to determine if the data set met the necessary assumptions.

Burr-Foster Q and Shapiro-Wilk W tests were used to test equality of variance and normality of the data, respectively, following description found in Anderson & McLean (1974). Transformation, when applied, was used according to the criteria suggested by Ostle & Mensing (1975). Regression analyses were also used to determine the relationship between spraying time and larval density, plant infestation, leaf damage and grain yield.

Results and Discussion

Results

Prior to the spraying of *B. spodoptera* (Table 1), the number of larvae per plot decreased over time, from 24 caterpillars obtained in the first assessment, to less than three larvae obtained in the last evaluation. Up to eight days after infestation, the relative number of larvae per plot was high, and no significant difference found among the mean number collected from plots taken during this period (three samples). From the fourth sample and thereafter the number of larvae collected per plot was significantly lower (4.4 larvae/plot on average) than the number obtained in previous assessments.

After virus application, the number of larvae collected at different sampling dates followed a trend similar to that observed prior to application. There was no significant difference among the number of larvae collected in samples taken in the first four samples, with an average

TABLE 1. Number of larvae, percentage of plants infested by *S. frugiperda*, presence of natural enemies before and 72 hours after spraying with *B. spodoptera* and leaf damage measured 19 days after infestation (mean ± SE).

Days after infestation	Larvae/plot[1]		Plants with larvae (%)[1]		Parasitism (%)[1]		Earwig/ plot[1,2]	Leaf damage[1]
	Before spraying	72 hours after spraying	Before spraying	72 hours after spraying	Before spraying	72 hours after spraying		
4	24.0 ± 7 A	19.6 ± 7 A	37.5 ± 5 A	55.0 ± 9 A	52.3 ± 14 A	37.4 ± 37.4 A	3.2 ± 2.3 B	0.42 ± 0.1 A
6	12.5 ± 3 A	12.0 ± 6 A	33.0 ± 12 A	33.0 ± 8 A	43.8 ± 12 A	35.6 ± 35.6 A	3.2 ± 1.5 B	0.68 ± 0.2 A
8	20.6 ± 2 A	7.4 ± 4 A	43.0 ± 4 A	28.0 ± 9 A	46.6 ± 6 A	27.9 ± 27.9 A	7.0 ± 2.8 A	0.78 ± 0.3 A
10	6.4 ± 3 B	9.0 ± 3 A	19.0 ± 7 B	19.0 ± 11 B	64.7 ± 21 A	53.5 ± 53.5 A	10.0 ± 4.5 A	0.84 ± 0.2 A
12	4.0 ± 1 B	1.8 ± 1 B	19.0 ± 6 B	7.0 ± 1 B	57.1 ± 20 A	20.0 ± 20.0 A	11.0 ± 3.0 A	0.78 ± 0.1 A
14	4.5 ± 2 B	1.7 ± 1 B	12.0 ± 5 B	5.0 ± 3 B	67.2 ± 18 A	33.3 ± 33.3 A	8.0 ± 2.2 A	0.82 ± 0.2 A
16	2.7 ± 1 B	1.0 ± 0 B	8.0 ± 6 B	4.0 ± 1 B			12.2 ± 2.9 A	0.82 ± 0.2 A

[1] Means followed by the same letter in column do not differ significantly according to Scott-Knott test (P ≤ 0.05).

[2] 72 hours after spraying with *B. spodoptera*.

of 12 larvae per plot (Table 1). In the following assessments the number of larvae fell to 1.5 per plot.

The percentage of infested plants (Table 1) occurring before virus application was relatively high in plots sampled four, six and eight days after infestation, with no significant difference among means, which were 37.5, 33 and 43% respectively. On subsequent assessments, the percentage of infested plants drops to an average of 14.5% (8 to 19%). In

the samples taken after virus application, the percentage of plants attacked by fall armyworm was 38.6% on average, across the first three samples, and no significant difference among means was observed. In the subsequent assessments the percentage of infested plants fell to an average of 8.8%, with no significant difference among means.

The presence of parasitoids in the experimental area was significant. The rate of parasitism (Table 1), in the samples carried out
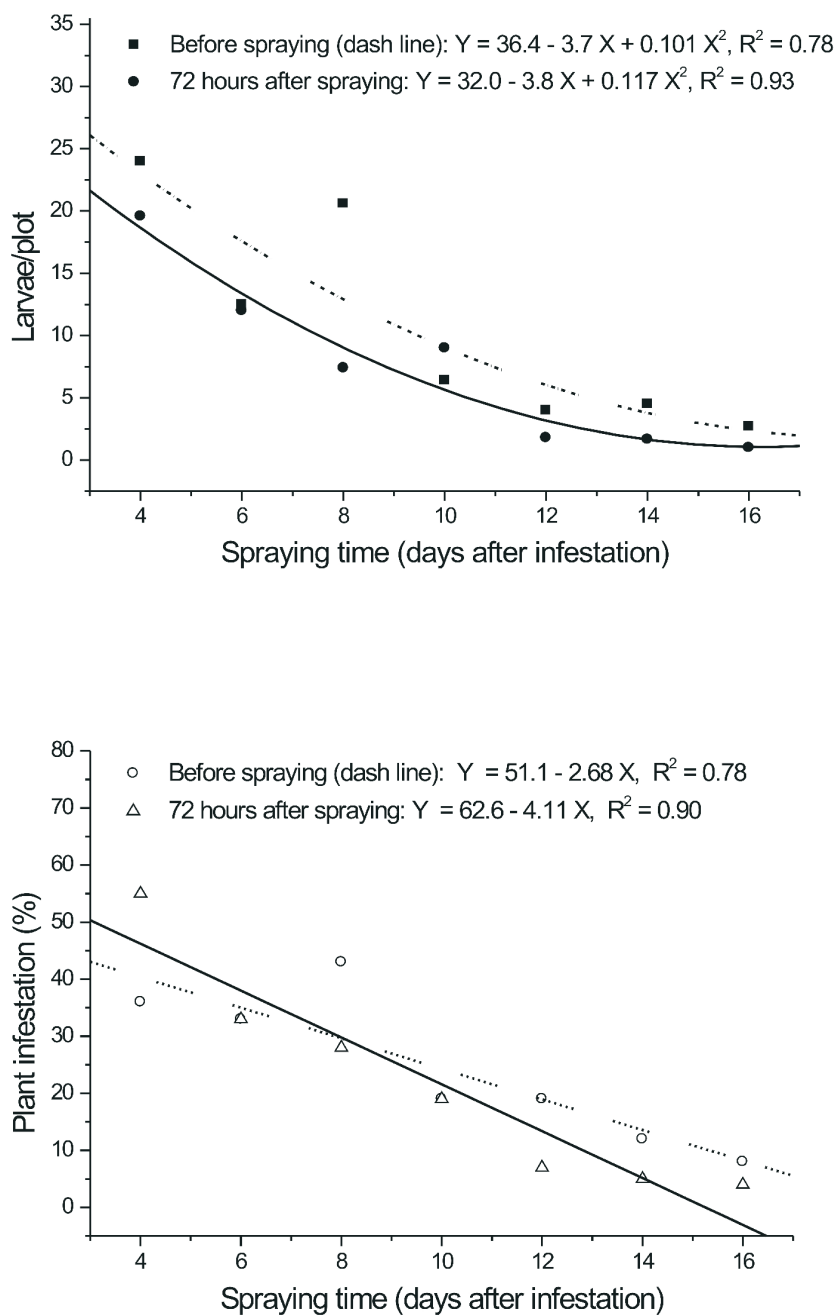
FIGURE 1. Adjusted curve for mean number of larvae and for infestation (%) of *S. frugiperda* with samples taken immediately before and 72 hours after application of *B. spodoptera*.

before spraying with the virus was 55.3% on average (43.8 to 67.2%), with no significant difference among treatments. Even after spraying the rate of parasitism was still high, and also there was no significant difference among different sampling dates, with an average parasitism rate of 34.7%. Among the parasitoids recovered from collected larvae there was the predominance of only two parasitoids, *Chelonus insularis* (Cresson) (Hymenoptera: Braconidae) and *Eiphosoma* *laphygmae* Costa Lima (Hymenoptera: Ichneumonidae).

In addition to parasitoids, there was a constant presence of the predator of eggs and larvae, the earwig *D. luteipes*, with highest densities in samples taken in more developed plants (Table 1).

Discussion

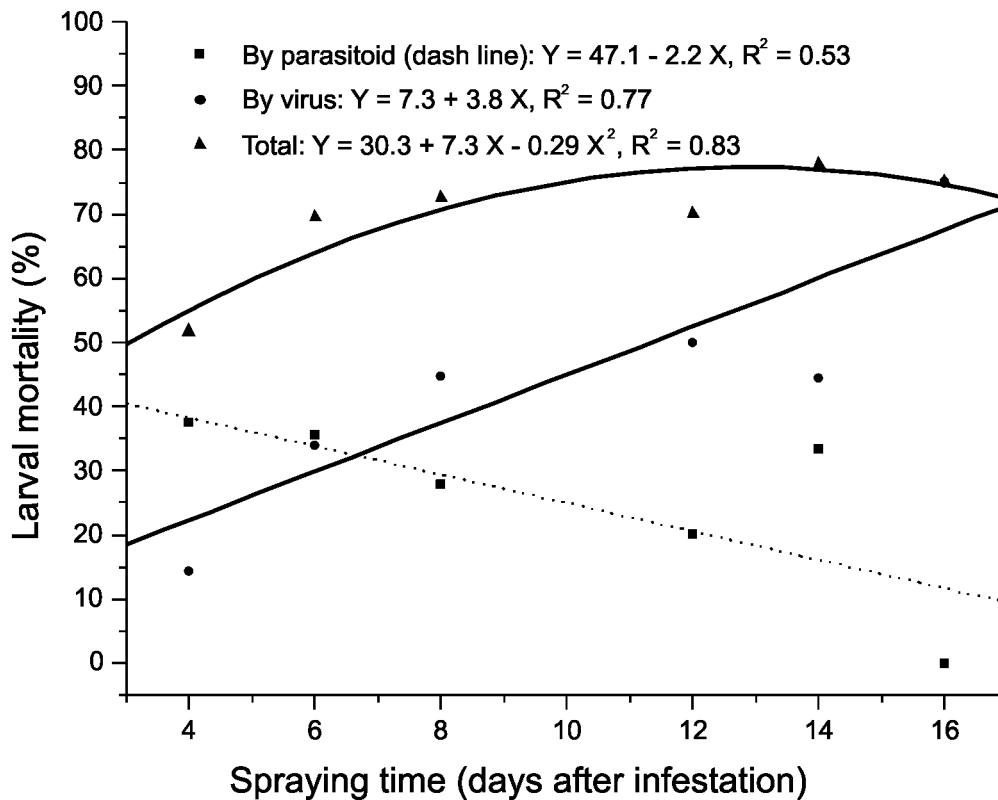The relationship between number of *S. frugiperda* larvae and time was negative and

FIGURE 2. Adjusted curve for fall armyworm larval mortality caused by parasitoid and/or virus according to different spraying time of *B. spodoptera* with samples taken 72 hours after application.

followed a polynomial model (quadratic) in samples taken before or after virus application (Figure 1). Infestation (%), on the other hand, followed a negative and linear model.

Reduction in the number of larvae (or infestation level) with the delaying in the time in which the samples were taken can not be attributed to the end of the larval stage of the pest, even on those plots where the samples were taken before virus application and 16 days after infestation (last evaluation period). It is well known that biological cycle of *S. frugiperda* depends mainly on temperature and, within a limit there is a decrease in larval life cycle with the increase of temperature (Cruz, 1995). During the experimental phase the average temperature was 25.3ºC. An average larval cycle of 15 days at a fix temperature of 25ºC was reported by Busato et al. (2002). Adding a three-days period for egg hatch (Cruz, 1995) by the time of the last evaluation, that is, 16 days after infestation, it can be expected that the insects were mostly still in the larval stage. Reduction on number of larvae then could be attributed to cannibalism inherent to the species and/or to biotic factors such as microorganisms and natural enemies (parasitoids and predators). Natural occurrence of larval disease was not verified in collected insect, in samples taken before virus application. However, occurrence of parasitoid in the area was high and constant. Before virus application, from the collected larvae 55.3% was parasitized. No significant difference was found among treatments (Table

1). Even with the application of virus, parasitism also contributed to suppress the insect pest with an average of 34.5% parasitism. However, larval mortality due to parasitoid decreased with time (Figure 2). The opposite occurred with the larval mortality caused by virus. In fact, there is a constant and positive relationship between larval mortality caused by both factor and spraying time of virus (Figure 2). According to Escribano et al. (2001) both parasitism and viral infection result in a marked reduction in host growth, specially when third instar fall armyworm larvae are dually parasitized by C. *insularis* and infected by virus compared to parasitized larvae. On the other hand, although Baculoviruses do not infect natural enemies, there is a possible impact on parasitoids through competitive or indirect effects. Escribano et al. (2000, 2001) reported that the survival of *C. insularis* was not possible in *S. frugiperda* larvae that ingested a lethal dose of NPV during the second, third or early fourth larval instars. Nakai & Cuc (2005) reported the fate of parasitoids developing within virus infected hosts especially in the case of *Chelonus* sp. dying during their larval stage before spinning cocoons, or failing to reach adult emergence compared to other parasitoids suggesting that the impact of virus application on the survival of parasitoids varies from species to species.

The parasitoids *C. insularis* and *E. laphygmae* were responsible for 77 and 18.5% of parasitism in the samples taken before spraying, respectively. The same species parasitoids
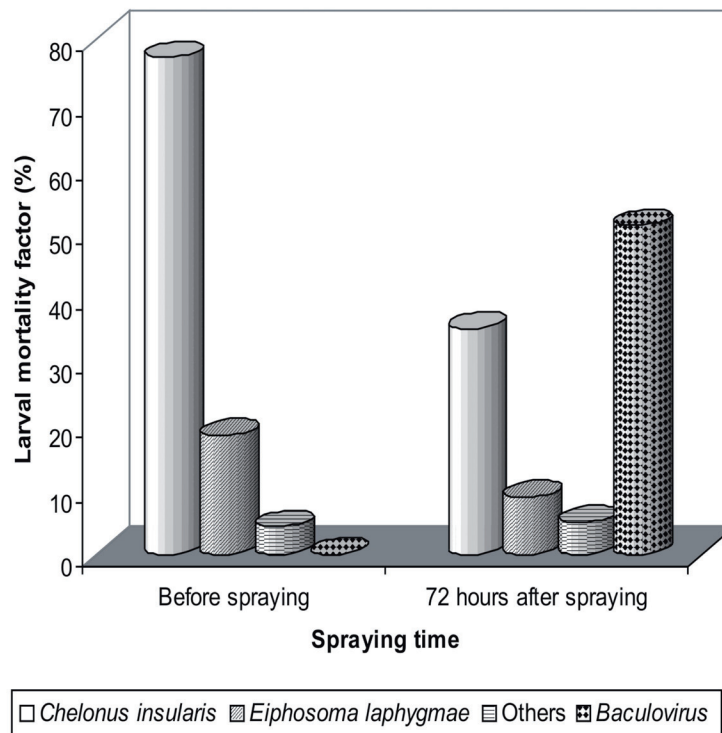
FIGURE 3. Causes of mortality of *S. frugiperda* larvae in maize plots observed in samples taken before and 72 hours after application of *B. spodoptera*.

were responsible for 35.1 and 8.9%, after virus application. Virus mortality accounted by 50% of larval death (Figure 3). The predator of egg and small larvae, *D. luteipes* was the main species found in the experimental area and also contributed to reduce pest density (Table 1).

The effect of the virus on the larvae of *S. frugiperda* can be noticed when they appear limp and blackened (Cruz, 1995). The efficiency of the virus tends to decrease with increasing age of larvae (Cruz et al., 2002; Matrangolo et al., 2007). Additional effect of natural enemy could be an important strategy to suppress

the population of *S. frugiperda* in maize, considering the specificity of virus. Synergistic effect of virus and natural enemies could be more economic and efficient than the unilateral use of a non-selective chemical pesticide. This is particularly true in the case of *C. insularis*, an egg/larva parasitoid very common in association to *S. frugiperda* in maize field in Brazil (Rezende et al., 1995a; Figueiredo et al., 2006ab).

Once *C. insularis* was the predominant species in the experimental area and due to the fact of parasitism initiate in the host eggs, by the time *B. spodoptera* was applied, since the

first virus applications it could be considered an interaction between the two *S. frugiperda* larval mortality factor.

According to Croft & Brown (1975), in general the effect of pathogens should be considered in similar away to the chemicals. For a parasitoid developing inside a larval host such as *C. insularis* and *E. laphygmae* it can be distinguished direct and indirect effect of a pathogen over the parasitoid. As a direct effect on the parasitoid through the host and an indirect effect by premature host killing and then causing the death of parasitoid larvae or even alter the physiology of the host so that it is no longer nutritionally suitable for the development of the parasitoid.

Escribano et al. (2000) demonstrated that all *S. frugiperda* larvae ingesting a lethal dose of multiple-enveloped nuclear polyhedrosis virus were unsuitable for *C. insularis* development. The same situation could partially explain the decrease of parasitism by *C. insularis* after virus application (Figure 3), once the parasitoid was still an important mortality factor of *S. frugiperda* larvae. The same tendency seems to be the case of *E. laphygmae,* a larval parasitoid. According to Murray et al. (1995) the parasitoids *Microplitis demolitor* Wilkinson (Hymenoptera: Braconidae), *Cotesia kazak* Telenga (Hymenoptera: Braconidae) and *Hyposoter didimator* Thunberg (Hymenoptera: Ichneumonidae), required a time advantage of at least three days at 25°C before host exposure to a NPV to ensure successful completion of

development within *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae. The parasitoid *Campoletis sonorensis* (Cameron) (Hymenoptera: Ichneumonidae) only survive inside *S. frugiperda* larvae when there is a 2-day interval between parasitism and viral infection (Escribano et al., 2000). Matrangolo et al. (2007) evaluating the interaction between the larval parasitoid *Campoletis flavicincta* Ashmead (Hymenoptera: Ichneumonidae) and virus concluded that the application of the virus to control *S. frugiperda* larvae can adversely affect the parasitism, since the virus can kill the parasitoid larvae developing within the host body. However, according to Vail et al. (1972) even when there is an effect of reducing the rate of parasitism due to application of a NPV, if the overall control is good, one should consider a normal completion between the virus and the parasitoid. And this seems to be the case, and NPV together with the action of *C. insularis* and *E. laphygmae* positively contributed to the reduction of pest population (Figure 2). According to Martínez et al. (2000) *S. frugiperda* larval mortality induced by virus was approximately 50% at the highest application rate tested of 1000 larval equivalents (LE) of virus/ha. However, when the impact of parasitism was taken into account, larval mortality increased to 45.0 - 90.7% in plots treated with virus at 250 LE/ha or more. Cruz et al. (2002) reported an average percentage of larvae killed by the NPV and natural biological control ranging from 88.2 to
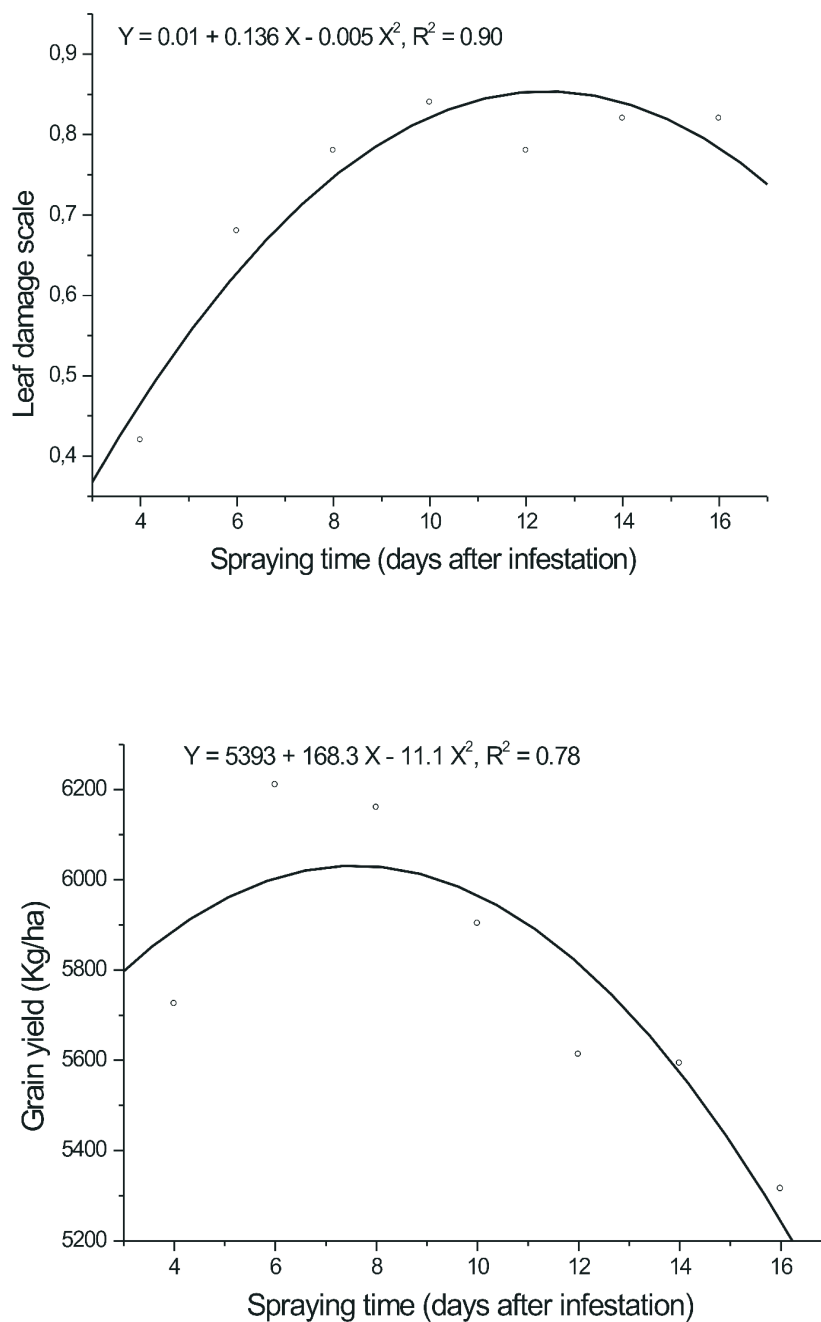
FIGURE 4. Adjusted curve for leaf damage and for maize yield in relation to application time of *B. spodoptera*.

96.1%. Parasitized fall armyworm larvae also reduce significantly the food consumption. For example, larva parasitized by *C. insularis* consumes only 6.8% of the total maize leaf consumption by a health larva (Rezende et al., 1995b). Similar reduction of food consumption was also verified for NPV infected larva (Cruz, 2000) or for a larval parasitoid such as the *C. flavicincta* (Cruz et al., 1997b).

Obviously, the presence of the earwig also contributed to reduce pest density once it is considered the most important insect predator in association to *S. frugiperda* in Brazil (Cruz & Oliveira, 1997; Guerreiro et al., 2003; Figueiredo et al., 2006ab) and may consume a large amount of egg and larvae of the insect pest during its life cycle (Reis et al., 1988).

The leaf damage caused by the fall armyworm larvae based in a visual scale from 0 (no damage) to 5 (dead plants) varied 0.42 to 0.82 (Table 1) indicating that the natural presence of parasitoid and predator besides the application of virus were enough to reduces de damage to the plant (Figure 4). According to Cruz & Turpin (1983) foliar damage has a linear and negative relationship with maize yield. Using the same visual scale, a score close to one was obtained by Figueiredo et al. (1999) in plots where the virus was applied alone or integrated to an egg parasitoid. Cruz et al. (2002) reported an average score of 1.87 on plots infested with larvae and sprayed with

B. spodoptera. The application of microbial agents in infested areas eliminates the insect host, interacts positively with the natural biological control and therefore reduces the damage to maize plants (Hopper & King, 1984).

In the plots sprayed earlier larvae were smaller, so the consequences of their damage on the plants were lower, giving them a quicker recovery with less effect on yield. Plots with larger larvae and sprayed later, somehow could be expected a reduction on the efficiency of *B. spodoptera*. The maize yield ranged from 5.315 to 6.210 kg/ha and there was no significant difference among treatments. However, adjusted curve for maize yield in relation to application time (Figure 4) indicates a tendency to the increases in yield in those plots sprayed earlier, that is, up to eight days after infestation, and a yield reduction after that point.

Considering that there was not statistical difference in maize yield regardless the time in which the virus was applied to suppress the fall armyworm larvae, it can be concluded that the presence of natural enemies in the target area was a positive factor contributing also to reduce pest population.

Acknowledgments

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