BIOLOGICAL CONTROL

Field Efficacy of the Nucleopolyhedrovirus of *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae): Effect of Formulations, Water pH, Volume and Time of Application, and Type of Spray Nozzle

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Eficiência do Nucleopoliedrovirus de *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) em Campo: Efeito de Formulações do Vírus, pH da Água, Volume e Horário de Aplicação, e Tipo de Bico de Pulverização

RESUMO – Avaliou-se o controle de *Anticarsia gemmatalis* Hübner através do seu nucleopoliedrovirus (AgMNPV), em função de diferentes parâmetros envolvidos com o uso desse inseticida biológico, visando elucidar alguns casos de baixa eficiência em determinadas regiões do País, principalmente no Rio Grande do Sul (RS). O trabalho foi conduzido em quatro safras, de 1994/95 a 97/98, em Cruz Alta – RS, avaliando-se os seguintes fatores sobre a eficiência do AgMNPV: formulações comerciais disponíveis; pH da calda; volume de calda; horário de aplicação; ponta de pulverização; e da mistura do vírus com óleo mineral. As parcelas consistiram de 28 fileiras de soja com 10 m de comprimento e espaçamento de 40 cm, adotando-se delineamento de blocos completamente casualizados, com quatro repetições. As amostragens foram realizadas pelo método do pano, determinando-se o número de lagartas infectadas pelo vírus (LI). As formulações comerciais produzidas pela Nitral, Nova Era, Coodetec e Embrapa controlaram adequadamente a praga, em comparação à testemunha; no entanto, foram significativamente inferiores à preparação do vírus obtida por maceração e filtragem de lagartas, demonstrando que essas formulações necessitam aperfeiçoamento. A eficácia do AgMNPV foi significativamente afetada pelo pH da água, pois parcelas tratadas com suspensão com pH 6 apresentaram número significativamente maior de LI que aquelas pulverizadas com suspensões com pH 2 e 10. O volume de aplicação também influenciou no controle de *A. gemmatalis* pelo vírus. Volumes de 300, 200 e 100 L/ha foram igualmente eficientes, mas a 50 L/ha o controle do inseto foi significativamente inferior aos obtidos com os demais volumes, aparentemente pelo fato de ter ocorrido entupimento de bicos no menor volume. Quanto ao horário de aplicação, as maiores eficiências foram observadas para pulverizações realizadas às 2:00 h e 20:00 h, em comparação com as efetuadas às 08:00 h e 14:00 h. Por outro lado, não houve diferença na eficácia do vírus quando aspergido com diferentes pontas de pulverização e tampouco a adição de óleo mineral à calda melhorou a performance do vírus formulado.

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

ABSTRACT – The efficacy of the nucleopolyhedrovirus of *Anticarsia gemmatalis* Hübner (AgMNPV) was evaluated in relation to different application parameters of this biological insecticide, to elucidate some problems of its low efficacy in certain regions in Brazil, mainly in the state of Rio Grande do Sul (RS). Each experiment was conducted during four consecutive soybean seasons (1994/95 to 97/98), in Cruz Alta – RS, to evaluate the following parameters on AgMNPV field efficacy: available commercial formulations of the virus; pH of the viral aqueous suspension; spray volume; application time; spray nozzle type; and the mixture of the virus with mineral oil. Each plot consisted of 28 soybean rows of 10 m in length, spaced of 40 cm. The statistical design was the completely randomized block design, with four replicates. Samplings of AgMNPV infected or dead larvae (IL) were periodically made by the shake cloth method. The AgMNPV formulations produced by Nitral, Nova Era, Coodetec and Embrapa were efficient in controlling larval populations of *A. gemmatalis* when compared to the control. However, these formulations were significantly inferior to the crude extract of AgMNPV (maceration and filtration of dead larvae), indicating that current commercial formulations of this virus need to be improved. The AgMNPV was significantly affected by the water pH in the spray tank, as plots treated with a viral suspension at pH 6 resulted in a significantly higher number of IL than plots treated with suspensions at pH 2 and 10. The spray volume also influenced the control of *A. gemmatalis* larval populations by the AgMNPV. Volumes of 300, 200 e 100 L/ha were equally efficient in controling the insect. However, at 50 L/ha, the virus efficacy was significantly lower than that provided by the other spray volumes, apparently due to nozzle clogging at the lower application volume. The AgMNPV performance to control the insect was also significantly affected by the time of application, with higher efficiencies when spraying at 2:00 a.m. or 8:00 p.m., in comparison to 8:00 a.m. or 2:00 p.m. On the other hand, no differences were detected on the efficacy of the virus when sprayed with different nozzle types, and mineral oil addition to the viral suspension did not improve the field performance of the formulated AgMNPV.

KEY WORDS: Insecta, velvetbean caterpillar, biological control, baculovirus, soybean.

The velvetbean caterpillar, *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), is a major defoliating insect of soybean in Brazil, being responsible for an average of two insecticide applications on this crop every season. In Rio Grande do Sul state (RS), around 70% of the insecticide applications on soybean are made against this pest. The use of a nucleopolyhedrovirus of *A. gemmatalis* (AgMNPV) as a component of the soybean integrated pest management program (Moscardi 1999) has been important in reducing the chemical insecticide applications on the crop and thus their negative environmental impact, crop protection costs and cases of human intoxications.

Initially, the AgMNPV was produced in the laboratory for further distribution of virus samples to soybean growers for its multiplication in the field on naturally occurring *A. gemmatalis* larval populations. Farmers would apply the virus in larger areas as crude preparations of the virus (homogenization in water and filtration through cloth), collect AgMNPV-dead larvae and store them in a freezer for use in the following soybean season (Moscardi 1983, 1986). In 1986 a formulation of this virus was made available to soybean growers, and from the early 1990's five private companies started production and commercialization of this formulation (Moscardi 1989, 1999), which is currently widely adopted among soybean growers (Gazzoni 1994, Moscardi 1999).

During the 1993/94 season, cases of low quality and efficacy of the biological product were reported, which could be related to different factors affecting its stability and efficacy under field conditions. These may include: solar radiation, particularly the UV spectrum (Jaques 1977, Moscardi *et al*. 1981, Silva 1987); relative humidity and precipitation (Jaques 1967, 1977); age and population intensity of the host insect (Boucias *et al*.1980, Moscardi 1983, Silva 1987); pH of the aqueous viral suspension in the spray tank (Young *et al*. 1977, Ignoffo & Garcia 1966, Keating *et al*. 1988, Batista 1997); temperature (Jaques 1977, Johnson *et al*. 1982, Ignoffo 1985); and viral formulation, equipment and application technology (Yearian 1978, Silva 1986). Due to cases of low efficacy, particularly in Rio Grande do Sul, the treated area with the AgMNPV stabilized and even decreased in this state from mid 1990's. However, overall treated area in Brazil kept increasing and currently the AgMNPV is used in 1.2 to 1.4 million ha annually (8-10% of the soybean cultivated area) (Moscardi 1999).

In light of the reported cases of low efficacy of the AgMNPV, especially of the commercial formulations of this virus in some regions, and the importance of maintaining or increasing its utilization and benefits, field studies were conducted to evaluate effects of the following parameters on field efficacy of this biological insecticide: commercial formulations of the virus; pH of the viral suspension in the spray tank; volume of application; time of application; spray nozzle type; and mixture of the AgMNPV with mineral oil.

Material and Methods

The experiments were conducted during four consecutive soybean seasons in the same sites at Fundacep Fecotrigo, in Cruz Alta, RS, adopting the same cultural practices each season. Sowing occurred on the second half of November of 1994/95 (cv. Cobb), 1995/96 (cv. IAS 5), 1996/97 (cv. FT Abyara), and 1997/98 (cv. Ocepar 14), at 18 seeds/row meter and row spacing of 40 cm. Each plot consisted of 28 rows with 10 m in length, with a border of 10 m between plots and blocks to reduce contamination by the AgMNPV through spray drift and other factors. The statistical design adopted was the completely randomized block design. Before application of the treatments, the number of *A. gemmatalis* larvae was evaluated in each plot through two samplings by the shake cloth method (Kogan & Pitre 1980). The treatments were applied when the majority of *A. gemmatalis* larvae were 1.5 cm or less in length (Moscardi 1983, 1986), and the soybean plants were at the flowering stage (R1 or R2) (Fehr & Caviness 1977). Except for the experiments involving different times of application or mixture with mineral oil (9:00h), treatments started at 18:00h, by using a coastal CO_2-propelled sprayer with a dispersing tube containing five spray nozzles, spaced 50 cm apart. The AgMNPV dosage for all treatments was equivalent to 1.5×10^{11} occlusion bodies/ha for the commercial formulations and the crude preparation of the virus (obtained by homogenization of virus-dead larvae in water and filtration through layers of cheese cloth). The shake cloth (two/plot) and plant examination methods were used to evaluate the number of *A. gemmatalis* larvae (> 2 cm) infected or dead (IL) by the AgMNPV at eight, 10, 12, 14 and 16 days after the application of treatments.

Due to the large amount of data for each experiment and year, data on mean total number of IL for each treatment were pooled, considering the information obtained in each plot/year as replications. This type of analysis was possible as all experiments had the same structure and were conducted in the same respective site each season. Furthermore, the previous analysis of each experiment per season indicated that there were no significant variations in climatic conditions and in LI from season to season to justify presentation of the data per year, as the outcome of the results would not be affected in relation to the pooled analysis. Data on mean total number of IL by the AgMNPV were submitted to ANOVA, and the means were compared by the confidence intervals and the Tukey test (P=0.05). The following experiments were conducted during the four consecutive soybean seasons:

Comparison of Commercial Formulations of the AgMNPV with the Crude Preparation of the Virus. The treatments based on the AgMNPV, with 16 replications (four/season), were: crude preparation, Embrapa formulation (Baculovirus AEE), Coodetec formulation (Coopervirus), Nitral formulation (Baculovirus Nitral), Nova Era formulation (Baculoviron), and check (water). The volume of the applied aqueous virus suspension was 100 L/ha, at 55 lbs/pol^2, using JD 10-1 conic spray nozzles.

Effect of the pH of Aqueous Spray Tank Suspensions on AgMNPV Efficacy. The treatments, with 20 replications (five/season) consisted of the following pHs of the AgMNPV aqueous spray suspensions: pH 2, pH 6, pH 10, and check (water). The volume of application was 100 L/ha + 20 g/ha of the Embrapa formulation of the AgMNPV, at 55 lbs/pol^2, using the cone spray nozzle JD 10. For the pH 10, water from Ijui (RS) was used. The other pHs in the spray tank suspension were obtained with appropriate amounts of two reagents (Sodium Citrate – $C_6H_5O_7Na_3$ and Citric acid – $C_6H_8O_7$), according to Colowick & Kaplan (1955).

Effect of the Spray Volume on AgMNPV Efficacy. The treatments, with 16 replications (four/season), consisted of the following application volumes, and the respective nozzles and application pressures: 50 L/ha - nozzle XR Teejet 11001, 8 lbs/pol^2; 100 L/ha - nozzle XR Teejet 11002, 10 lbs/pol^2; 200 L/ha - nozzle XR Teejet 11003, 30 lbs/pol^2 ; 300 L/ha - nozzle XR Teejet 11004, 50 lbs/pol^2; and check (water). The Embrapa formulation of the AgMNPV was used in this test.

Effect of the Time of Application on AgMNPV Efficacy. The treatments, with 16 replications (four/season), consisted of the following times of application: 2:00 a.m.; 8:00 a.m.; 2:00 p.m.; 8:00 p.m.; and check (water). The spray volume used was 100 L/ha + the Coodetec (Coopervirus) formulation of the AgMNPV, at 55 lbs/pol^2, using the conic nozzle JD 10 – 1.

Effect of the Spray Nozzle Type on AgMNPV Efficacy. The treatments, with 16 replications (four/season), consisted of the following nozzle types and respective application volumes and spray pressures: Cone JD 10 – 1, 109 L/ha, 60 lbs/ pol^2; Cone JA 02 - 104 L/ha, 15 lbs pol^2; XR Teejet 110 02, 107 L/ha, 10 lbs/pol^2; Twinjet 110 02, 105 L/ha, 10 lbs/ pol^2; Turbo Teejet TT 11003 VP, 110 L/ha, 6 lbs/pol^2; and check (water). The virus inoculum used was the Nova Era formulation (Baculoviron).

Effect of the AgMNPV Mixture with Mineral Oil. The treatments, with 20 replications (five/season), consisted of: AgMNPV – Nitral formulation (Baculovirus Nitral), mineral oil at 0.5% v/v, AgMNPV – Nitral formulation + mineral oil at 0.5% v/v, and check (water). The spray volume was 100 L/ha, at 55 lbs/pol^2, using the conic nozzle JD 10 – 1.

Results and Discussion

Comparison of Commercial Formulations of the AgMNPV with the Crude Preparation of the Virus. The highest efficiency of the AgMNPV was obtained with the crude preparation, as the plots receiving this treatment had a significantly higher number of virus-infected or dead larvae (IL) than plots receiving the other treatments (Fig. 1). The formulations of AgMNPV from Nitral, Nova Era, Coodetec and Embrapa were also significantly efficient when compared to the check (water). The present results are similar to those reported by Silva (1991), who observed higher speed of kill of larvae of *A. gemmatalis* and a tendency of higher yield of soybean in plots treated with a crude preparation of AgMNPV than in plots treated with available commercial formulations of the virus. However, the results with the formulations are in disagreement with those reported by Batista (1997), who did not find significant differences regarding virulence of the pathogen and number of larvae in plots treated with the different formulations and the crude extract.

The superior performance of the crude extract in relation to the commercial formulations of the virus was consistent through the four seasons of the test (data not shown), indicating that the commercial formulations available at the beginning of the experiment were probably not the most appropriate in terms of certain characteristics, such as particle size, suspension in water, adherence to the foliar substrate, and UV protection, among others. Current AgMNPV commercial formulations lack surfactants, adhesives and UV protectants, which may, in part, help to explain the superior performance of the crude extract in relation to the commercial formulations of this biological insecticide. Crude extracts of baculoviruses have natural protectants against the UV radiation and they adhere well to the plant subtracts, due to the hemolymph contents of larvae containing proteins and other substances acting as stickers and UV protectants (Cherry *et al.* 2000, and papers cited therein). A crude preparation and a formulation of AgMNPV containing an UV protectant presented a half-life of approximately seven and eight days, respectively, compared to a half-life of about three days for the partially purified AgMNPV (Moscardi 1983, 1986). Studies with protectants against UV radiation and formulations have been conducted in Brazil with AgMNPV (Batista Filho *et al.* 1986, Batista Filho & Augusto 1987, Alves *et al.* 1992, Lessa & Medugno 2000, 2001, Morales *et al.* 2001), showing that the current commercial formulations of this virus can be significantly improved. However, these technological developments have not been adopted yet by the private companies producing the AgMNPV in Brazil, probably because they either may not have been brought to the attention of these companies or they may imply in higher costs of the final product compared to the current procedures for processing and formulating this biological insecticide.

Effect of the pH of Aqueous Spray Tank Suspensions on AgMNPV Efficacy. The efficacy of AgMNPV was significantly affected by the pH of the aqueous spray suspension (Fig. 2). The number of virus-infected and dead larvae (IL) was significantly higher (P<0.05) in plots treated with a virus suspension at pH 6 in relation to plots treated

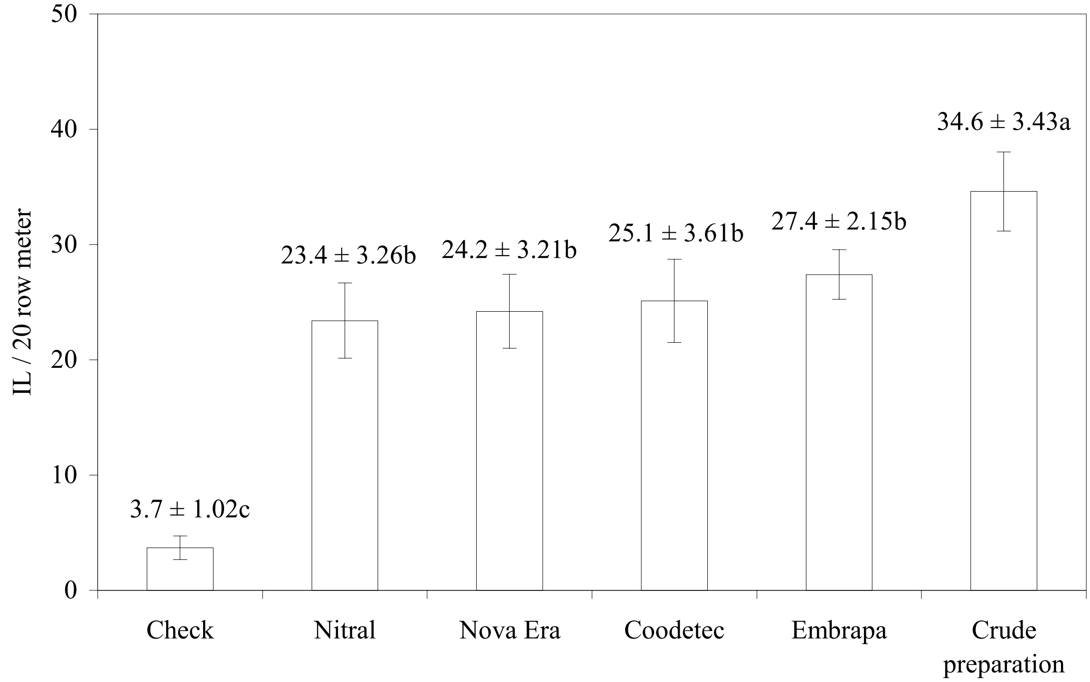

Figure 1. Mean number of *A. gemmatalis* larvae infected or dead (IL) by the AgMNPV in plots treated with commercial formulations and a crude preparation of the virus. Means (± 95%CI) followed by different letters differ significantly by the Tukey test at the 5% level (CV = 21.1%).

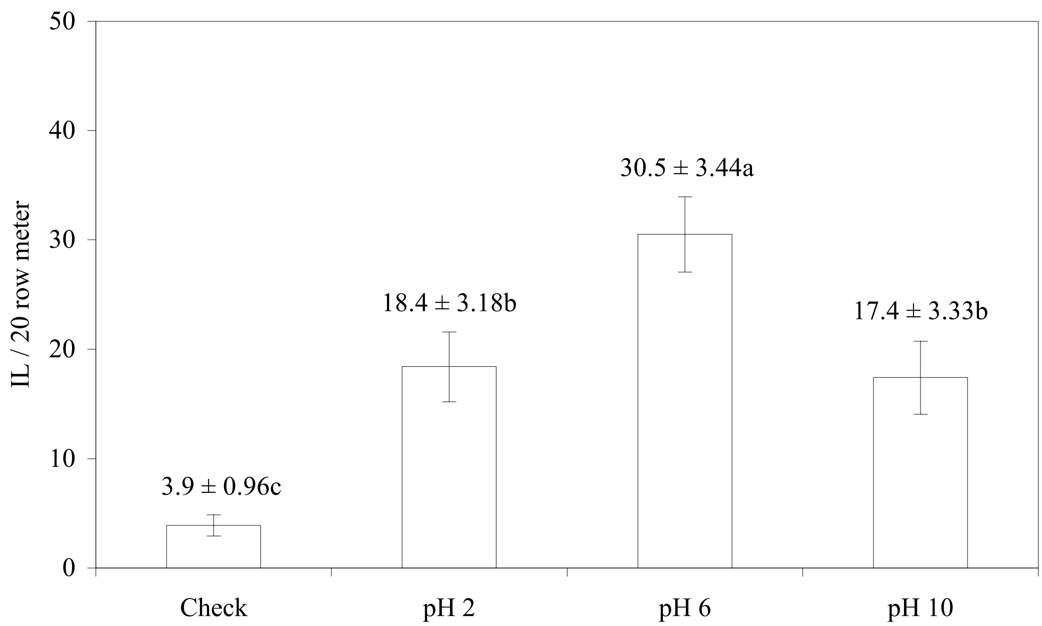

Figure 2. Mean number of *A. gemmatalis* larvae infected or dead (IL) by the AgMNPV in plots treated with viral aqueous suspensions at different pH. Means (± 95%CI) followed by different letters differ significantly by the Tukey test at the 5% level (CV = 26.2%).

with viral suspensions at pHs 2 and 10, but these had significant more IL than those plots treated only with water (check). These results are similar to those obtained by other authors (Ignoffo & Garcia 1966, Young *et al*. 1977, Keating *et al*. 1988, Batista 1997). The lower efficacy of AgMNPV at pHs 2 e 10 is probably due to the dissolution of the occlusion bodies as a result of an excess of hidrogen (H^+) ions and of oxydril (OH) in acid and basic water, respectively (Ignoffo & Garcia 1966, Batista 1997). In general, the baculoviruses are stable between pHs 4 and 9 (Ignoffo &

Garcia 1966). Rheinheimer & Souza (2000) reported that about 15% of the samples taken from water supplies in the state of Rio Grande do Sul presented pH over 8. Those from the municipalities of Tapera, Sarandi, Santo Angelo and Itaqui had a maximum pH over 9. Although these authors reported the importance of these findings for the care that should be taken when using water from different sources for the application of herbicides, their results indicate that application efficacy of AgMNPV might have been influenced by the high pH of water used for its application in some regions.

Effect of the Spray Volume on AgMNPV Efficacy. There was a significant difference (P<0.05) on AgMNPV field efficacy to control *A. gemmatalis* larvae for the different volumes of viral aqueous suspension utilized during the sprays (Fig. 3). There were no significant differences (P>0.05) among the treatments 300, 200 and 100 L/ha, which resulted in higher efficiency of AgMNPV. At 50 L/ha the virus was more efficient in relation to the check, but was less efficient when compared to the other three spray volumes. Although a pre-mixture of the formulated product in water was performed before diluting it in the specified volumes in the spray tank, clogging of nozzles was observed at 50 L/ha, in part explaining the lower efficiency of AgMNPV at this application volume. Yearian (1978) obtained similar results as he observed higher efficacy of baculovirus deposition on plants at higher aqueous spray volumes, which reduced losses of virus due to spray drift and evaporation. Silva (1986) also found that ground spraying of AgMNPV was more efficient at higher aqueous spray volumes.

The formulated AgMNPV product is obtained by homogenizing dead larvae in water in blenders, filtering the suspension through a screen, with the resulting aqueous suspension being mixed with kaolin (1:1 v/w) to be air dried overnight in thin layers spread in plastic trays. The dried material is then milled into a powder to be mixed with water for field application of the biological insecticide (Moscardi 1989). However, this process results in a high amount of particulate organic material from dead larvae, leading to the problem of nozzle clogging, especially in spray volumes below 80 L/ha. In the last three years, the AgMNPV formulation process has been improved by at least one of the companies (Coodetec), related to the homogenization of dead larvae, the filtering process of the homogenate, and the size of the final particles in the formulation, solving the problem of nozzle clogging under low application volumes (B. Santos, pers. commun.).

Effect of the Time of Application on AgMNPV Efficacy. The performance of AgMNPV on *A. gemmatalis* larval populations was significantly dependent on the time of application (Fig. 4). The highest efficacy of the virus was observed for applications made at 2:00 a.m. or 8:00 p.m. compared to those made at 8:00 a.m. and 2:00 p.m. The latter times of application, however, resulted in significantly higher number of IL compared to the check. The differences observed among the different times of application were probably due to solar radiation, mainly the UV spectrum and temperature, which are usually intense during daylight. Moscardi *et al.* (1981), Moscardi (1986) and Silva (1987) showed that solar radiation was detrimental to AgMNPV activity if the virus occlusion bodies were not protected from UV radiation. To avoid this problem, Ignoffo (1985) recommended application of baculoviruses at sundown to reduce the effects of UV on the activity of these biological insecticides. UV is reported to be the major factor contributing to the deactivation of baculoviruses (Jaques 1977). Furthermore, baculovirus infections on host insects may be

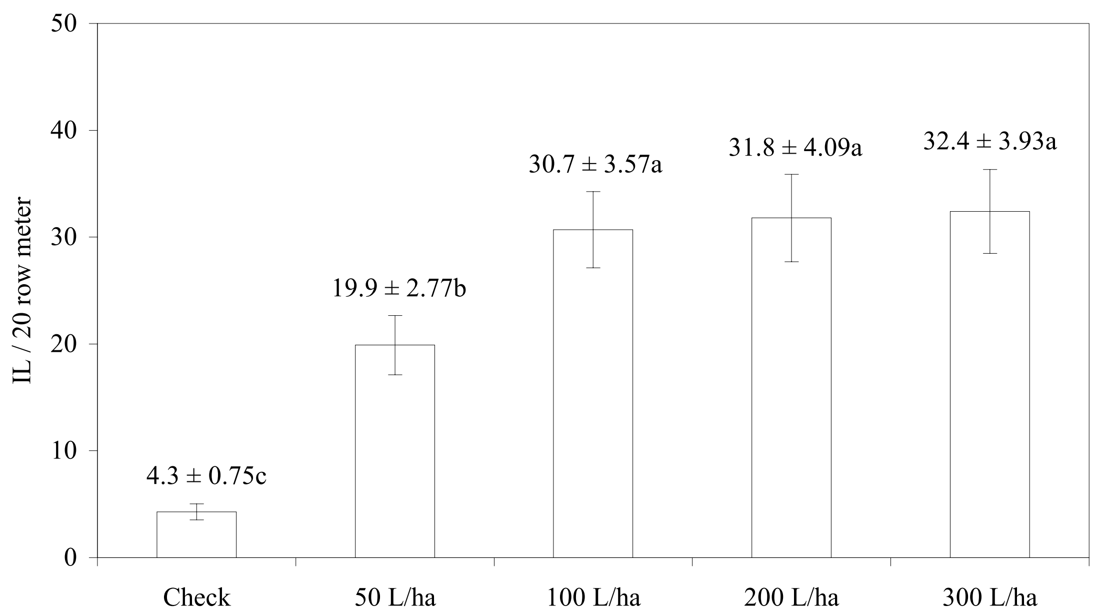

Figure 3. Mean number of *A. gemmatalis* larvae infected or dead (IL) by the AgMNPV in plots treated with different spray volumes of the AgMNPV suspension. Means (± 95%CI) followed by different letters differ significantly by the Tukey test at the 5% level (CV = 19.0%).

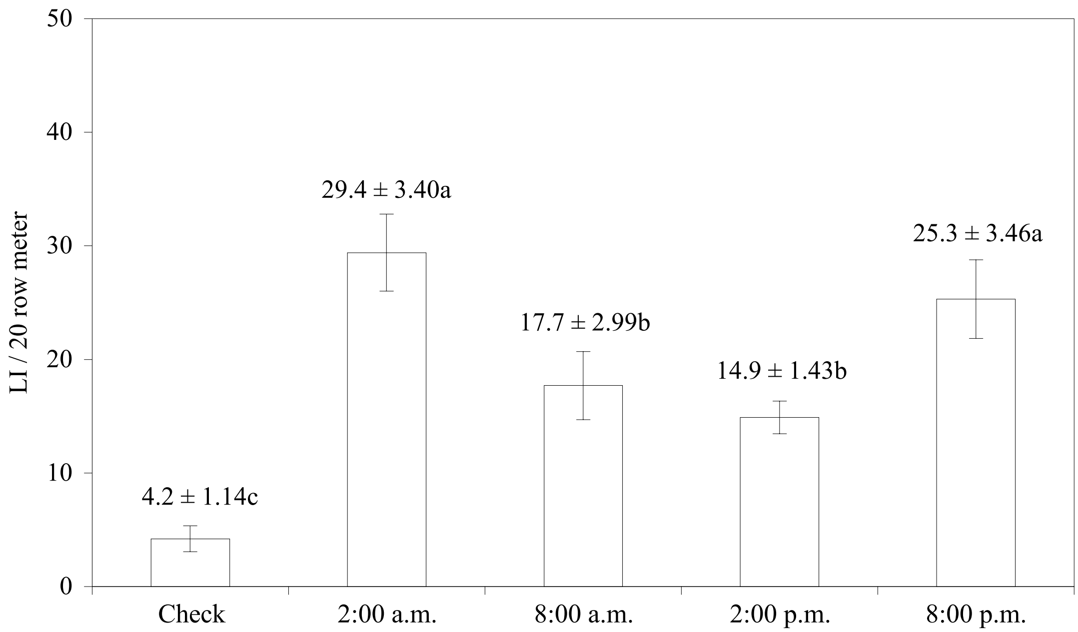

Figure 4. Mean number of *A. gemmatalis* larvae infected or dead (IL) by the AgMNPV in plots treated at different times of application of the AgMNPV. Means (± 95%CI) followed by different letters differ significantly by the Tukey test at the 5% level (CV = 25.2%).

inhibited in low (<10°C) as well as high (>40°C) temperatures (Johnson *et al*. 1982). These authors reported that the effect of temperature may be related to the invasion and infection of the host insect by the virus or also to the increase in host cellular immunity due to low and high metabolic levels of the insect at low and high temperatures.

Effect of the Spray Nozzle Type on AgMNPV Efficacy. The different types of spray nozzles evaluated did not affect the field efficacy of AgMNPV, since there were no differences among the nozzles used, which in turn differed significantly only from the check (Fig. 5). These results may be related to high plant height/foliar density when the treatments were applied, which might have blocked high intensity of solar radiation penetrating in the plant canopy and thus allowing higher persistence of activity of the applied virus, mainly on internal leaves (Johnson *et al*. 1982). According to Yearian (1978), the degree of plant coverage and spray drift in applications of baculoviruses are influenced by the droplet size and the application volume, which in turn are affected by the type of spray equipment used, the type and quality of the spray nozzles, and the environmental conditions. In the present study, even considering the different climatic conditions in each of the four seasons during implementation and evaluation, AgMNPV was effective to control *A. gemmatalis* larval populations independently of the nozzle types.

Effect of the AgMNPV Mixture with Mineral Oil. AgMNPV used solely performed as well as its mixture with mineral oil to control *A. gemmatalis* larval populations, and these two treatments were significantly superior to the check and the mineral oil used solely, with the latter having no effect whatsoever on the insect mortality (Fig. 6). Therefore, the addition of mineral oil to the spray suspension did not contribute to improve AgMNPV field efficacy, which is in disagreement with Smith & Bouse (1981), who suggested the use of molasses and vegetable or mineral oil to increase the viscosity of viral suspensions, under adverse climatic conditions, to increase field efficacy of baculoviruses.

Concluding Remarks

Although the application pressure was not the object of the different experiments, its variation was necessary to obtain the different volumes of application or equilibrium among spray nozzle/volume of application/spray pressure. However, these variations apparently did not affect the outcome of these experiments.

The efficacy of the different AgMNPV commercial formulations tested to control field populations of *A. gemmatalis* larvae did not differ significantly. However, these formulations were inferior to the crude extract of the virus in controlling the insect, indicating that the current formulations need improvement. The different types of spray nozzles utilized or the mixture of the AgMNPV with mineral oil did not affect efficacy of the virus. However, treatments involving the spray suspension at pH 6, application volumes of 100, 200 e 300 L/ha, and time of application at 2:00 a.m. e 8:00 p.m. improved the efficacy of the virus against *A gemmatalis* larvae compared to the other respective treatments. Therefore, the three latter factors associated to a possible inadequacy of commercial formulations may be responsible for failure cases of the AgMNPV in the field, and should be taken into

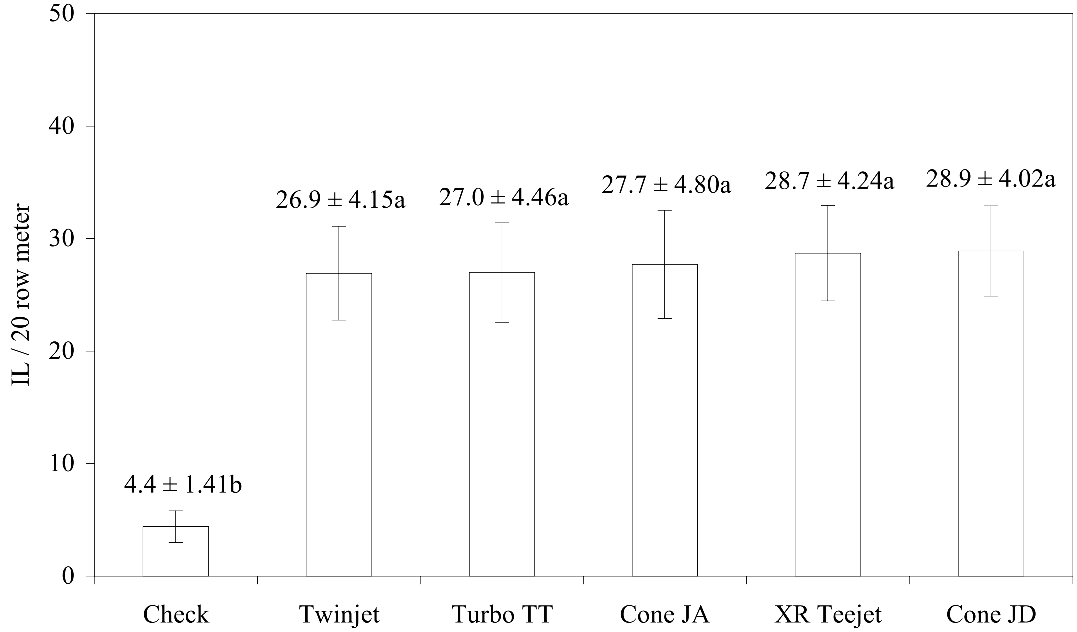

Figure 5. Mean number of *A. gemmatalis* larvae infected or dead (IL) by the AgMNPV in plots treated with the virus using different spray nozzle types. Means (± 95%CI) followed by different letters differ significantly by the Tukey test at the 5% level (CV = 23.7%).

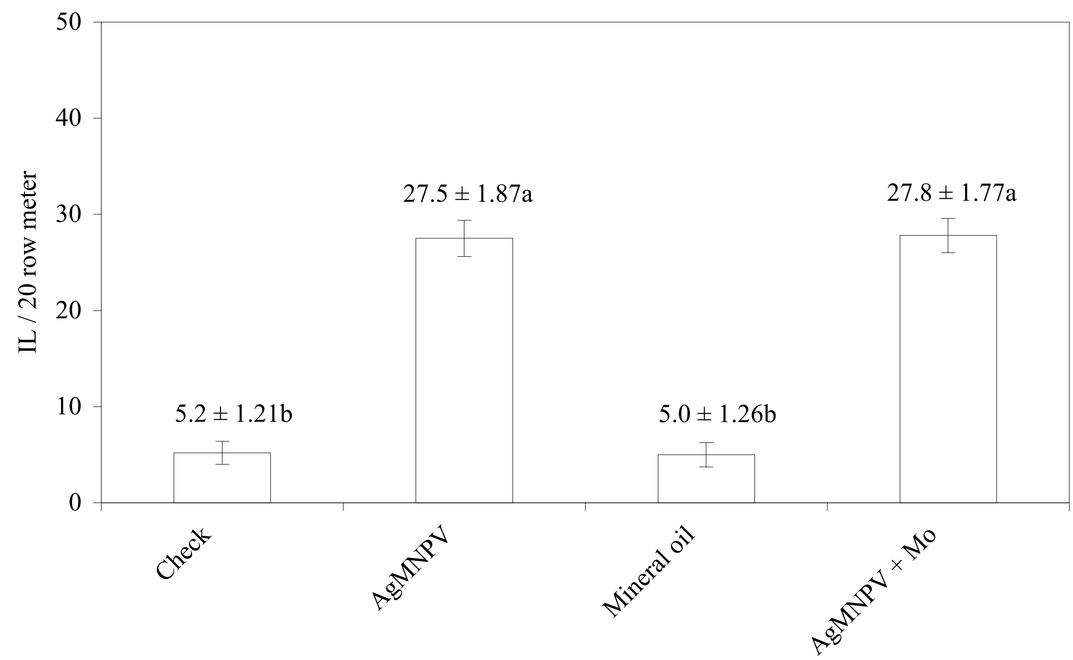

Figure 6. Mean number of *A. gemmatalis* larvae infected or dead (IL) by the AgMNPV in plots treated with a mixture of the virus with mineral oil and the virus used solely. Means (± 95%CI) followed by different letters differ significantly by the Tukey test at the 5% level (CV = 33.1%).

consideration in applications of this biological insecticide.

Other factors involved in cases of failure of the AgMNPV in the field may be related to low mean temperatures in some regions, which result in a longer time to kill the host, and improper timing of application, in disagreement with the recommendations for AgMNPV use (Moscardi 1983, 1986), associated with drought spells that magnify the intensity of insect occurrence on soybean (Moscardi 1999). With current improvements in AgMNPV formulations, part of the problems of AgMNPV unsuccesses will be solved. However, additional efforts will be needed by research, extension and private companies producing this virus on educating farmers about using this biological insecticide properly and on the factors that may affect its efficacy in the field.

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Literature Cited

Alves, L.F.A., A.E.F. Leitão, N.T. Augusto, L.G. Leite & A. Batista Filho. 1992. Utilização de adjuvante protetor contra radiação solar e fagoestimulante, em mistura com um vírus de poliedrose nuclear de *Anticarsia gemmatalis* (Lepidoptera: Noctuidae). Arq. Inst. Biol. 59: 23-27.

Batista Fº, A. & N.T. Augusto. 1987. Eficiência do *Baculovirus anticarsia* formulado como óleo emulsionável, no controle de *Anticarsia gemmatalis* Hübner, 1818. Biológico 53: 57-59.

Batista Fº, A., N.T. Augusto, B.P.B. Cruz & L.A. Machado. 1986. Utilização de *Baculovirus anticarsia* formulado como pós molháveis, no controle de *Anticarsia gemmatalis* Hübner, 1818, no campo. Biológico 52: 75-78.

Batista, T.F.C. 1997. Fatores que limitam a eficiência de *Baculovirus anticarsia* sobre *Anticarsia gemmatalis* Hübner, 1818. Tese de mestrado, FAEM/UFPEL, Pelotas, 68p.

Boucias, D.G., D.W. Johnson & G.E. Allen. 1980. Effects of host age, virus dosage, and temperature on the infectivity of a nucleopolyhedrosis virus against velvetbean caterpillar, *Anticarsia gemmatalis*, larvae. Environ. Entomol. 9: 59-61.

Cherry, A.J, R.J. Rabindra, M.A. Parnell, N. Geetha, J.S. Kennedy & D. Grzywacz. 2000. Field evaluation of *Helicoverpa armigera* nucleopolyhedrovirus formulations for control of the chickpea pod-borer, *H. armigera* (Hubn.), on chickpea (*Cicer arietinum* var. Shoba) in southern India. Crop Protection 19: 51-60.

Colowick, S.P. & N.O. Kaplan. 1955. Methods in enzimology. New York: Academic Press, 835p.

Fehr, W.R. & L.E. Caviness. 1977. Stages of soybean development. Ames, Iowa State University – Cooperative Extension Service. Special Report 80, 11p.

Gazzoni, D. 1994. Manejo de pragas da soja: uma abordagem histórica. EMBRAPA-CNPSo., Londrina, Documentos 8, 58p.

Ignoffo, C.M. 1985. Manipulating enzootic-epizootic diseases of arthropods, p. 243-261. In M.A Hoy & D.C. Herzog (eds.), Biological control in agricultural IPM systems. Orlando, Academic Press, 589p.

Ignoffo, C.M. & C. Garcia. 1966. The relation of pH to the activity of inclusion bodies of a *Heliothis* nuclear polyhedrosis. J. Invertebr. Pathol. 8: 426-427.

Jaques, R.P. 1967. The persistence of a nuclear-polyhedrosis virus in the habitat of the host insect, *Trichoplusia ni*. I. Polyhedra deposited on foliage. Can. Entomol. 99: 785-794.

Jaques, R.P. 1977. Stability of entomopathogenic viruses. Misc. Publ. Entomol. Soc. Amer. 10: 99-116.

Johnson, D.W., D.G. Boucias, C.S. Barfield & G.E. Allen. 1982. A temperature-dependent developmental model for a nucleopolyhedrosis virus of the velvetbean caterpillar, *Anticarsia gemmatalis* (Lepidoptera: Noctuidae). J. Invertebr. Pathol. 40: 292-298.

Keating, S.T., W.G. Yendol & J.C. Shultz. 1988. Relationship between susceptibility of gypsy moth larvae (Lepidoptera: Lymantriidae) to a *Baculovirus* and host plant foliage constituents. Environ. Entomol. 17: 952-958.

Kogan, M & H.N. Pitre Jr. 1980. General sampling methods for above-ground populations of soybean arthropods, p. 30-60. In M. Kogan & D.C. Herzog (eds.), Sampling methods in soybean entomology. New York, Springer-Verlag, 587p.

Lessa, M.M. & C.C. Medugno. 2000. Heteroflocculation of amidine polystyrene latex and *Anticarsia gemmatalis* nucleopolyhedrovirus as a model system for studying sunlight protection. J. Colloids Interf. Sci. 225: 317-322.

Lessa, M.M. & C.C. Medugno. 2001. Heteroflocculation of sulfate polystyrene latex and *Anticarsia gemmatalis* nucleopolyhedrovirus as a model system for studying sunlight protection. J. Colloids Interf. Sci. 239: 328-333.

Morales, L., F. Moscardi, D.R. Sosa-Gómez, F.E. Paro & I.L. Soldorio. 2001. Fluorescent brighteners improve *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) nucleopolyhedrovirus (AgMNPV) activity on AgMNPV-susceptible and resistant strains of the insect. Biol. Control 20:247-253.

Moscardi, F. 1983. Utilização de *Baculovirus anticarsia* para o controle da lagarta da soja, *Anticarsia gemmatalis*. EMBRAPA-CNPSo, Londrina, Com. Téc. 23, 13p.

Moscardi, F. 1986. Utilização de vírus para o controle da lagarta da soja, p. 188-202. In S.B.Alves (coord.), Controle microbiano de insetos. São Paulo, Manole, 407p.

Moscardi, F. 1989. Use of viruses for pest control in Brazil: the case of the nuclear polyhedrosis virus of the soybean caterpillar, *Anticarsia gemmatalis*. Mem. Inst. Oswaldo Cruz 84: 51-56.

Moscardi, F. 1991. Vírus entomopatogênicos. Inf. Agropec. 15: 5-20.

Moscardi, F. 1999. Assessment of the application of baculoviruses for control of Lepidoptera. Annu. Rev. Entomol. 44: 257-289.

Moscardi, F., G.E. Allen & G.L. Greene. 1981. Control of the velvetbean caterpillar by nuclear polyhedrosis virus and insecticides and impact of treatments on the natural incidence of the entomopathogenic fungus *Nomuraea rileyi*. J. Econ. Entomol. 74: 480-85.

Rheinheimer, D. dos S. & R.O. de Souza. 2000. Condutividade elétrica e acidificação de águas usadas na aplicação de herbicidas no Rio Grande do Sul. Ci. Rur. 30: 97-104.

Silva, M.T.B. da. 1986. Estudo comparativo sobre o uso de *Baculovirus anticarsia* por diversos equipamentos de aplicação. Trigo e Soja 87: 22-29.

Silva, M.T.B. da. 1987. *Baculovirus anticarsia*: época de aplicação e efeito residual sobre plantas de soja. Rev. Centr. Ci. Rur. 17: 339-350.

Silva, M.T.B. da. 1991. Avaliação de *Baculovirus anticarsia* formulado. Ci. Rur. 21: 1-11.

Smith, D.B. & L.F. Bouse. 1981. Machinery and factors that affect the application of pathogens, p. 635-653. In H.D. Burges (ed.), Microbial control of pests and plant diseases 1970-1980. New York, Academic Press, 949p.

Yearian, W.C. 1978. Application technology to increase effectiveness of entomopathogens, p. 100-110. In G.E. Allen, C.M. Ignoffo & R.P. Jaques (eds.), Microbiol control of insect pests: future strategies in pest management systems. Gainesville: NSF-USDA, University of Florida, 290p.

Young, S.Y., W.C. Yearian & K.S. Kim. 1977. Effect of dew from cotton and soybean foliage on activity of *Heliothis* nuclear polyhedrosis virus. J. Invertebr. Pathol. 30: 237-241.

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