# Impact of a Brazilian Nucleopolyhedrovirus Release on *Anticarsia gemmatalis* (Lepidoptera: Noctuidae), Secondary Insect Pests, and Predators on Soybean in Mexico[1]

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**Abstract** A 4-yr study (2000-2003) in southern Tamaulipas, Mexico, compared the impact of a Brazilian nucleopolyhedrovirus (AgNPV) and cypermethrin on *Anticarsia gemmatalis* Hübner, secondary pests, and arthropod predators in soybean fields. Areas treated with AgNPV had consistently low levels of *A. gemmatalis* beginning soon after application, while other arthropods within the agroecosystem remained in balance. Cypermethrin was highly effective against *A. gemmatalis*, but caused its rapid resurgence and increased secondary pests, mainly *Pseudoplusia includens* (Walker), as a result of elimination of the beneficial fauna. Densities of *Nezara viridula* (L.) were higher in the AgNPV plot than in the cypermethrin plot only during 2000, with no significant differences in the subsequent years. Overall (2000-2003), numbers of the predators *Chrysoperla carnea* (Stephens), *Olla v-nigrum* (Mulsant), hemipterans and lynx spiders were generally five times higher in the AgNPV plots than in the cypermethrin plots. Additional observations made only during 2003 showed a moderate presence of other predators (*Calosoma* sp. and *Polistes* sp.) and parasitism by *Copidosoma truncatellum* (Dalman) on *A. gemmatalis* and *P. includens* up to 40% in the AgNPV plot, in contrast with the cypermethrin plot, where these beneficials were not detected. This study showed the potential of AgNPV as a biocontrol-based IPM strategy for soybeans in Mexico.

**Key Words** Biological control, natural enemies, microbial pesticides, cypermethrin, pest resurgence

The velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, is the most important defoliator of soybean, *Glycine max* (L.), in Mexico (Maldonado et al. 1991). Numbers of *A. gemmatalis* generally comprise up to 80% of the total soybean defoliating insect complex, which also includes the soybean looper, *Pseudoplusia includens* (Walker), and the cabbage looper, *Trichoplusia ni* Hübner (Maldonado et al. 1991). The latter two caterpillars and the stink bug, *Nezara viridula* (L.), are considered as secondary insect pests, but may become troublesome when insecticides are applied against *A. gemmatalis*. Both the presence of secondary pests and *A. gemmatalis* resurgence are a result of the elimination of the beneficial fauna (Maldonado et al. 1991, Avila and Rodríguez-del-Bosque 2003).

Preliminary field observations in the northern states of Tamaulipas and Sinaloa, Mexico, yielded promising results with the use of a Brazilian *A. gemmatalis* nucleo-

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polyhedrovirus (AgNPV), with infection up to 100% of larvae (Avila 1999, Cortez et al. 2003). The use of AgNPV in Brazil represents a remarkable case of a large-scale biocontrol implementation program; over 1.7 million ha are treated annually, with considerable economic, ecological and social benefits (Moscardi 1999, Moscardi et al. 2002). This virus is indigenous to tropical America and has been introduced into USA (Fuxa and Ritcher 1993) and, more recently, into northern Mexico (Avila 1999, Cortez et al. 2003).

The risks associated with biocontrol agents have been increasingly considered and evaluated worldwide (Ruberson et al. 1991, de Nardo et al. 2001). Microbial pesticides have the potential to survive in the environment, to evolve and to infect nontarget organisms (Cook et al. 1996). In the present study, the Brazilian AgNPV was tested in southern Tamaulipas, Mexico, under field conditions during 4 yrs (2000-2003) to evaluate the impact on *A. gemmatalis*, secondary pests, and beneficial arthropods on soybeans, as compared to insecticide applications, the main current control method against insect defoliators in Mexico.

## Materials and Methods

Field studies were conducted in the "Huastecas" area of Mexico, a tropical agricultural region in southern Tamaulipas, during each of the fall growing seasons from 2000 to 2003. Main crops in this area include soybeans, cotton, maize, grain sorghum, pepper, tomato, and onions (Maldonado 1999). Each year, the experiment was conducted in different commercial soybean fields of cooperative growers, at least 5 km apart from each other, all within Altamira Co.

In all years, soybean cultivar 'Huasteca-200' was planted and grown under rainfed conditions. Planting dates were 4 July 2000, 20 July 2001, 27 July 2002 and 16 July 2003. The experiment was established and conducted similarly during all years and consisted of two treatments: (a) AgNPV at 20 g/ha ($7.5 \times 10^9$ polyhedra/g), applied once during the vegetative stage when *A. gemmatalis* density first reached 10 larvae (>1.5 cm large)/m (Avila 1999); and (b) cypermethrin at 66 g A.I./ha, applied as many times as needed, when *A. gemmatalis* density reached 15 larvae (>1.5 cm large)/m, simulating local recommendations for insecticide usage (Maldonado et al. 1991,

**Table 1. Chi-square values obtained in contingency tables to test independence between treatments (AgNPV and cypermethrin) and either insect pests or predators (*df* = 1, 3). Altamira, Tam., México 2000-2003**

| Year | Insect pests* | Predators** |
|---|---|---|
| 2000 | 25.3† | 5.3 n.s. |
| 2001 | 12.3† | 1.9 n.s. |
| 2002 | 0.5 n.s. | 1.8 n.s. |
| 2003 | 11.9† | 1.8 n.s. |
| All (2000–2003) | 41.3† | 3.5 n.s. |

* *A. gemmatalis, P. includens, T. ni,* and *N. viridula.*
** *C. carnea, O. v-nigrum,* hemipterans, lynx spiders.
† = Variables are not independent ($P < 0.01$).
n.s. = Variables are independent ($P < 0.01$).

**Table 2. Number of *A. gemmatalis* and secondary insect pests (Average ± SE of individuals/m during *n* weekly samples*) on soybean plots treated with either AgNPV or cypermethrin. Altamira, Tam. México. 2000-2003**

| Pest | Year | AgNPV | Cypermethrin |
|---|---|---|---|
| *A. gemmatalis* | 2000 | 8.2 ± 4.2 b | 22.2 ± 7.2 a |
| | 2001 | 4.5 ± 0.9 b | 9.9 ± 2.6 a |
| | 2002 | 6.1 ± 2.0 a | 9.5 ± 2.7 a |
| | 2003 | 4.3 ± 1.3 b | 10.3 ± 3.7 a |
| | Mean | 5.78 b | 12.98 a |
| *P. includens* | 2000 | 1.4 ± 0.3 b | 4.6 ± 1.3 a |
| | 2001 | 1.7 ± 0.3 b | 7.4 ± 2.0 a |
| | 2002 | 1.1 ± 0.3 a | 1.9 ± 0.4 a |
| | 2003 | 1.3 ± 0.5 b | 6.7 ± 2.2 a |
| | Mean | 1.38 b | 5.15 a |
| *T. ni* | 2000 | 1.0 ± 0.2 a | 1.6 ± 0.6 a |
| | 2001 | 0.9 ± 0.2 a | 1.1 ± 0.2 a |
| | 2002 | 0.4 ± 0.1 b | 0.6 ± 0.1 a |
| | 2003 | 0.1 ± 0.1 a | 0.2 ± 0.1 a |
| | Mean | 0.60 b | 0.88 a |
| *N. viridula* | 2000 | 1.5 ± 0.1 a | 0.5 ± 0.2 b |
| | 2001 | 0.3 ± 0.1 a | 0.4 ± 0.1 a |
| | 2002 | 0.2 ± 0.1 a | 0.2 ± 0.1 a |
| | 2003 | 0.6 ± 0.1 a | 0.4 ± 0.2 a |
| | Mean | 0.65 a | 0.38 a |

* Weekly samples during 2000, 2001, 2002 and 2003 were 11, 15, 13 and 12, respectively. Values within rows followed by the same letter are not significantly different (paired *t*-test; $P < 0.05$).

Garza et al. 2001). A commercial formulation of AgNPV was supplied by EMBRAPA Soybean Research Center in Brazil (Moscardi et al. 2002); cypermethrin was obtained locally. Each treatment was established in 3-ha adjacent plots, surrounded by commercial soybeans. All applications were made with a 800-L tractor-mounted sprayer using 200 L/ha at 2.8 kg/cm$^2$ pressure. Other agricultural practices were the same for both treatments, according to local recommendations (Maldonado et al. 1991).

When the crop reached the V-10 (10 nodes) vegetative stage (Fehr et al. 1971), plants were inspected weekly for insect pests and predators in 10 m of row at each of 10 randomly chosen sites within 1 ha at the center of each treatment (plot), using the ground cloth method (Studebaker et al. 1991). Sampling was discontinued at R-7 stage (beginning maturity). Insect pests sampled included larvae of *A. gemmatalis*, *P. includens* and *T. ni*, and nymphs and adults of *N. viridula*. Sampling of arthropod

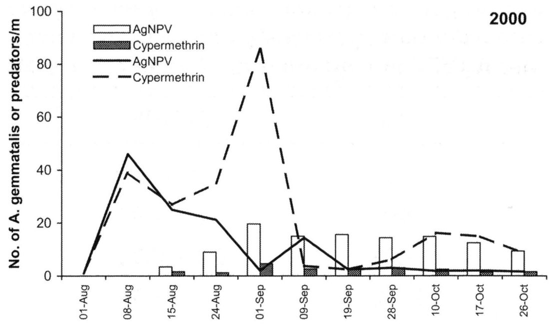


Fig. 1. Number of *A. gemmatalis* larvae (lines) and all-predators (bars) on soybeans after application with either AgNPV or cypermethrin. In their respective plots, AgNPV was applied only on 10-Aug, whereas cypermethrin was applied four times: 10-Aug, 24-Aug, 2-Sep, and 15-Oct. Altamira, Tam. México. 2000.

predators included the following four groups: the chrysopid *Chrysoperla carnea* (Stephens) (larvae and adults), the coccinellid *Olla v-nigrum* (Mulsant) (larvae and adults), hemipterans (pooled numbers of *Sinea* sp., *Zelus* sp., *Repipta* sp., *Nabis* sp., and *Podisus* sp.) and lynx spiders (pooled numbers of *Oxyopes* sp., *Peucetia* sp., and *Misumenops* sp.). Unpublished previous observations indicated these four predator groups were predominant on soybeans in this region. Additional counts made only during 2003 included two other predators: a carabid (*Calosoma* sp.) and a vespid (*Polistes* sp.); and parasitism by the encyrtid *Copidosoma truncatellum* (Dalman) on *A. gemmatalis* and *P. includens*. Only living and active individuals (pests and predators) were counted throughout the study.

To stabilize the variances, data were transformed to square root prior to statistical analyses (Ott 1984). The interaction between treatments and either insect pests or

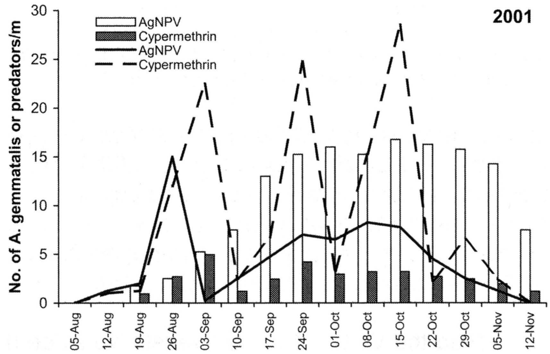


Fig. 2. Number of *A. gemmatalis* larvae (lines) and all-predators (bars) on soybeans after application with either AgNPV or cypermethrin. In their respective plots, AgNPV was applied only on 27-Aug, whereas cypermethrin was applied three times: 4-Sep, 25 Sep, and 16-Oct. Altamira, Tam. México. 2001.

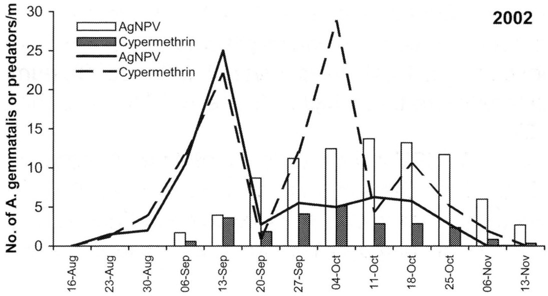


Fig. 3. Number of *A. gemmatalis* larvae (lines) and all-predators (bars) on soybeans after application with either AgNPV or cypermethrin. In their respective plots, AgNPV was applied only on 10-Sep, whereas cypermethrin was applied three times: 14-Sep, 5-Oct, and 19-Oct. Altamira, Tam. México. 2002.

predators was tested by chi-square contingency tables ($df = 1,3$; $P < 0.01$) by year and pooled data (2000-2003) (SAS Institute 1999). Means of insect pests and natural enemies between treatments were compared by paired *t*-test, $P < 0.05$ (SAS Institute 1999).

## Results and Discussion

Except in 2002, a significant interaction between treatments and insect pests was detected (Table 1), suggesting the effects of treatments were not constant among all insect pests. Overall, densities of all insect pests were 2.3 times higher in the cypermethrin plots than in AgNPV plots (Table 2). However, cypermethrin caused a mean 3.7-fold increase in *P. includens* as compared to AgNPV, with significant differences in 3 yrs (2000, 2001, and 2003). In contrast, higher numbers of *N. viridula* were found in the AgNPV plot than the insecticide treatment in only one year (2000). No signifi-

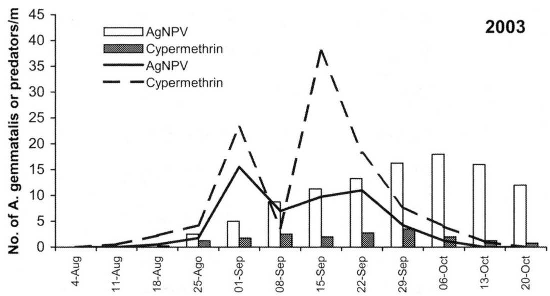


Fig. 4. Number of *A. gemmatalis* larvae (lines) and all-predators (bars) on soybeans after application with either AgNPV or cypermethrin. In their respective plots, AgNPV was applied only on 1-Sep, whereas cypermethrin was applied three times: 2-Sep, 15-Sep, and 23-Sep. Altamira, Tam. México. 2003.

cant interaction was detected between treatments and predators (Table 1), with effects of treatments being constant among all predators. Thus, the sum of all predators for graphical purposes (Figs. 1-4) is valid. Overall (2000-2003), numbers of arthropod predators were generally five times higher in the AgNPV plots than in the cypermethrin plots (Table 3).

Regardless of year, AgNPV consistently maintained *A. gemmatalis* at low levels after application (Figs. 1-4). Diseased larvae were commonly observed throughout the season, suggesting the virus could persist and continue to infect new hosts, as demonstrated elsewhere (Young and Yearian 1989, Fuxa and Ritcher 1994). Cyper-

**Table 3. Number of arthropod predators (Average ± SE of individuals/m during *n* weekly samples*) on soybean plots treated with either AgNPV or cypermethrin. Altamira, Tam. México. 2000-2003**

| Predator | Year | AgNPV | Cypermethrin |
|---|---|---|---|
| *C. carnea*** | 2000 | 5.3 ± 0.6 a | 0.7 ± 0.1 b |
| | 2001 | 5.2 ± 0.7 a | 1.1 ± 0.2 b |
| | 2002 | 4.2 ± 0.7 a | 0.9 ± 0.3 b |
| | 2003 | 3.5 ± 0.3 a | 0.5 ± 0.1 b |
| | Mean | 4.55 a | 0.80 b |
| *O. v-nigrum*** | 2000 | 1.6 ± 0.4 a | 0.2 ± 0.1 b |
| | 2001 | 0.3 ± 0.2 a | 0.1 ± 0.1 a |
| | 2002 | 0.2 ± 0.1 a | 0.1 ± 0.1 a |
| | 2003 | 1.0 ± 0.3 a | 0.0 ± 0.0 b |
| | Mean | 0.78 a | 0.10 b |
| Hemipterans† | 2000 | 0.7 ± 0.1 a | 0.0 ± 0.0 b |
| | 2001 | 0.2 ± 0.1 a | 0.1 ± 0.1 a |
| | 2002 | 0.7 ± 0.1 a | 0.1 ± 0.1 b |
| | 2003 | 2.2 ± 0.3 a | 0.3 ± 0.1 b |
| | Mean | 0.95 a | 0.13 b |
| Lynx spiders‡ | 2000 | 5.3 ± 0.7 a | 1.5 ± 0.2 b |
| | 2001 | 5.7 ± 0.9 a | 1.4 ± 0.2 b |
| | 2002 | 3.7 ± 0.7 a | 1.2 ± 0.3 b |
| | 2003 | 6.5 ± 1.2 a | 1.0 ± 0.1 b |
| | Mean | 5.30 a | 1.28 b |

* Weekly samples during 2000, 2001, 2002 and 2003 were 11, 15, 13 and 12, respectively.

** Larvae and adults.

† Total individuals from the Genera *Sinea, Zelus, Repipta, Nabis* and *Podisus.*

‡ Total individuals from the Genera *Oxyopes, Peucetia* and *Misumenops.* Values within rows followed by the same letter are not significantly different (paired *t*-test; $P < 0.05$).

methrin applications were highly effective against *A. gemmatalis* in the short time, but caused its rapid resurgence (Figs. 1-4), and increased secondary pests, particularly *P. includens* (Table 2), by affecting the beneficial fauna (Figs. 1-4, Table 3). In the AgNPV-treated plots, arthropod predators increased soon after the first *A. gemmatalis* peak and remained at significantly higher densities thereafter as compared to cypermethrin plots (Figs. 1-4). Evidently, such higher levels of predators in the AgNPV treatment contributed to maintain other arthropods within the agroecosystem in balance. In contrast, cypermethrin applications were evidently highly disruptive, favoring *A. gemmatalis* resurgence, lower numbers of predators, and higher densities of secondary insect pests throughout the study. Arthropod predators are highly mobile and spend considerable time searching for prey, thus insecticides generally discriminate against predaceous species, which are active as they search for prey or when they recolonize fields previously treated with insecticides (Boyd and Boethel 1998).

Densities of *A. gemmatalis* were significantly higher during 2000 peak of 86 larvae/m, requiring four applications of cypermethrin during the season (Fig. 1). In the remaining years, *A. gemmatalis* peaks were <40 larva/m, and required three insecticide applications in each year (Fig. 2-4). Regardless of year and treatment, the second-most-important insect pest was *P. includens*, with an overall average of 5.2 larva/m in the cypermethrin plots (Table 2). Overall mean densities of *T. ni* and *N. viridula* were <1 individual/m (Table 2). In Brazil, insecticide application against *A. gemmatalis* generally favored population increases of *P. includens* and *N. viridula* (Moscardi 1993). We also observed increases in *P. includens* density, but not *N. viridula* (Table 2). In fact, *N. viridula* density was significantly higher in the AgNPV plot than in the cypermethrin plot during 2000. The remaining years (2001-2003), there was not significant difference for *N. viridula* between the two treatments.

The most-abundant arthropod predators in the soybean habitat were *C. carnea* and lynx spiders, with overall averages of 4.6 and 5.3 individuals/m, respectively, in the AgNPV plots (Table 3). Densities of *O. v-nigrum* and predaceous hemipterans averaged <1 individual/m (Table 4). However, we did not measure the individual predation rate of each predator, and comparison of their densities may not reflect their real impact in the agroecosystem (Brown and Goyer 1984, ÓNeil and Stimac 1988, Clements and Yeargan 1997). In addition to the prey consumption capabilities, arthropod predators are important disseminators of pest entomopathogens, including AgNPV (Young and Yearian 1987, 1992, Kring et al. 1988, Fuxa and Ritcher 1994).

The additional counts of the predators *Calosoma* sp. and *Polistes* sp. made during 2003 showed a mean density of 0.4 and 0.5 individuals/m, respectively, during the season in the AgNPV plot. The same year, parasitism by *C. truncatellum* on *A. gemmatalis* and *P. includens* varied from 4.7 (8 Sep) to 40.0% (6 Oct) in the AgNPV-treated plot. In contrast, these beneficials were not detected in the cypermethrin plot, further demonstrating the negative impact of insecticides on natural enemies.

The data presented here show the potential of AgNPV as a biocontrol-based IPM strategy for soybean pests in Mexico. The effectiveness against *A. gemmatalis* and the preservation of the beneficial arthropod fauna using AgNPV offer important economic and environmental advantages as compared to the conventional chemical methods. The AgNPV is highly specific to Lepidoptera and is safe for mammals and other nontarget organisms, including natural enemies (de Nardo et al. 2001). In fact, AgNPV has been proven to be excreted in the feces of hemipteran predators over several days as intact viable virus particles, and neither replicates nor has any adverse effect on predators (Young and Yearian 1987). Some adverse effects of AgNPV to predators by consuming exclusively infected *A. gemmatalis* in the laboratory might be due to some inert components present in the commercial formulation (de Nardo et al. 2001). Such potential negative effects observed under laboratory conditions are likely to be diluted in the field, where other prey are available (Ruberson et al. 1991). In addition, laboratory studies have shown a high potential in *A. gemmatalis* for developing resistance to AgNPV in Brazil, where the virus occurs naturally and is extensively used as a microbial pesticide. In contrast, populations of *A. gemmatalis* elsewhere have a lower potential for AgNPV resistance (Abot et al. 1996). Recent studies have shown fluorescent brighteners combined with AgNPV resulted in reversal of resistance to the virus in a laboratory-selected highly resistant Brazilian *A. gemmatalis* population (Morales et al. 2001).

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