Journal of Agricultural Science; Vol. 11, No. 14; 2019
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Chrysodeixis includens nucleopolyhedrovirus (ChinNPV) Affects on Immature Stages of *Chrysodeixis includens* (Lepidoptera: Noctuidae)

Ana Beatriz Riguetti Zanardo Botelho[1], Ivana Fernandes da Silva[2] & Crébio José Ávila[3]

[1] Department of Entomology and Acarology, "Luiz de Queiroz" School of Agriculture, University of Sao Paulo, Piracicaba, SP, Brazil

[2] Department of Entomology and Biodiversity Conservation, Federal University of Grande Dourados, Dourados, MS, Brazil

[3] Embrapa Western Agriculture, Dourados, MS, Brazil

Correspondence: Ana Beatriz Riguetti Zanardo Botelho, Department of Entomology and Acarology, "Luiz de Queiroz" School of Agriculture, University of Sao Paulo, Piracicaba, SP, Brazil. E-mail: biazanbotelho@gmail.com

Received: March 3, 2019 Accepted: June 7, 2019 Online Published: August 31, 2019

doi:10.5539/jas.v11n14p47 URL: https://doi.org/10.5539/jas.v11n14p47

Abstract

Soybean looper (*Chrysodeixis includens*) is an important defoliating pest, which has caused significant losses in Brazilian soybean crops. The present study evaluated the foliar consumption, feeding period and mortality of small (< 1.0 cm), medium (1.0 to 2.0 cm) and large (> 2.0 cm) *C. includens* larvae after infection by the virus *Chrysodeixis includens nucleopolyhedrovirus* (ChinNPV), isolate Chin-IA (I-A). The bioassay was performed in a completely randomized design organized in a 3 × 2 factorial combination (three size of larvae fed on soybean leaf discs, either treated or not with 4.0×10^{11} PIB ha^{-1} suspension of virus) with ten replicates per treatment. The average consumption of all three sizes *C. includens* larvae were significantly reduced after ingestion of soybean discs treated with virus, compared to the larvae from control treatment. The total consumption reduction was 95.6%, 69.4% and 45.9% for the small, medium and large larvae, respectively. Feeding period was significatively reduced for small and medium larvae infected by the virus, but not for large larvae. The corrected mortality level of soybean loopers ranged from 70 to 90% and was not significant different between the three larval sizes. The behavior and physiological alterations of larvae started on the third day, and the mortality occurred between fifth and sixth day after ingestion of infective particles of virus, therefore reducing their damage abilities. Based on the results obtained, ChinNPV can be considered as an important tool within integrate management to control *C. includens,* mainly when small larvae were predominant in soybean crops.

Keywords: integratet pest management (IPM), larvae, soybean, soybean looper, virus

1. Introduction

Brazil is the largest soybean exporter in the world [*Glycine max* (L). Merr.], and the second largest grain producer with an estimated production of 115 million ton within an area of 35 million ha in 2018/2019 season (Conab, 2019; Lima et al., 2019). The presence of arthropod pests attacking soybean plants during its phenological phases have been a constant concern to the farmers. *Chrysodeixis includens* (Walker, 1858) (Lepidoptera: Noctuidae: Plusiinae) is a polyphagous insect, which is found from Northern USA to Southern South America (Wagner et al., 2011). It is an economically important pest, which causes damage in soybean crops by feeding on its leaves, especially between the veins, displaying a lacy appearance, which is also the pest's fingerprint (Bueno et al., 2011; Ávila & Grigolli, 2014). In the last years, the soybean looper has become a serious problem in Brazil, especially in Cerrado's region, where population outbreaks were reported causing significant losses in the productivity of soybean (Baldin et al., 2014; Bortolotto et al., 2015; Specht et al., 2015). This might be associated with incorrect and indiscriminate use of chemical products that decreased the incidence of natural enemies (pathogens, predators and parasitoids), causing a biological imbalance in soybean agro-ecosystems. Additionally, control of this species has been difficult because of its resistance to some synthetic insecticides and less exposure of larvae to the insecticides sprays due to their location on the low and middle part of the plants canopy (Oliveira et al., 2010).

The development of control methods alternative to chemical insecticides are extremely important for a sustainable production (Togni et al., 2019). Two genera of entomopathogenic virus, Nucleopolyhedrovirus (NPVs) and Granulovirus (GVs) belonging to the *Baculoviridae* family, represent an alternative and promising method within integrate management programs (IPM). These virus groups have been widely studied and investigated as biological products in Brazil and worldwide, due to safety for human and non-target organisms, specificity and high virulence provided to the several pest species, including noctuids (Moscardi et al., 2011; Haase, Sciocco-Cap, & Romanowski, 2015). NPVs acts through ingestion, causing infection in the larval stage and several behavioral and morphological changes, such as reduction of feeding time, developmental retardation, integument discoloration and migration towards the top of plants (negative geotropism) (Eberle et al., 2012). *Chrysodeixis includens nucleopolyhedrovirus* (ChinNPV: *Baculoviridae*: *Alphabaculovirus*) was initially isolated in the 70's and recently characterized morphologically and genetically by Brazilian groups of scientists (Alexandre et al., 2010; Craveiro et al., 2013, 2015).

During the entomopathogenic virus' infection, the susceptibility, consumption and mortality of insect hosts may change accordingly to their larval stages (Harrison & Hoover, 2012). These parameters are important during the monitoring and to establish the control level for the pest's population as well. Studies with *Anticarsia gemmatalis* (Hübner, 1818) (Lepidoptera: Eribidae) and the virus *Anticarsia gemmatalis multiple nucleopolyhedrovirus* (AgMNPV: *Baculoviridae*: *Alphabaculovirus*), have demonstrated that larval susceptibility of this insect decreased after AgMNPV infection (Moscardi & Zonta-de-Carvalho, 1993). For this reason, they recommend applying the virus until 20 velvetbean caterpillars with less than 1.5 cm exists per linear meter in the soybean crops, and other parameters such as the stage of crop development and climatic conditions were also considered. Early larval instars of *Trichoplusia ni* (Hübner, 1802) (Lepidoptera: Noctuidae) and *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) were also more susceptible to their respectives NPVs (Harper, 1973; Valicente & Tuelher, 2009). However, there are limited studies in Brazil, which uses this approach against *C. includens*.

We hypothesize that the virus *Chrysodeixis includens nucleopolyhedrovirus* (ChinNPV) affects foliar consumption, feeding period and mortality rate of immature stages of *C. includens*. We performed this study with an aim to evaluate these parameters in three *C. includens* larval sizes, after ingestion of soybean leaf discs containing polyhedral bodies of one isolate of ChinNPV, named Chin-IA (I-A). This information will improve the knowledge about the virus infection in different immature stages of soybean looper, which will help us to employ this method properly and efficiently in field conditions, in future.

2. Material and Methods

The bioassays were performed at Embrapa Western Agriculture in the Entomology's Laboratory located in Dourados city, Mato Grosso do Sul State, Brazil (22º16′30″ S, 54º49′00″ W, 408 m).

2.1 Chrysodeixis includens Colony

C. includens larvae were collected in soybean fields near to Dourados. Larvae were taken to the laboratory where they were reared at 25±2 ºC, 70±10% relative humidity, and photoperiod 14:10 (light:dark) using artificial diet (adapted from Greene et al., 1976) until pupation. These pupae were used to start the colony. Adults were kept in wooden cages (70 × 60 × 60 cm) with newsprint paper sheets used as substrate for oviposition. 10% honey solution (Hoffmann-Campo et al., 1985) was provided in hydrophilic cotton pad inside of glass containers (5 mL) for adult feeding. The moths were allowed to oviposit for 2-3 days, and after this period, the paper sheets containing the eggs were placed in plastic containers (capacity of 4.5 liters) with the bottom containing pieces of artificial diet for freshly hatched larvae (about 3 days). Then, 1st instar larvae were transferred to transparent and sterile plastic cups (100 mL) for 5 to 7 days. After this period, larvae were individualized in sterile and transparent plastic cups (50 mL) with 5g of artificial diet where they remained until the pupation and emergence of adults. Adults were transferred to wooden cages and the *C. includens* larvae obtained were used in the experiments.

2.2 Bioassay

The isolate Chin-IA (I-A) from virus *Chrysodeixis includens nucleopolyhedrovirus* (ChinNPV), provided by Embrapa Soybean (Londrina/PR, Brazil), was considered one of the most pathogenic to *C. includens* according to Alexandre et al. (2010) and for this reason, it was used in the present study. A suspension containing 4.0×10^{11} PIB ha^{-1} of this virus was prepared considering a spray volume of 150 L ha^{-1} similar to that is commonly used in field conditions.

Soybean plants from BRS 255 RR cultivar, adapted to edaphoclimatic conditions of region, were maintained in plastic vases (8 L) containing a mixture of land, sand and cattle manure (1:1:1) under greenhouse conditions until the flowering stage (R2). In the laboratory, fresh leaves from the middle third of the plants, portion where soybean loopers usually remains in field conditions (Czepak & Albernaz, 2014), were surface sterilized in 0.1% sodium hypochlorite solution for two minutes and washed three times in sterile distilled water. Soybean leaf discs with an area of 12.56 cm² were prepared using a metal hole-puncher (approximately 4 cm of diameter) and treated with the virus suspension. *C. includes* larvae with small (< 1.0 cm), medium (1.0 to 2.0 cm) and large (> 2.0 cm) sizes were allowed to feed on two ChinNPV treated leaf discs for 48 hours. Larvae fed on leaf discs treated with only sterile distilled water served as control. Fresh untreated leaves (surface sterilized in 0.1% sodium hypochlorite for 2 min and washed three times in sterile distilled water) were fed to all the larvae from day 3 of the experiment. Larvae were individualized in Petri dishes (6.0 cm diameter × 1.3 cm height) containing filter paper moistened with distilled water.

Size instead of instar was considered in this study because are terms commonly used during evaluations of population survey in the soybean fields.

Bioassay was conducted at 25±2 ºC, 70±10% relative humidity, and photoperiod 14:10 (light:dark) and larval mortality was recorded every 24 hours until the larvae either died or pupated.

A completely randomized design was considered in a factorial scheme 3 × 2 (three larval sizes fed on soybean leaf discs either treated or not in the virus suspension) and ten replicates (one larvae/plate) per treatment. Foliar area consumed, feeding period, and mortality (larvae that were unable to move and feed were proclaimed dead) for the three *C. includens* larvae sizes studied. Dead larvae were stored in eppendorf vials at -20 ºC to confirm the presence of polyhedral bodies. The soybean foliage area consumed (cm^2) was determined in a leaf area meter (Model LI-3100; Li-Cor, Lincoln, NE) after larval feeding (Bueno et al., 2011).

2.3 Data Analysis

The mortality data were corrected according to Schneider-Orelli's formula (Püntener, 1981), and normalized with the control treatment. Foliar area consumption, feeding period, and mortality data were tested for analysis of variance and the means within each factor were compared by the Tukey's test at 5% of probability. Tests were conducted using ASSISTAT software (Silva & Azevedo, 2002).

3. Results and Discussion

A significant interaction for the average foliar consumption, between the factors size of *C. includens* larvae and treatments (larvae either infected or not by the virus ChinNPV) were found, $F(3, 2) = 7.75$, $p < 0.01$ (Table 1). Furthermore, there was also significative effect of each factor analysed individually ($p < 0.01$). In this way, the average foliar consumption of all three *C. includens* larvae sizes were significative reduced after ingestion of soybean leaf discs containing polyhedral bodies of Chin-IA (I-A), when compared with the consumption of larvae in the control treatments (Table 2).

Table 1. Summary for analysis of variance for average foliar consumption of small, medium and large *Chrysodeixis includens* larvae, after ingestion of soybean leaf discs treated with sterilized distilled water (control) and with suspension of 4.0×10^{11} PIB ha^{-1} of virus isolate Chin-IA (I-A)

Source	DF	Mean square	F-value
Size of *C. includens* larvae	2	147.15130	92.2394**
Treatments (larvae either infected or not by the virus)	1	426.45443	267.3160**
Interaction Larval size × Treatments	2	12.36584	7.7513**
Treatments	5	149.09774	93.4595**
Experimental error	54	1.59532	
Total	59		

Note. **Significant F-value at the 0.01 level. Coefficient of variation = 17.6%.

Table 2. Average foliar consumption (cm²/day) of small, medium, and large *Chrysodeixis includens* larvae after ingestion of soybean leaf discs treated with sterilized distilled water (control) and with suspension of 4.0×10^{11} PIB ha^{-1} of virus isolate Chin-IA (I-A)

Size (cm)	Treatment (cm²/day)	
	Control [(1)]	Chin-IA (I-A) [(1)]
Small larvae	7.3±0.49 cA	0.7±0.17 bB
Medium larvae	9.5±0.30 bA	5.3±0.55 aB
Large larvae	12.2±0.37 aA	6.9±0.87 aB

Note. [(1)] Mean±Standard Error followed by the same lower-case letter in the column and by the same upper-case letter in the row are not statistically different by the t-test ($p < 0.05$).

After the larvae intake the viral particles together with leaves, the occlusion bodies from the virus are dissolved in the highly alkaline environment of the larval midgut, releasing occlusion-derived virions (ODV), which attach to the microvillar membranes of midgut epithelial cells via proteins, found in the ODV envelope. Infected midgut epithelial cells produce then, BV (budded virus), which bud from the basal side spreading the infection throughout the insect (Clem & Passarelli, 2013). Thus, the interaction of larval midgut with virus negatively influenced the consumption of the larvae, in agreement with the results obtained in our study.

The three sizes of *C. includens* larvae, in both virus [Chin-IA (I-A)] and control treatments, have shown a similar daily foliar consumption for the first two days of evaluation, with medium larvae size keeping this same pattern until the third day (Figure 1).

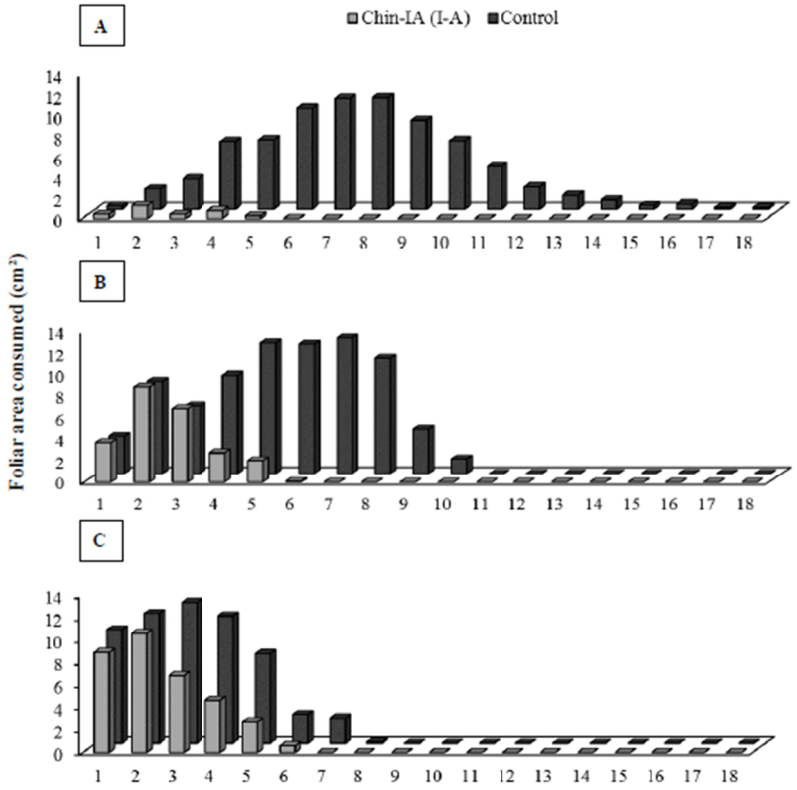

Figure 1. Foliar area consumed daily by small (A), medium (B), and large (C) *Chrysodeixis includens* larvae after ingestion of soybean leaf discs treated with sterilized distilled water (control) and with suspension of 4.0×10^{11} PIB ha^{-1} of virus isolate Chin-IA (I-A)

However, the consumption of infected larvae was drastically reduced from third day of evaluation for small and large larvae (fourth day for medium larvae), with small larvae showing a greater reduction compared to the others larval size, confirming the previously obtained results (Table 2) where small larvae from virus [Chin-IA

(I-A)] treatment, had an average consumption five and seven times lower than the medium and large infected larvae. This might be related with the fact of last larval instars consume a largest amounts of foliage (Trichilo & Mack, 1989).

Soybean loopers infected by the virus, from the third day, started showing external virus symptoms such as feeding reduction, loss of movement and changes of integument color to light brown and progressing to dark. These results are in accordance with Volkman and Keddie (1990), who reported that Nucleopolyhedrovirus (NPVs) take, on average, 48 to 72 hours to cause morphological changes in the immature phase of lepidopterans. All these changes identified in our study, occur as a cascade due to baculovirus' gene expression (Clem & Passarelli, 2013). According to the authors, the fat bodies, tracheal matrix, and epidermis of insects are severely damaged by the virus with the peak infection occurring generally from the fifth day when the cell nuclei burst and releases the polyhedral inclusion bodies into the cells. Other studies also reported similar results to noctuides from different species after being infected by their respective NPVs (Moscardi, 1999; Valicente & Tuelher, 2009; Ali et al., 2019).

The total soybean leaf consumption of small, medium and large larvae was reduced to 95.6; 69.4 and 33.0%, respectively, after ingestion of viral particles, in relation to the consumption of larvae from control treatments (Figure 2). Moscardi and Zonta de Carvalho (1993) also reported that *A. gemmatalis* caterpillars in the second instar, which the size is very similar with that used in this study for small larvae, also had 95% of their consumption reduced after intake soybean leaves with polyhedral bodies from AgMNPV, while caterpillars from third, fourth and fifth instar have shown reductions around 60, 50 and 40, respectively, in agreement with the results obtained in our study. These findings confirm that, indeed *C. includens* become less susceptible to the virus ChinNPV with development of its larval stage. Thus, application of virus ChinNPV should have targeted the early stages of larval development in order to obtain the full potential of virus to control this insect pest at field level.

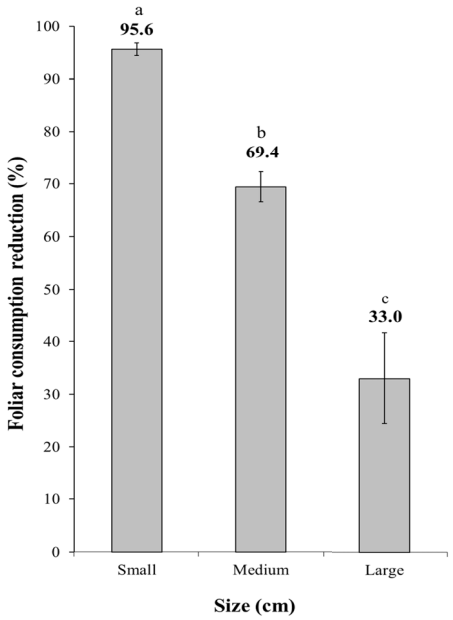

Figure 2. Percentage of foliar consumption reduction for small, medium, and large *Chrysodeixis includens* larvae, after ingestion of soybean leaf discs treated with a suspension of 4.0×10^{11} PIB ha^{-1} of virus isolate Chin-IA (I-A), compared to the consumption of larvae from control treatment

Note. *Different letters over the bars indicate values statistically different by the t-test ($p < 0.05$). Bars inside the columns indicate the Mean Standard Error.

The infection process caused by the virus studied, also significantly reduced the feeding period of small and medium *C. includens* larvae (Table 3). However, no statistical difference between large infected and uninfected larvae was found. This probably happened because during the period when the virus was acting on large infected larvae, larvae from control treatment were at the end of their larval stage and were already preparing for the

pre-pupation stage, as also observed by Dezianian et al. (2010). All larval sizes evaluated in the treatment with the virus Chin-IA (I-A) had similar feeding period. On the other hand, the feeding period of larvae from control treatment was statistically different among the size ranging from 4.8 to 10 days for large and small larvae, respectively (Table 3).

Table 3. Average feeding period (days) of small, medium, and large *Chrysodeixis includens* larvae, after ingestion of soybean leaf discs treated with sterilized distilled water (control) and with suspension of 4.0×10^{11} PIB ha^{-1} of virus isolate Chin-IA (I-A)

Size	Treatment (days)	
	Control [(1)]	Chin-IA (I-A) [(1)]
Small larvae	10.0±0.55 aA	4.0±0.22 aB
Medium larvae	8.4±0.31 bA	4.6±0.10 aB
Large larvae	4.8±0.40 cA	5.0±0.31 aA

Note. [(1)] Mean±Standard Error followed by the same lower-case letter in the column and by the same upper-case letter in the row are not statistically different by the t-test ($p < 0.05$).

Small and medium infected *C. includens* larvae have shown a percentage of corrected mortality of 90%, and for large infected larvae 70%, without significant difference among three larvae sizes considered in this study (Figure 3).

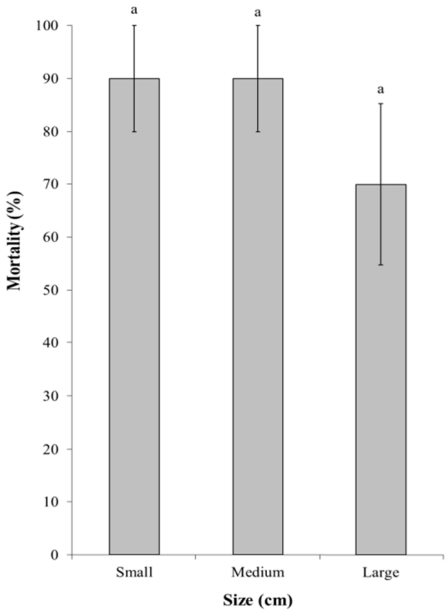

Figure 3. Corrected mortality of small, medium, and large *Chrysodeixis includens* larvae after ingestion of soybean leaf discs treated with suspension of 4.0×10^{11} PIB ha^{-1} of virus isolate Chin-IA (I-A)

Note. [*]Same letters over the bars indicate that the values are not statistically different by the t-test ($p < 0.05$) probability level. Bars inside the columns indicate the Mean Standard Error.

The mortality levels (superior to 70%) obtained in laboratory conditions are considered satisfactory and efficient to control lepidopteran population in the field (Tomquelski & Martins, 2007). Ramos et al. (2017) found similar levels when insecticides from different active ingredients were used to control *C. includens* larvae, confirming that the virus is a promising candidate to be used as a control tool to this pest. Furthermore, in natural conditions its also possible to find a constant reposition of infective particles of the virus that will provide new infection cycles, reducing then, the number of virus' application, and more importantly the use of chemical insecticides during the period of occurrence of this insect in soybean fields (Moscardi, 1999). Larvae of *S. frugiperda* of small (≤ 1.5 cm), medium (1.5 and 2.5 cm) and large (> 2.5 cm) sizes produced 1.46×10^9; 1.95×10^9 and $3.4 \times$

10^9 PIB per insect, respectively, after infection by the *Spodoptera frugiperda multiple nucleopolyhedrovirus* (Cruz, 2000). These results indicate the potential increment of the virus in field.

In addition, when selective control tactic, such as the virus ChinNPV is used, the action of other biological control agents (natural enemies) like parasitoids also become possible. As an example, parasitoids *Copidosoma floridanum* (Hymenoptera: Encyrtidae), *Microcharops anticarsiae* (Hymenoptera: Ichneumonidae), *Hypomicrogaster* sp. (Hymenoptera: Braconidae) and *Lixophaga* sp. (Diptera: Tachinidae) in larval stages, and *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) in the eggs of soybean looper, were found providing natural control of soybean looper population during the previous experiments evaluating parasitism on this pest (personal observation). Pereira et al. (2018) also reported the occurrence of other factors as rainfall and predators such as spiders, ants (Hymenoptera: Formicidae), *Orius* sp. (Hemiptera: Anthocoridae), *Geocoris* sp. (Hemiptera: Lygaeidae), *Franklinothrips* sp. (Thysanoptera: Aeolothripidae), and Vespidae (Hymenoptera) causing mortality in soybean loopers in the field.

4. Conclusions

All three larval sizes of *C. includens* had significantly reduced foliar consumption after 3rd day of ingestion of soybean leaf discs, which were treated with 4.0×10^{11} PIB ha^{-1} suspension of virus isolate Chin-IA (I-A), and this reduction was more expressive in the small larvae;

The Chin-IA (I-A) reduced the feeding period of small and medium infected larvae of *C. includens* only, while large larvae's feeding period was not affected by the virus.

Corrected mortality of *C. includens* ranged from 70 to 90% without a significant difference between the three larval sizes.

Acknowledgements

We would like to acknowledge FUNDECT (Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado do Mato Grosso do Sul) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the financial support to this study.

References

Alexandre, T. M., Ribeiro, Z. M. A., Craveiro, S. R., Cunha, F., Fonseca, I. C., Moscardi, F., & Castro, M. E. B. (2010). Evaluation of seven viral isolates as potential biocontrol agents against *Pseudoplusia includens* (Lepidoptera: Noctuidae) caterpillars. *Journal of Invertebrate Pathology, 105*, 98-104. https://doi.org/10.1016/j.jip.2010.05.015

Ali, G., van der Werf, W., & Vlak, J. M. (2019). Infection with *Spodoptera litura* NPV reduces food consumption and weight gain of *Spodoptera litura* larvae. *Pakistan Journal of Zoology, 51*, 495-501. https://doi.org/10.17582/journal.pjz/2019.51.2.495.501

Ávila, C. J., & Grigolli, J. F. (2014). Pragas da soja e seu controle. In A. L. F. Lourenção, J. F. Grigolli, A. M. Melotto, C. Pitol, D. C. Gitti, & E. R. Rosco (Eds.), *Tecnologia e Produção* (pp. 109-169). Soja 2013/2014.

Baldin, E. L. L., Lourenção, A. L., & Schlick-Souza, E. C. (2014). Outbreaks of *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae) in common bean and castor bean in São Paulo State, Brazil. *Bragantia, 73*, 458-461. https://doi.org/10.1590/1678-4499.0277

Bortolotto, O. C., Pomari-Fernandes, A., Bueno, R. C. O. F., Bueno, A. F., Kruz, Y. K. S., Queiroz, A. P., ... Ferreira, R. B. (2015). The use of soybean integrated pest management in Brazil: a review. *Agronomy Science and Biotechnology, 1*, 25-32.

Bueno, R. C. O. F., Bueno, A. F., Moscardi, F., Parra, J. R. P., & Hoffmann-Campo, C. B. (2011). Lepidopteran larva consumption of soybean foliage: Basis for developing multiplespecies economic thresholds for pest management decisions. *Pest Management Science, 67*, 170-174. https://doi.org/10.1002/ps.2047

Clem, R. J., & Passarelli, A. L. (2013). Baculoviruses: Sophisticated Pathogens of Insects. *PLOS Pathogens*, *9*(11), e1003729. https://doi.org/10.1371/journal.ppat.1003729

Conab. Companhia Nacional de Abastecimento (2019). *Acompanhamento de safra brasileira: Grãos* (pp. 1-125). Safra 2018/19 - Quinto levantamento, Brasília.

Craveiro, S. R., Inglis, P. W., Togawa, R. C., Grynberg, P., Melo, F. L., Ribeiro, Z. M. A., ... Castro, M. E. B. (2015). The genome sequence of *Pseudoplusia includens* single nucleopolyhedrovirus and an analysis of p26 gene evolution in the baculoviruses. *BMC Genomics, 16*, 127-136. https://doi.org/10.1186/s12864-015-1323-9

Craveiro, S. R., Melo, F. L., Ribeiro, Z. M. A., Ribeiro, B. M., Báo, S. N., Inglis, P. W., & Castro, M. E. B. (2013). *Pseudoplusia includens* single nucleopolyhedrovirus: Genetic diversity, phylogeny and hypervariability of the pif-2 gene. *Journal of Invertebrate Pathology, 114*, 258-267. https://doi.org/10.1016/j.jip.2013.08.005

Cruz, I. (2000). Utilização do baculovírus no controle da lagarta-do-cartucho do milho, *Spodoptera frugiperda*. In I. S. Melo, & J. L. Azevedo (Eds.), *Embrapa Meio Ambiente* (pp. 201-230).

Czepak, C., & Albernaz, K.C. (2014). Manejo avançado: Surtos de falsa-medideira. *Cultivar Grandes Culturas, 178*, 20-24.

Dezianian, A., Sajap, A. S., Lau, W. H., Omar, D., Kadir, H. A., Mohamed, R., & Yusoh, M. R. M. (2010). Morphological characteristics of *P. xylostella* granulovirus and effects on its larval host Diamondback moth *Plutella xylostella* L. (Lepidoptera, Plutellidae). *American Journal of Agricultural and Biological Sciences, 5*, 43-49. https://doi.org/10.3844/ajabssp.2010.43.49

Eberle, K. E., Wennmann, J. T., Kleespies, R. G., & Jehle, J. A. (2012). *Basic techniques in insect virology* (2nd. ed.). In A. L. Lacey (Ed.), *Manual of Techniques in Invertebrate Pathology* (pp. 15-74). Oxford: Elsevier. https://doi.org/10.1016/B978-0-12-386899-2.00002-6

Greene, G. L., Leppla, N. C., & Dickerson, W. A. (1976). Velvetbean Caterpillar: A Rearing Procedure and Artificial Medium. *Journal of Economic Entomology, 69*, 487-488. https://doi.org/10.1093/jee/69.4.487

Haase, S., Sciocco-Cap, A., & Romanowski, V. (2015). Baculovirus Insecticides in Latin America: Historical Overview, Current Status and Future Perspectives. *Viruses*, 7, 2230-2267. https://doi.org/10.3390/v7052230

Harper, J. D. (1973) Food consumption by cabbage loopers infected with nuclear polyhedrosis virus. *Journal of Invertebrate Pathology, 21*, 191-197. https://doi.org/10.1016/0022-2011(73)90201-2

Harrison, R., & Hoover, K. (2012). Baculoviruses and Other Occluded Insect Viruses. In F. E. Vega, & K. K. Harry (Eds.), *Insect Pathology* (pp. 73-131). Oxford: Elsevier. https://doi.org/10.1016/B978-0-12-384984-7.00004-X

Hoffmann-Campo, C. B., Oliveira, E. B., & Moscardi, F. (1985). *Criação massal da lagarta-da-soja (Anticarsia gemmatalis)* (p. 23). Londrina: Embrapa Soja.

Lima, M., Junior, C. A. S., Rausch, L., Gibbs, H. K., & Johann, J. A. (2019). Demystifying sustainable soy in Brazil. *Land Use Policy, 82*, 349-352. https://doi.org/10.1016/j.landusepol.2018.12.016

Moscardi, F. (1999). Assessment of the application of baculoviruses for control of Lepidoptera. *Annual Review of Entomology, 44*, 257-289. https://doi.org/10.1146/annurev.ento.44.1.257

Moscardi, F., & Zonta-De-Carvalho, R. C. (1993). Consumo e utilização de folhas de soja por *Anticarsia gemmatalis* Hüb. (Lepidoptera: Noctuidae) infectada, em diferentes estádios larvais, por seu vírus de poliedrose nuclear. *Anais da Sociedade Entomológica do Brasil, 22*, 267-280.

Moscardi, F., Souza, M. L., Castro, M. E. B., Moscardi, M., & Szewczyk, B. (2011). Baculovirus pesticides: present state and future perspectives. In I. Ahmad, F. Ahmad, & J. Pichtel (Eds.). *Microbes and Microbial Technology* (pp. 415-445). New York, NY: Springer. https://doi.org/10.1007/978-1-4419-7931-5_16

Oliveira, J. R. G., Ferreira, M. C., & Román, R. A. A. (2010). Diameter of droplets and different equipments for the application of insecticide to control *Pseudoplusia includens*. *Engenharia Agrícola, 30*, 92-99. https://doi.org/10.1590/S0100-69162010000100010

Pereira, R. R., Neves, D. V. C., Campos, J. N., Santana Júnior, P. A., Hunt, T. E., & Picanço, M. C. (2018). Natural biological control of *Chrysodeixis includens*. *Bulletin of Entomological Research, 108*, 831-842. https://doi.org/10.1017/S000748531800007X

Püntener, W. (1981). *Manual for field trials in plant protection* (2nd ed., p. 205). Agricultural Division: Ciba-Geigy Limited.

Ramos, R. S., Sediyama, C. S., Queiroz, E. A., Costa, T. L., Martins, J. C., Araújo, T. A., & Picanço, M. C. (2017). Toxicity of insecticides to *Chrysodeixis includens* and their direct and indirect effects on the predator *Blaptostethus pallescens*. *Journal of Applied Entomology, 141*, 677-689. https://doi.org/10.1111/jen.12382

Silva, F. A. S., & Azevedo, C. A. V. (2002). Versão do programa computacional ASSISTAT para o sistema operacional Windows. *Revista Brasileira de Produtos Agroindustriais, 4*, 71-78. https://doi.org/10.15871/1517-8595/rbpa.v4n1p71-78

Specht, A., Paula-Moraes, S. V., & Sosa-Gómez, D. R. (2015). Host plants of *Chrysodeixis includens* (Walker) (Lepidoptera, Noctuidae, Plusiinae). *Revista Brasileira de Entomologia, 59*, 343-345. https://doi.org/10.1016/j.rbe.2015.09.002

Togni, P. H. B., Venzon, M., Lagôa, A. C. G., & Sujii, E. R. (2019). Brazilian Legislation Leaning Towards Fast Registration of Biological Control Agents to Benefit Organic Agriculture. *Neotropical Entomology, 48*, 175-185. https://doi.org/10.1007/s13744-019-00675-8

Tomquelski, G. V., & Martins, G. L. M. (2007). Eficiência de inseticidas sobre *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) em milho na Região dos Chapadões. *Revista Brasileira de Milho e Sorgo, 6*, 26-39. https://doi.org/10.18512/1980-6477/rbms.v6n1p26-39

Trichilo, P. J, & Mack, T. P. (1989). Soybean leaf consumption by the soybean looper (Lepidoptera: Noctuidae) as a function of temperature, instar, and larval weight. *Economic Entomology, 82*, 633-638. https://doi.org/10.1093/jee/82.2.633

Valicente, F. H. & Tuelher, E. de S. (2009). *Controle Biológico da Lagarta do Cartucho, Spodoptera frugiperda, com Baculovírus*. Retrieved from https://ainfo.cnptia.embrapa.br/digital/bitstream/CNPMS-2010/22431/1/Circ-114.pdf

Volkman, L. E., & Keddie, B. A. (1990). Nuclear polyhedrosis virus pathogenesis. *Seminars in Virology, 1*, 249-256.

Wagner, D. L., Schweitzer, D. F., Sullivan, J. B., & Reardon, R.C. (2011). *Owlet caterpillars of eastern North America (Lepidoptera: Noctuidae)* (p. 576). Princeton University Press.