Notas

Susceptibility of *Helicoverpa gelotopoeon* (Lepidoptera: Noctuidae) to generalist AcMNPV and comparative pathogenicity of a specific nucleopolyhedrovirus

Susceptibilidad de *Helicoverpa gelotopoeon* (Lepidoptera: Noctuidae) al generalista AcMNPV y patogenicidad comparada de un nucleopoliedrovirus específico

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Revista de la Sociedad Entomológica Argentina vol. 84 núm. 4 e0406 2025

Sociedad Entomológica Argentina
Argentina

Recepción: 20 Mayo 2025
Aprobación: 20 Septiembre 2025

Abstract: *Helicoverpa gelotopoeon* Dyar (Lepidoptera: Noctuidae) is a major agricultural pest in southern South America. *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is a generalist baculovirus that was proven to be highly pathogenic to several lepidopteran species, some of which co-occur in time and space with *H. gelotopoeon*. Here, the infectivity of AcMNPV to *H. gelotopoeon* larvae was assessed through bioassays, molecular methods and microscopy. AcMNPV infection in H. gelotopoeon did occur, but the speed of kill was extremely slow. For comparative purposes, bioassays using specific *Helicoverpa gelotopoeon* single nucleopolyhedrovirus (HegeSNPV) showed a relatively fast lethal effect. Both viruses had similar tissue tropism, however symptom development differed: AcMNPV-infected larvae retained an intact integument, whereas HegeSNPV caused tissue liquefaction. HegeSNPV, rather than AcMNPV, could serve as a biocontrol agent against this pest.
Keywords: Baculovirus, Biological control, Bollworm, Heliothinae, Pathology.

Resumen: *Helicoverpa gelotopoeon* Dyar (Lepidoptera: Noctuidae) es una importante plaga de la agricultura en el sur de América del Sur. El virus de la poliedrosis multiple de *Autographa californica* (AcMNPV) es un baculovirus generalista de alta patogenicidad en varias especies de lepidópteros, entre ellas algunas que coocurren en tiempo y espacio con *H. gelotopoeon*. En este trabajo se investigó la infectividad de AcMNPV en larvas de *H. gelotopoeon* mediante bioensayos, métodos moleculares y microscopía. Si bien se constató la infección de *H. gelotopoeon* por AcMNPV, se observó una muy lenta velocidad de acción. En ensayos comparativos usando el nucleopoliedrovirus simple específico de *H. gelotopoeon* (HegeSNPV), se comprobó un efecto letal relativamente rápido. Ambos virus infectaron los mismos tejidos, pero ocasionaron distinta sintomatología en el hospedador: no se produjo desintegración del tegumento en larvas infectadas con AcMNPV, mientras que se observó licuefacción en aquellas infectadas con HegeSNPV. HegeSNPV, más que AcMNPV, podría servir como agente de control biológico de la plaga.

Palabras clave: Baculovirus, Control biológico, Heliothinae, Oruga bolillera, Patología.

Autographa californica multiple nucleopolyhedrovirus (AcMNPV) infects dozens of species across at least 11 lepidopteran families, including Noctuidae (Harrison 2009; Rohrmann 2019). This virus has proven to be a successful biocontrol agent against several agricultural pests (Haase et al., 2015; Sun, 2015; Lei et al., 2019). Interestingly, within the *Helicoverpa*/*Heliothis* complex, dissimilar effects were observed after AcMNPV ingestion, depending on the species evaluated. Previous works reported that while AcMNPV was highly pathogenic to *Heliothis* (=*Chloridea*) *virescens* Fab., low mortality rates and prolonged time to death were observed in *Helicoverpa zea* Boddie (Vail et al., 1978; Arne & Nordin, 1995; Volkman, 2007). Haemocyte resistance, melanization reactions and elevated immune responses have been signalled as possible reasons for decreased susceptibility (Trudeau et al., 2001; Volkman, 2007). The absence of a common infection pattern implies that the nature of the AcMNPV/Heliothinae interactions needs to be studied case by case, especially if envisaging the development of a bioinsecticide.

The South-American bollworm *Helicoverpa gelotopoeon* Dyar (Lepidoptera: Noctuidae) is an important pest of extensive and horticultural crops in central Argentina and neighbouring countries. The use of microbial agents against *Helicoverpa* spp. is gaining consideration globally because of the remarkable ability of this genus to develop resistance to chemical insecticides and/or transgenic crops (Rowley et al., 2011; Bolloki et al., 2024; Huang et al., 2025). For the geographically restricted *H. gelotopoeon*, however, basic and applied research on this matter is still incipient (Russo et al., 2019). A highly virulent *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) variant has been described from *H. gelotopoeon* larvae collected in Argentina (Arneodo et al., 2016; Ferrelli et al., 2016). The isolate, named *Helicoverpa gelotopoeon* single nucleopolyhedrovirus (HegeSNPV), stands as a promising active ingredient for a baculovirus-based insecticide. As the other HearNPV variants, HegeSNPV is supposed to have a narrow host range restricted to the subfamily Heliothinae (Ferrelli et al., 2016). In addition to this, a local isolate of AcMNPV from *Rachiplusia nu* Guenée (Lepidoptera: Noctuidae) larvae, was characterised (Rodríguez et al., 2012) and preserved for future testing on Heliothinae and other potential noctuid hosts.

The infectivity of entomopathogens needs to be determined to select the best candidates for developing a bioinsecticide. In this paper, the susceptibility of *H. gelotopoeon* to AcMNPV was investigated using bioassays, microscopy and molecular tools. Furthermore, different aspects of the pathogenic process in *H. gelotopoeon* larvae (including speed of kill, symptomatology and tissue tropism) were compared between generalist AcMNPV and specific HegeSNPV.

Baculoviral pathogenesis in lepidopteran larvae is heavily age-dependent. Therefore, strict precision is required regarding larval instar at inoculation. As no biometric data were available for *H. gelotopoeon*, these parameters were established prior to the bioassays. Healthy insects used for these and subsequent studies were reared at 25±1 °C and 16:8 (light:dark) photoperiod, and fed with artificial diet (slightly modified from Greene et al., 1976). A cohort of 30 individuals was supervised daily between 07:30 a.m. and 12:00 a.m. from hatching to pupation. Moultings were evidenced by the detachment of the head capsules, which were recovered and measured under an Olympus SZX9 stereomicroscope (Olympus Optical Co. LTD, Tokyo, Japan) using Image-Pro Plus (Media Cybernetics Inc., Bethesda, Maryland, USA). Five larval instars were recorded. Head capsule widths (HCW) exhibited a regular geometric progression across successive larval instars, in accordance with Dyar's rule (Dyar, 1890), albeit with a relatively high average growth ratio of 1.74 between instars. Mean HCW values were 0.26±0.01, 0.42±0.03, 0.75±0.05, 1.34±0.10, and 2.38±0.16 mm for first to fifth instars, respectively. At least under the artificial rearing conditions described, low standard deviations and the absence of overlap among developmental groups supported HCW as a reliable indicator for discriminating instars. The near-perfect fit to the adjusted equation corroborated the consistency of the data. (Fig. 1).

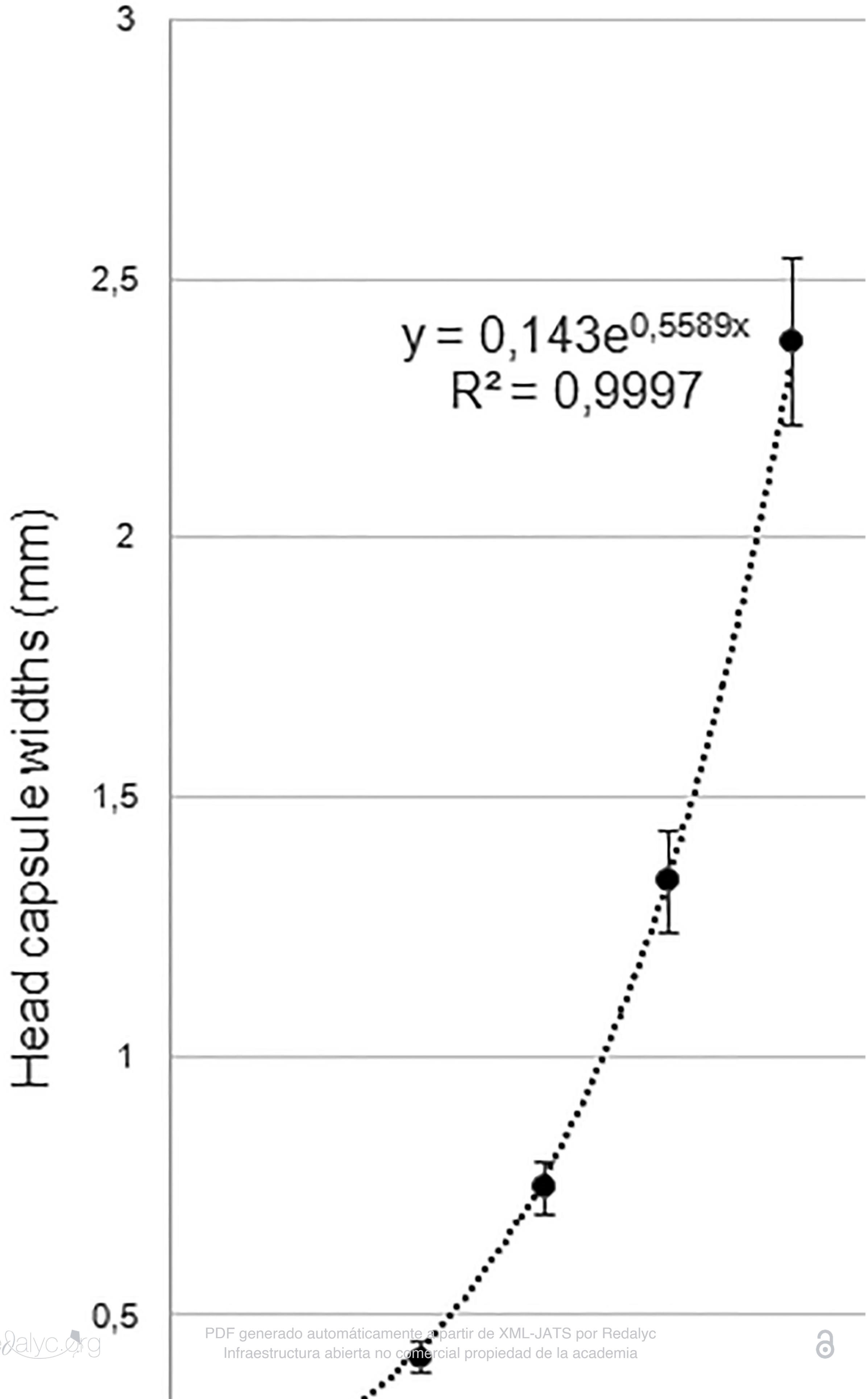
3
2,5
2
1,5
1
0,5
Head capsule widths (mm)
y = 0,143e0,5589x
R² = 0,9997

Figure 1.

Head capsule widths across larval instars of artificially reared *Helicoverpa gelotopoeon*.

Error bars represent standard deviation from the mean values. The equation describing the head capsule width increase across instars, along with the coefficient of determination (R^2), is shown.

Occlusion bodies (OBs) of the local AcMNPV isolate were produced and purified from its original host (*R. nu* larvae). They were checked by scanning electron microscopy (FEI Quanta 250, FEI Co., Eindhoven, Netherlands) to discard possible contaminations (Fig. 2a) and counted on a Neubauer chamber. In a first experiment, groups of 37-44 neonate *H. gelotopoeon* larvae were supplied with different doses of AcMNPV OBs (0, 2, 5, 10 and 50), one group per dose, by droplet-feeding (Hughes & Wood, 1981), assuming a mean ingestion of 0.014 µl per specimen (Ferrelli et al., 2016). Larvae were then maintained individually on artificial diet as referred earlier and observed daily until death or pupation. AcMNPV caused 100 % mortality to *H. gelotopoeon* larvae only at the highest dose of 50 OBs. In this case, half of the tested insects were dead by day 8 post inoculation (p. i.). Irrespective of the dose, speed of kill was consistently slow (Fig. 3a). Accordingly, though a different methodology was used, more than 9 days were required to attain 50 % mortality in *H. zea* neonates (Vail et al., 1978). For biocontrol purposes, these results were rather poor: a similar study on nenonate *H. gelotopoeon* infected with HegeSNPV revealed that 18 OBs/larva were sufficient to induce 100 % mortality, while the median lethal dose was below 2 OBs and the average time to death slightly above 4 days (Ferrelli et al., 2016). AcMNPV infection was verified in all dead larvae by direct observation of cubic OBs by phase-contrast microscopy. A transmission electron microscopy confirmation was also carried out. Two larvae were infected in parallel to the main experiment; at 7 days p. i. they were processed as previously reported (Ferrelli et al., 2016) and the ultrathin sections examined in a TEM JEOL 1200 EX II (JEOL, Tokyo, Japan). AcMNPV was relatively sparse across the host tissues. A few heavily infected cells displayed intranuclear OBs. Multiple nucleocapsids per envelope were observed (Fig. 2b). The shape, size and general ultrastructure of OBs and virions were typical of AcMNPV. To further corroborate disease etiology, total DNA was extracted (CTAB method) from two dead larvae, and the polyhedrin and gp64 genes amplified by PCR using primer pairs 5’-NRCNGARGAYCCNTT-3’ / 5’-DGGNGCRAAYTCYTT-3’ and 5’-ACTGCAGTTAATATTGTCTATTACGG-3’ / 5’-ACTGCAGCGGCCGCCCACTTGGCGCC-3’, respectively. Amplicons were sequenced in an ABI PRISM 3500 XL genetic analyzer (Applied Biosystems, USA) at Instituto de Biotecnología-CICVyA-INTA (Hurlingham, Argentina). Both sequences, available at GenBank under accession numbers MF066628 (polyhedrin) and

MF066627 (gp64), were identical to those of AcMNPV-*R. nu* (DQ345451, polyhedrin gene) and AcMNPV isolates WP10 (KM609482, complete sequence) from *Trichoplusia ni* (France) and E2 (KM667940, complete sequence) from *A. californica* (USA).

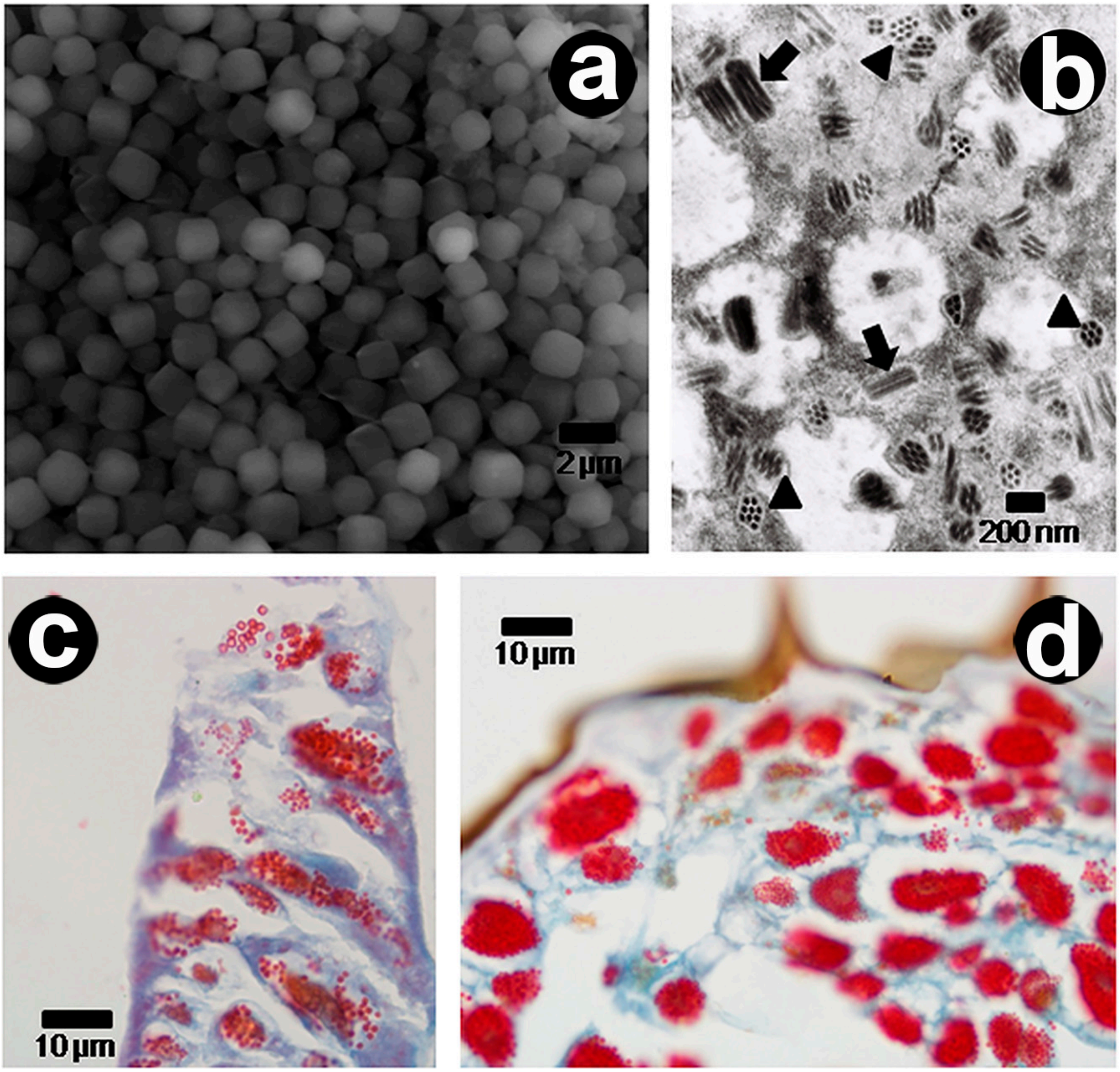

Figure 2.

Micrographs obtained using different microscopy techniques.

a. Purified AcMNPV cubic OBs under the scanning electron microscope. b. Ultrathin section of a nucleus of an AcMNPV-infected *Helicoverpa gelotopoeon* larva. Virions containing multiple nucleocapsids per envelope in longitudinal (arrows) and transversal (arrowheads) view. c. Epidermis of an AcMNPV-infected *H. gelotopoeon* larva after Azan staining, showing bright-red OBs. d. Same as before, but in a HegeSNPV-infected larva.

A second experiment was performed to simultaneously evaluate the pathogenicity of AcMNPV and HegeSNPV to 3rd instar *H. gelotopoeon* larvae. The AcMNPV isolate used was the same as above, while HegeSNPV OBs (also checked by microscopic and molecular analyses) were propagated and purified from *H. gelotopoeon* larvae.

Because older *H. gelotopoeon* larvae were not attracted by the sugar solution, the diet surface contamination method (Eberle et al., 2012) was used to deliver the virus isolates to two groups of 40 larvae each. Based on prior results showing low susceptibility of *H. gelotopoeon* to the generalist baculovirus, a considerably high dose of 400 OBs/mm^2 was selected in order to induce detectable effects. Starved, newly moulted larvae were exposed to one or the other virus for 48 h and transferred to individual cups containing artificial diet for further rearing. Negative (healthy) controls (n = 32) were also considered. The effect of AcMNPV and HegeSNPV on larval development and survival, and the associated symptomatology, was strikingly different. In HegeSNPV-inoculated individuals, 100 % mortality was achieved by day 7 p. i. (Fig. 3b). As in most baculoviral infections, post-mortem liquefaction of the integument was consistently observed. In contrast, specimens supplied with AcMNPV developed in an apparently normal manner until the prepupal stage. However, 90 % of tested larvae failed to pupate and died between 12 and 31 days p. i. (Fig. 3b). The typical disintegration of insect cadavers was not recorded. Such retarded larval development and unusual symptomatology had also been noticed by Vail & Jay (1973) when assessing the susceptibility of *H. zea* to AcMNPV.

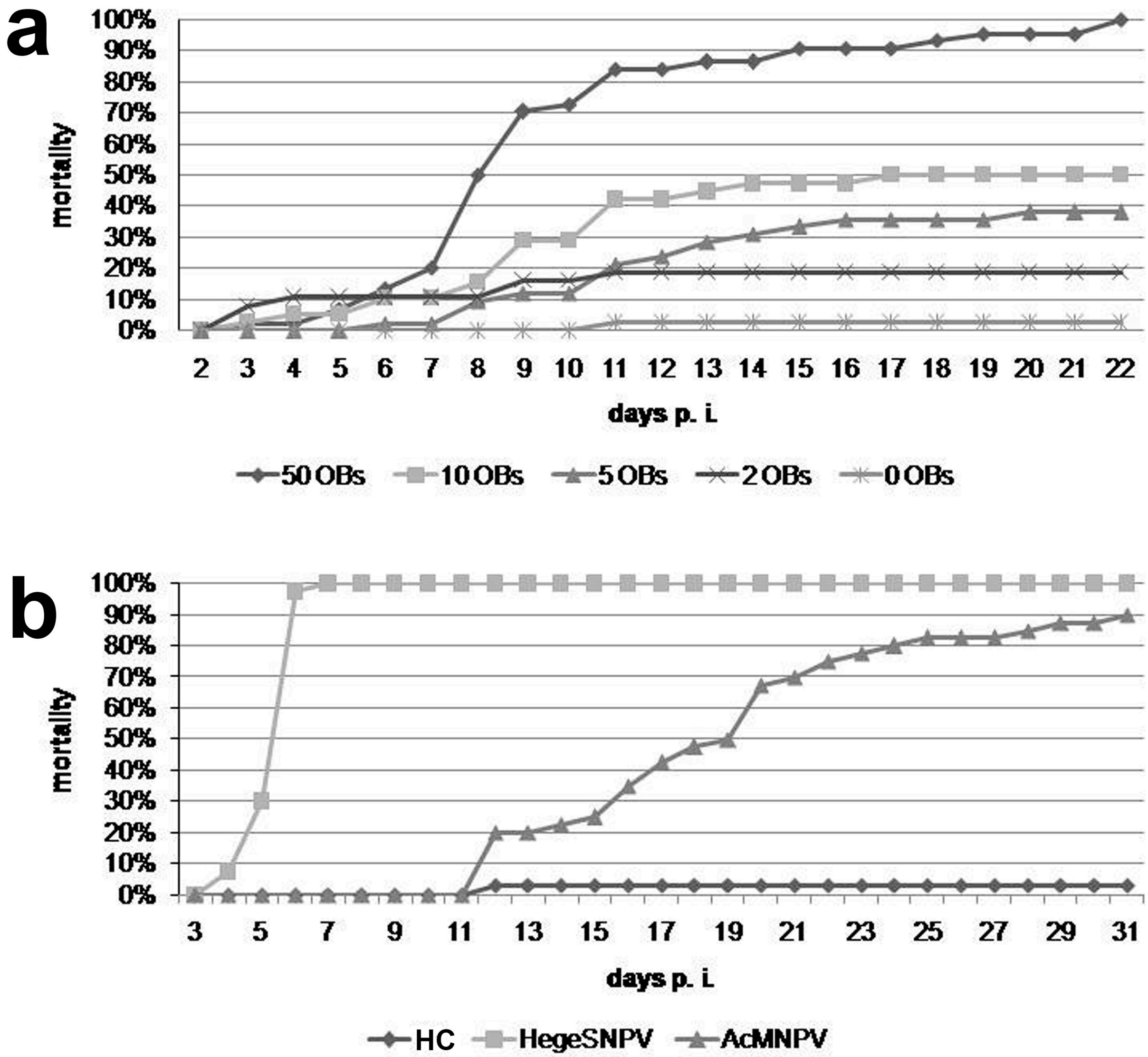

Figure 3.

Bioassays with *Helicoverpa gelotopoeon*.

a. Neonate larvae inoculated with different doses of AcMNPV through the droplet-feeding method. b. Third-instar larvae exposed to 400 OBs/mm^2 of HegeSNPV or AcMNPV by diet surface contamination. HC: healthy (uninfected) controls.

Larval liquefaction favours the release of OBs from the cadavers into the environment and, therefore, the dissemination and horizontal transmission of the virus. The disruption of the cuticle is promoted by viral enzymes, which have been identified in several baculovirus species, including AcMNPV (Rohrmann, 2019). Such fragility could be boosted by the viral replication in the epithelial cells following systemic infection. Hence, a histopathological analysis was conducted on AcMNPV- and HegeSNPV-infected *H. gelotopoeon* larvae, aiming to detect possible differences in tissue tropism. Third-instar larvae were inoculated by diet contamination as explained above. At 3 (HegeSNPV) or 5 (AcMNPV) days p. i., larvae were fixed, embedded, sectioned and stained with Azan (Hamm, 1966). A preliminary sampling of AcMNPV-infected larvae at 10 days p. i. appeared to be inappropriate for histology because of tissue reorganisation during metamorphosis (not shown). Light microscopic examinations of semithin sections disclosed bright-red stained OBs in AcMNPV- (Fig. 2c) and HegeSNPV-infected (Fig. 2d) larval tissues. No obvious differences were found with respect to the main sites of virus colonisation which were, in both cases, the fat bodies, epidermis and tracheal matrix. Thus, AcMNPV was polyorganotropic in *H. gelotopoeon*, as in full permissive lepidopteran hosts (Barret et al., 1998). The same was true for HegeSNPV. Larvae treated with HegeSNPV showed, however, higher OB proliferation in a comparatively larger proportion of infected cells than those exposed to AcMNPV. In addition, the nuclear hypertrophy associated to baculoviral infections was more clearly appreciated in the former case. No OBs were seen in the healthy controls (not shown). Again, regarding tissue specificity and OB production, the gross pathology produced by AcMNPV in *H. gelotopoeon* resembled that described for *H. zea* (Vail & Jay, 1973).

The present work demonstrated that *H. gelotopoeon* is a semi-permissive host for AcMNPV. As for *H. zea*, an extremely slow speed of kill, together with the relatively high doses required to produce mortality, makes this virus an ineffective biocontrol agent for *H. gelotopoeon*. Such response is particularly noteworthy since *H. gelotopoeon* often co-occurs in space and time with other polyphagous noctuid pests for which AcMNPV-based formulations are available or in development, *e. g.Spodoptera* spp and *Rachiplusia nu* (Haase et al., 2015; Decker-Franco et al., 2021). For the latter species, AcMNPV was shown to act even faster than its specific baculovirus, RanuNPV (Decker-Franco et al., 2021). On the other hand, the experiments provided additional evidence on the biocontrol potential of HegeSNPV, which deserves further research for its effective use in IPM strategies. Although HearNPV-based bioinsecticides are already available in the market (Rowley et al., 2011; Knox et al., 2015), the incorporation of new, virulent isolates may help improve current management of Heliothinae and delay the emergence of resistance.

Acknowledgments

To former laboratory director Alicia Sciocco for sharing knowledge and materials; and to Roberto Carlos Igarza (IMyZA-CICVyA-INTA) for *H. gelotopoeon* rearing. This work was funded by Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina. JDA is member of the CONICET research staff.

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Notes

COMPETING INTERESTS

The authors have declared that no competing interests exist.

AUTHORS CONTRIBUTIONS

Conceptualization: JDA; Methodology: JDA and CT; Formal analysis and investigation: JDA and CT; Writing: JDA; Funding acquisition: JDA.

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Información adicional

redalyc-journal-id: 3220

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Revista de la Sociedad Entomológica Argentina
vol. 84, núm. 4, e0406, 2025
Sociedad Entomológica Argentina, Argentina
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ISSN-E: 1851-7471

DOI: https://doi.org/10.25085/rsea.840406