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Original article (Orijinal araştırma)

Bioactivity of a betabaculovirus, *Hyphantria cunea* granulovirus, in six lepidopteran insects as potential hosts[1]

Betabaculovirüs, *Hyphantria cunea* granulovirüs'ün potansiyel konukçu olarak altı lepidopter böcekteki biyoaktivitesi

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Abstract

The aim of this study, conducted in 2018 and 2020, was to investigate the bioactivity of a local baculovirus isolate, *Hyphantria cunea* granulovirus (HycuGV), in seven lepidopteran pests. Based on data collected 10 days after exposure, HycuGV was found to infect *Malacosoma neustria* (L., 1758) (Lepidoptera: Lasiocampidae), *Lymantria dispar* (L., 1758) (Lepidoptera: Erebidae), *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae) and *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae) larvae as well as its host *Hyphantria cunea* (Drury, 1773) (Lepidoptera: Erebidae). However, it did not infect *Spodoptera littoralis* (Boisduval, 1883) (Lepidoptera: Noctuidae) and *Cydia pomonella* (L., 1758) (Lepidoptera: Tortricidae). A HycuGV dose rate experiment indicated LC_{50} of $4.7x10^5$ occlusion bodies (OBs)/ml in *H. cunea*, $5.6x10^6$ OBs/ml in *L. dispar*, $7x10^7$ OBs/ml in *S. exigua*, $1.5x10^9$ OBs/ml in *M. neustria* and $7.7x10^9$ OBs/ml in *H. armigera*. HycuGV was infectious to *S. exigua* and *L. dispar*, but only provided effective control in *M. neustria* and *H. armigera* at high dose rates. These findings demonstrate that HycuGV can be highly effective for control of *S. exigua*, *L. dispar* and *H. cunea*.

Keywords: Baculovirus, bioactivity, biological control, host range, *Hyphantria cunea* granulovirus

Öz

2018 ve 2020 yıllarında yürütülen bu çalışmanın amacı, yerel bir bakülovirüs izolatı *Hyphantria cunea* granulovirus (HycuGV)'ün yedi lepidopter zararlısı üzerindeki biyoaktivitesinin araştırılmasıdır. Denemeden sonraki 10. günde elde edilen verilere göre HycuGV'nin kendi konukçusu olan *Hyphantria cunea* (Drury, 1773) (Lepidoptera: Erebidae)'nın yanı sıra *Malacosoma neustria* (L., 1758) (Lepidoptera: Lasiocampidae), *Lymantria dispar* (L., 1758) (Lepidoptera: Erebidae), *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae) ve *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae) larvaları üzerinde de enfeksiyon oluşturma kabiliyetine sahip olduğu belirlenirken, *Spodoptera littoralis* (Boisduval, 1883) (Lepidoptera: Noctuidae) ve *Cydia pomonella* (L., 1758) (Lepidoptera: Tortricidae) larvalarında enfeksiyon oluşturmadığı tespit edildi. HycuGV'nin doz denemelerinde LC_{50} değeri, *H. cunea*'da $4.7x10^5$ OBs/ml, *L. dispar*'da $5.6x10^6$ OBs/ml, *S. exigua*'da $7x10^7$ OBs/ml, *M. neustria*'da $1.5x10^9$ OBs/ml ve *H. armigera*'da $7.7x10^9$ OBs/ml olarak hesaplandı. Bu sonuçlar HycuGV'nin *S. exigua* ve *L. dispar* için bulaşıcı olduğunu, ancak *M. neustria* ve *H. armigera*'da ise yüksek doz oranlarında etkili olduğunu gösterdi. Bu bulgular, HycuGV'nin *S. exigua*, *L. dispar* ve *H. cunea*'nın mücadelesi için oldukça etkili olabileceğini göstermektedir.

Anahtar sözcükler: Bakülovirüs, biyoaktivite, biyolojik mücadele, konukçu aralığı, *Hyphantria cunea* granulovirus

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Introduction

Baculoviruses are generally characterized by a narrow host range, which can be important when compared to synthetic chemical insecticides or other microbial control agents. However, there are significant differences between Baculoviridae host ranges. Due to limited knowledge of their host ranges, it is difficult to predict the infectiveness of a particular isolate under laboratory conditions, let alone in the field (Cory & Entwistle, 1990; Cory et al., 1997).

Baculoviruses have been isolated from more than 700 insect species in the Lepidoptera, Hymenoptera and Diptera (Moscardi, 1999; Herniou & Jehle, 2007). They are often preferred as agents for integrated pest management due to their high specificity (Ahmad et al., 2011). Baculoviruses are categorized into two groups based on morphology: nucleopolyhedrovirus (NPV) and granulovirus (GV). The Baculoviridae family is divided into four genera: *Alphabaculovirus* (nucleopolyhedroviruses isolated from Lepidoptera), *Betabaculovirus* (nucleopolyhedroviruses isolated from Lepidoptera), *Gammabaculovirus* (nucleopolyhedroviruses isolated from Hymenoptera) and *Deltabaculovirus* (nucleopolyhedroviruses isolated from Diptera) (Jehle et al., 2006). More than 150 insect species (Lepidoptera) are known to be susceptible to granuloviruses (Rohrmann, 2013).

Host range information is important for determining the properties of a microbial biocontrol agents. The host ranges of NPVs were reported to be broader than GVs (Ignoffo, 1968); however, one recent study did not support this view (Hamm, 1982). Although cross-infection tests are commonly used in the study of baculoviruses, comparative test data are rarely reported for different hosts. Nevertheless, knowing the effect of baculoviruses on different hosts is important for recommending the deployment of microbial biocontrol agents. Standard bioassays are needed to determine the host range and specificity of NPVs (Cory et al., 1997). NPVs are prevalent among 400 arthropod species in seven insect genera (Murphy et al., 1995). Mostly, the host range of NPVs are limited to one or more genera, or the host species from which it was isolated (Moscardi, 1999). Host range and cross infectivity of baculoviruses have been reviewed by Gröner (1986). The infectivity of NPV and GV in alternate hosts was typically determined on virus infection and mortality of the test larvae after oral virus application. However, these examinations are biased towards Lepidopteran species and other economically important insects. To date, no standardized bioassays have been developed to determine the host range and specificity of baculoviruses (Cory, 2003).

Baculoviruses are often reported to have various host ranges. Certain baculoviruses such as *Autographa californica multicapsid nucleopolyhedrovirus* and *Mamestra brassicae multicapsid nucleopolyhedrovirus* have broad host ranges and are infectious in certain insect species in different families. Also, the host range of certain baculoviruses, such as *Spodoptera exigua multiple nucleopolyhedrovirus*, are limited to a single (or a few) insect species (Federici, 1997; Goulson, 2003). High host specificity provides advantages for biocontrol; however, it limits the commercialization of baculoviruses as bioinsecticides. The production of a baculovirus with a broad host range is more economically attractive compared to producing many host-specific baculovirus that control only one (or a few) target species (Haase et al., 2015).

Host range is important in the determination of the persistence of the baculovirus isolates in an ecosystem based on the presence of primary and alternative hosts. Similarly, a broad host range is significant for the development of effective commercial biocontrol agents (Brodeur, 2012).

Hyphantria cunea granulovirus (HycuGV) has been isolated from *Hyphantria cunea* (Drury, 1773) (Lepidoptera: Erebidae) larvae and characterized by Bayramoglu et al. (2018) and Gencer et al. (2020). The aim of present study is to investigate the bioactivity and the host ranges of this virus in a range of pest species: *Malacosoma neustria* (L., 1758) (Lepidoptera: Lasiocampidae), *Lymantria dispar* (L., 1758) (Lepidoptera: Erebidae), *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae), *Spodoptera littoralis* (Boisduval, 1883) (Lepidoptera: Noctuidae), *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae) and *Cydia pomonella* (L., 1758) (Lepidoptera: Tortricidae).

Materials and Methods

Virus preparation

A single isolate of Betabaculovirus (HycuGV) was obtained from *H. cunea* larvae and identification, phylogeny, biological activity and whole-genome sequence determined in previous studies (Bayramoglu et al., 2018; Gencer et al., 2020). The present study was conducted at Karadeniz Technical University, Department of Biology between 2018 and 2020. For propagation of the virus, *H. cunea* larvae collected from the field in Trabzon and Rize Provinces, Turkey and then cultured in the laboratory for use in bioassays. Fresh mulberry leaves (*Morus* sp., Rosales: Moraceae) were surface sterilized initially with 70% ethanol then 2% sodium hypochlorite before rinsing with sterile dH_2O. A suspension of $1x10^7$ occlusion bodies (OBs)/ml HycuGV was applied to the leaves along with 100 *H. cunea* third-stage larvae. The larvae infected with HycuGV were collected and the viruses were reisolated by the method of Opoku-Debrah et al. (2013). Individual infected larvae were homogenized in 1 ml 0.1% sodium dodecyl sulfate (SDS) and filtered through cheesecloth. An equal volume of 0.1% SDS was added and the process was repeated. The resulting filtrate was centrifuged at 7,840 × g for 30 min at 4°C. The supernatant was discarded and the pellet suspended in dH_2O. A sucrose gradient (30-80% w/v) was prepared in Beckman Avanti centrifuge tubes (Beckman Coulter Inc., CA, USA) and kept overnight at 4°C. Test virus suspensions was loaded on top of a sucrose gradient and centrifuged for 30 min at 29,774 × g at 4°C in a Beckman Avanti (J-30I) ultracentrifuge. The visible OB band in the middle of the tube was extracted using a pipette, placed into a new sterile tube, filled to the brim with dH_2O and centrifuged again for 30 min at 29,774 × g at 4°C. The final pellet was resuspended with dH_2O and the OBs were quantified visually with a Neubauer hemocytometer under a phase-contrast microscope (Nikon Eclipse LH-M100C1, Tokyo, Japan) at 400X. The purified HycuGV stock was stored at -20°C until the bioassays were conducted.

Insects

The third larval stages of *H. cunea*, *M. neustria* (L., 1758) (Lepidoptera: Lasiocampidae), *S. exigua* (Hübner, 1808), *S. littoralis* (Boisduval, 1883) (Lepidoptera: Noctuidae), *C. pomonella* (L., 1758) (Lepidoptera: Tortricidae), *H. armigera* (Hübner, 1805) (Lepidoptera: Noctuidae) and *L. dispar* (L., 1758) (Lepidoptera: Erebidae) larvae were tested in bioassays. *Spodoptera exigua*, *S. littoralis* and *H. armigera* were cultured on an artificial diet under laboratory conditions at 60-70% RH and 16:8 h L:D photoperiod at 26±1°C. The other (*M. neustria*, *L. dispar* and *C. pomonella*) larvae were collected in Gümüşhane, Bingöl and Trabzon, and fed with fresh rose leaves (*Rosa canina* L., Rosales: Rosaceae), oak leaves (*Quercus petraea* L., Fagales: Fagaceae) and apples (*Malus domestica* Borkh, Rosales: Rosaceae), respectively. Only apparently healthy larvae were selected for inclusion in the bioassays.

Insecticidal activity trials on the hosts

The pathogenicity of HycuGV was tested at five concentrations (10^{5-9} OBs/ml) on the seven-lepidopteran species (*H. cunea*, *M. neustria*, *S. exigua*, *S. littoralis*, *C. pomonella*, *H. armigera* and *L. dispar*). Thirty third-stage larvae were used in tests and all tests were repeated three times at different times. For bioassays, five concentrations ($1x10^5$, 10^6, 10^7, 10^8 and 10^9 OBs/ml) were applied on 3 x 3 cm leaf disks and artificial diet disks. Larvae incubated in a 16:8 h L:D photoperiod at 26±1°C and those that consumed the entire disks were supplied with fresh food as needed. The control groups for each pest species, only water was added to each leave and placed into box. Mortality was recorded daily for 10 days. Dead larvae were examined for symptoms of viral infection and then the presence of viral structures under a phase-contrast microscope.

Confirmation of HycuGV infection with polymerase chain reaction

HycuGV infection in dead larvae (*M. neustria*, *L. dispar*, *H. armigera* and *S. exigua*) was confirmed using a PCR kit (Phire Animal Tissue Direct PCR Kit, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The presence of granulovirus was confirmed by polymerase chain reaction (PCR) using granulin primers (forward, ATG GGA TAY AAC ARA KCW YTR MGK TAY AGY MRH CAC, and reverse, TTA RTA VGC BGG DCC DGT RWA YAR WGG YAC RTC). The PCR products were analyzed on 1% agarose gel electrophoresis. The bands detected after PCR were considered positive for insect infection.

Statistical analysis

Mortality data were corrected by Abbott's formula (Abbott, 1925). Lethal concentrations (LC_{50}) for the virus against third-stage larvae of hosts were calculated by probit analysis using MS Excel (Finney, 1952).

Results

The mortalities of all insects infected with HycuGV are shown in Figure 1. The highest mortalities with HycuGV treatment were 90, 80 and 70% for *H. cunea, L. dispar* and *S. exigua*, respectively, at the 10^9 OBs/ml. Although, *L. dispar* larvae had a mortality, they do not have the soft body tissues like the other host larvae (Figure 2). The mortalities were similar for *M. neustria* (46%) and *H. armigera* (33%) larvae but low than the *L. dispar* and *S. exigua*. Based on the results, it was determined that HycuGV can infect and kill *H. cunea, M. neustria*, *L. dispar*, *H. armigera* and *S. exigua* larvae to different degrees. However, it was not lethal to *S. littoralis* and *C. pomonella* hosts. The LC_{50} values calculated in the experiments are presented in Table 1. The LC_{50} calculated by probit analysis were lower at $5.6x10^6$ OBs/ml for L. dispar and $4.7x10^5$ OBs/ml for *H. cunea* host, but higher in *S. exigua*, *M. neustria* and *H. armigera* at 7.0, 1.5 and $17.7x10^9$ OBs/ml, respectively.

During the bioassays, viral disease symptoms such as the soft body tissues were observed in dead larvae (Figure 2). Dead tissue samples were examined under a phase-contrast microscopy observing GV structures and PCR confirmed granulin gene region amplification bands (~800 bp) (Figure 3). Control groups of each insect species remained in healthy throughout the bioassay period.

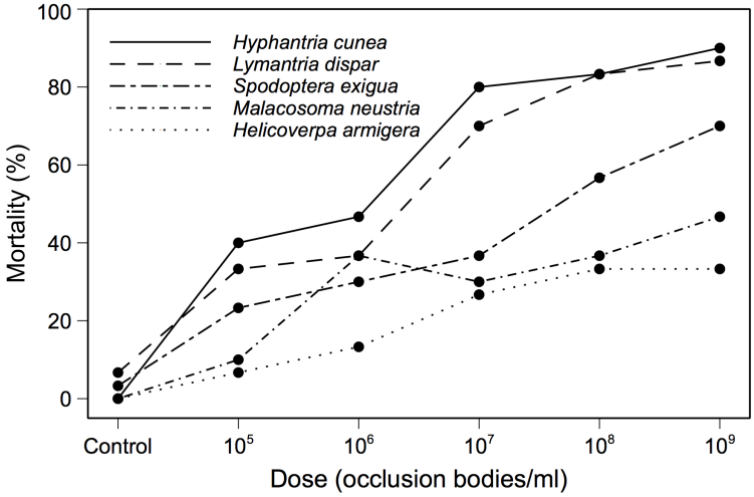

Figure 1. Mortality of insect larvae resulting from HycuGV application.

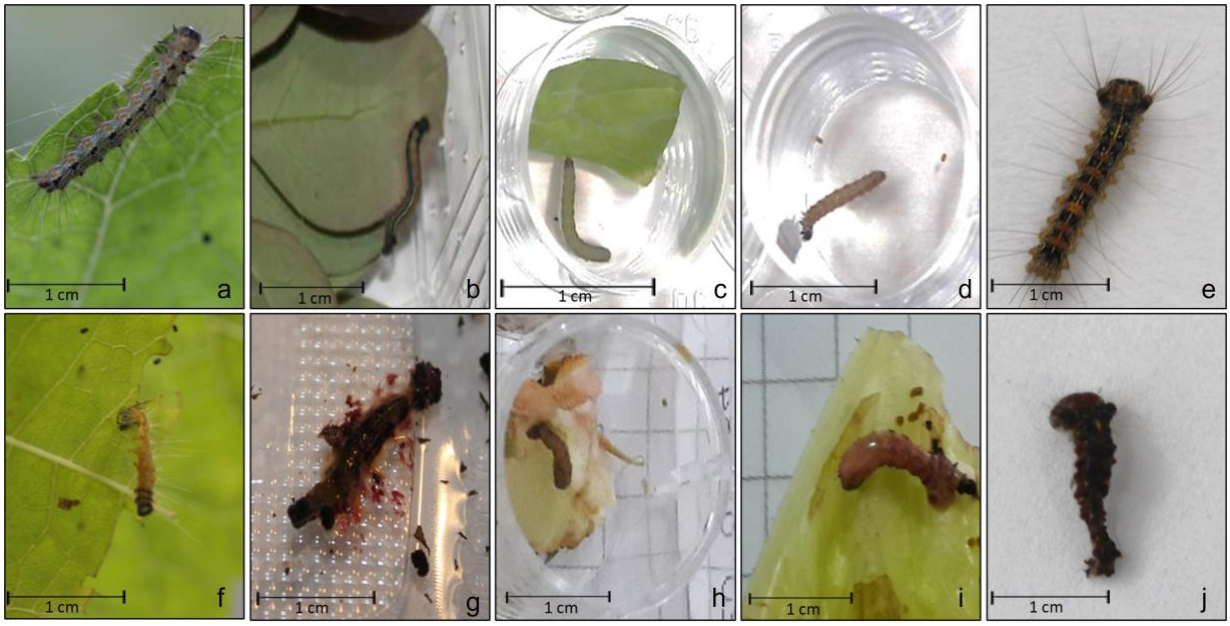

Figure 2. Pre- and post-*Hyphantria cunea* granulovirus treated larvae: a) *Hyphantria cunea*, b) *Malacosoma neustria*, c) *Spodoptera exigua*, d) *Helicoverpa armigera*, and e) *Lymantria dispar* healthy larvae; f) *Hyphantria cunea*, g) *Malacosoma neustria*, h) *Spodoptera exigua*, i) *Helicoverpa armigera*, and j) *Lymantria dispar* infected larvae.

Table 1. Median lethal concentrations (LC_{50}) of HycuGV on seven lepidopteran pests

Hosts	LC_{50} (OBs/ml)	Slope±*SE*	LC_{95} (OBs/ml)	*df*	X^2
Helicoverpa armigera	$7.7x10^9$ ($2.9x10^8$ - $2x10^{11}$)	0.324±0.521	$5.3x10^{15}$	3	0.847
Malacosoma neustria	$1.5x10^9$ ($4.3x10^7$ - $5.4x10^{10}$)	0.237±0.764	$2.9x10^{16}$	3	0.446
Lymantria dispar	$5.6x10^6$ ($5.6x10^5$ - $3.3x10^7$)	0.471±0.452	$4.0x10^{10}$	3	0.893
Spodoptera exigua	$7x10^7$ ($5.2x10^6$ - $9.3x10^8$)	0.345±0.578	$8.3x10^{12}$	3	0.970
Hyphantria cunea	$4.7x10^5$ ($5.5x10^4$ - $4x10^6$)	0.427±0.480	$4.7x10^9$	3	0.961

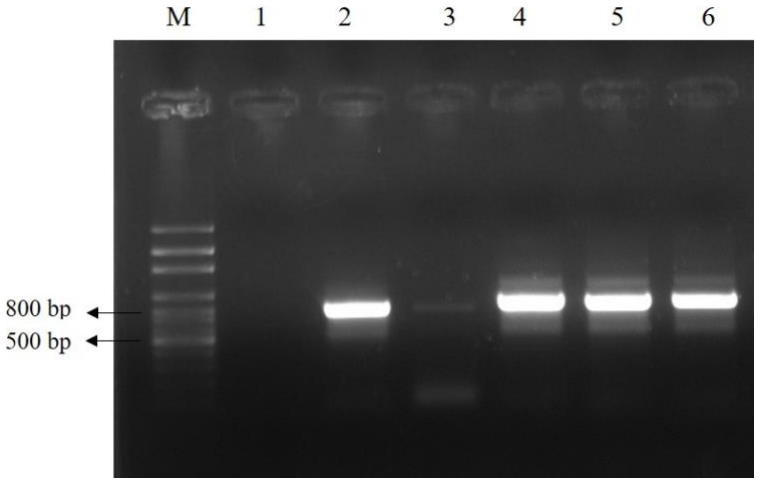

Figure 3. Agarose gel image of the granulin gene region (~800 bp) obtained from dead larvae by PCR. (M: 1 kb; 1: negative control; 2: positive control (from *Hyphantria cunea granulovirus* DNA sample); 3: from *Malacosoma neustria* cadaver; 4: from *Lymantria dispar* cadaver; 5: from *Spodoptera exigua* cadaver; 6: from *Helicoverpa armigera* cadaver).

Discussion

We conducted bioassays with *H. cunea*, *M. neustria*, *S. exigua*, *S. littoralis*, *C. pomonella*, *H. armigera* and *L. dispar* larvae to obtain a better insight into the of host range of HycuGV. Different rates of mortality were determined for *L. dispar*, *S. exigua*, *M. neustria* and *H. armigera*, but *S. littoralis* and *C. pomonella* were not susceptible to this virus. The mortality of *H. cunea* was higher than the others. Other studies have investigated the effects of HycuGV on some other hosts (Vasiljevic, 1968; Ignoffo, 1968; Hukuhara et al., 1969; Vaughn, 1974; Tomita & Ebihara, 1982). Vasiljevic (1968) examined the effect of GV from *H. cunea* on *Bombyx mori* (L., 1758) (Lepidoptera: Bombycidae) and *Pieris rapae* (L., 1758) (Lepidoptera: Pieridae) with HycuGV lethal to only *P. rapae* larvae. Vasiljevic (1968) also reported that HycuGV was infective *P. rapae* larvae but not to domestic *B. mori* larvae. Tomita & Ebihara (1982) tested HycuGV on eight species, *Euproctis pseudoconspersa* (Strand, 1923) (Lepidoptera: Erebidae), *Euproctis similis* (Füssli, 1775) (Lepidoptera: Erebidae), *Numenes disparilis* (Staudinger, 1887) (Lepidoptera: Erebidae), *Clostera anastomosis tristis* (L., 1758) (Lepidoptera: Notodontidae), *Diaphania pyloalis* (Hampson, 1859) (Lepidoptera: Crambidae), *B. mori*, *Spilarctia subcarnea* (Walker, 1855), *Lemyra imparilis* (Butler, 1877) (Lepidoptera: Erebidae) and *H. cunea*. The lepidopteran species, *S. subcarnea* and *L. imparilis*, in the same family (Erebidae) as *H. cunea*, were found to be susceptible to HycuGV. However, HycuGV was not lethal to *E. pseudoconspersa*, *N. disparilis albofascia*, *E. similis*, *C. anastomosis tristis*, *D. pyloalis* and *B. mori* (Tomita & Ebihara, 1982). In the present study, HycuGV provided useful control of *L. dispar* larvae, also in the Erebidae. Similarly, Hukuhara et al. (1969) reported that HycuGV was not lethal to *B. mori*. Ignoffo (1968) and Vaughn (1974) also reported that HycuGV is highly host specific.

Given these results, it is clear that the host range of HycuGV is not limited to a single species and this should be further investigated. In the present study, it was determined that the impact of HycuGV on *L. dispar* and *S. exigua* was higher than on *M. neustria* and *H. armigera*. Therefore, HycuGV could potentially be used as a biocontrol agent for *L. dispar* and *S. exigua*. HycuGV had some impact on *M. neustria* and *H. armigera,* however, but a higher dose was required for 50% mortality. Thus, the effect of HycuGV on these two insects was limited. No effect was observed on *S. littoralis* and *C. pomonella*. The two *Spodoptera* species used in this study had quite different mortality when exposed to HycuGV. HycuGV gave more than 80% mortality of *S. exigua* but did not affect *S. littoralis*. This may be because *S. littoralis* is a more highly resistant species. Some studies have shown that *S. littoralis* larvae are resistant to baculovirus infection (Riwkin et al., 2006; Ghulam et al., 2017).

GV infections have been recorded in more than 100 insects, but only in Lepidoptera (Murphy et al., 1995). Unlike NPVs, the host range of the GVs is narrower and mostly limited to a single species. In several studies, baculoviruses were tested in various species without any pathogenicity observed (Del Rincon-Castro & Ibarra, 1997). In the present study, it was determined that HycuGV was a weaker pathogen of *M. neustria*, *S. littoralis*, *C. pomonella* and *H. armigera* larvae than the species from which it was originally isolated. In contrast, however, *L. dispar* and *S. exigua* larvae were susceptible to HycuGV infection.

In conclusion, it was demonstrated that HycuGV was infectious and lethal to various lepidopteran species in addition to its source host. These results show that HycuGV has a wider host range confirming some earlier studies.

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References

Abbott, W. S., 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18 (2): 265-267.

Ahmad, I., F. Ahmad & J. Pichtel, 2011. Microbes and Microbial Technology: Agricultural and Environmental Applications. Springer, Media LLC, New York, 516 pp.

Bayramoglu, Z., R. Nalcacioglu, Z. Demirbag & I. Demir, 2018. Characterization of a Betabaculovirus from the fall webworm, *Hyphantria cunea* Drury. (Lepidoptera: Erebidae), in Turkey. Biocontrol Science & Technology, 28 (12): 1178-1190.

Brodeur, J., 2012. Host specificity in biological control: insights from opportunistic pathogens. Evolutionary Applications, 5 (5): 470-480.

Cory, J. S., 2003. "Ecological Impacts of Virus Insecticides: Host Range and Non-Target Organisms, 73-91". In: Environmental Impacts of Microbial Insecticides (Eds. M. T. Heikki, H. Hokkanen & A. Hajek). Springer, Dordrecht, 269 pp.

Cory, J. S. & P. F. Entwistle, 1990. Assessing the risks of releasing genetically manipulated baculoviruses. Aspects of Applied Biology, 24 (1): 187-194.

Cory, J. S., R. S. Hails & S. M. Sait, 1997. "Baculovirus Ecology, 301-339". In: The Baculoviruses (Ed. L. K. Miller). Plenum, New York, 450 pp.

Del Rincon-Castro, M. & J. E. Ibarra, 1997. Genotypic divergence of three single nuclear polyhedrosis virus (SNPV) strains from the cabbage looper, *Trichoplusia ni*. Biochemical Systematics and Ecology, 25 (4): 287-295.

Federici, B. A., 1997. "Baculovirus Pathogenesis, 39-59". In: The Baculoviruses (Ed. L. K. Miller). Plenum Press, New York, 450 pp.

Finney, D. J., 1952. Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve. Cambridge University Press, Cambridge, England, 256 pp.

Gencer, D., Z. Bayramoglu, R. Nalcacioglu, Z. Demirbag & I. Demir, 2020. Genome sequence analysis and organization of the *Hyphantria cunea* granulovirus (HycuGV-Hc1) from Turkey. Genomics, 112 (1): 459-466.

Ghulam, A., W. van der Wopke & J. M. Vlak, 2017. Biological and genetic characterization of a Pakistani isolate of *Spodoptera litura* nucleopolyhedrovirs. Biocontrol Science and Technology, 28 (1): 20-33.

Goulson, D., 2003. Can host susceptibility to baculovirus infection be predicted from host taxonomy or life history? Environmental Entomology, 32 (1): 61-70.

Gröner, A., 1986. "Specifity and Safety of Baculoviruses, 177-202". In: The Biology of Baculoviruses (Eds. R. R. Granados & B. A. Federici). CRC Press, Boca Raton, 275 pp.

Haase, S., A. Sciocco-Cap & V. Romanowski, 2015. Baculovirus Insecticides in Latin America: Historical Overview, Current Status and Future Perspectives. Viruses, 7 (5): 2230-2267.

Hamm, J. J., 1982. Extension of the host range for a granulosis virus from *Heliothis armiger* from South Africa. Environmental Entomology, 11 (1): 159-160.

Herniou, E. A. & J. A. Jehle, 2007. Baculovirus Phylogeny and Evolution. Current Drug Targets, 8 (10): 1043-1050.

Hukuhara, T., H. Aruga & M. Kobayashi, 1969. On the Granulosis of *Hyphantria cunea* Drury. Japanese Journal of Applied Entomology and Zoology, 13 (1): 1-4.

Ignoffo, C. M., 1968. Specificity of insect viruses. Bulletin of the Entomological Society of America, 14 (4): 265-276.

Jehle, J. A., M. Lange, H. Wang, Z. Hu, Y. Wang & R. Hauschild, 2006. Molecular identification and phylogenetic analysis of baculoviruses from Lepidoptera. Virology, 346 (1): 180-193.

Moscardi, F., 1999. Assessment of the application of baculoviruses for control of Lepidoptera. Annual Review of Entomology, 44 (1): 257-289.

Murphy, F. A., C. M. Fauquet, D. H. L. Bishop, S. A. Ghabrial, A. W. Jarvis, G. P. Martelli, M. A. Mayo & M. D. Summers, 1995. Virus Taxonomy: The Classification and Nomenclature of Viruses. Sixth Report of the International Committee on Taxonomy of Viruses, Springer-Verlag, Wien, 586 pp.

Opoku-Debrah, J. K., M. P. Hill, C. Knox & S. D. Moore, 2013. Overcrowding of false codling moth, *Thaumatotibia leucotreta* (Meyrick) leads to the isolation of five new *Cryptophlebia leucotreta* granulovirus (CrleGV-SA) isolates. Journal of Invertebrate Pathology, 112 (3): 219-228.

Riwkin, H., J. S. Kroemer, A. Bronshtein, E. Belausov, B. A. Webb & N. Chejanovsky, 2006. Response of immunocompetent and immunosuppressed *Spodoptera littoralis* larvae to baculovirus infection. Journal of General Virology, 87 (1): 2217-2225.

Rohrmann, G., 2013. Baculovirus Molecular Biology. Bethesda (MD), National Library of Medicine (US), National Center for Biotechnology Information, 154 pp.

Tomita, K. O. & T. Ebihara, 1982. Cross-transmission of the Granulosis virus of the *Hyphantria cunea* Drury (Lepidoptera: Arctiidae), to other Lepidopterous insect's species. Japanese Journal of Applied Entomology and Zoology, 26 (4): 224-227.

Vasiljevic, L., 1968. Utijac virusa granuloze dudovca (*Hyphantria cunea* DRURY) na gusenice svilene bube (*Bombyx mori* L.) i kupusara (*Pierris rapae* L.). Plant Protection Beograd, 98 (1): 29-40 (in Croatian with abstract in English).

Vaughn, J. L., 1974. "Virus and Richettsial Diseases, 59-62". In: Insect Diseases (Ed. G. L. Cantwell). Marcel Dekker, New York, 595 pp.