Diversity of Iberian nucleopolyhedrovirus wild-type isolates infecting *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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**A B S T R A C T**

Larvae of the tomato fruitworm, *Helicoverpa armigera*, were surveyed for nucleopolyhedrovirus (NPV) infection (referred to as HearNPV) in three different locations from the Iberian Peninsula: Olivenza and Toledo in Spain and the Oeste region in Portugal. Twenty HearNPV isolates were obtained from single field-collected larval cadavers. Restriction endonuclease (REN) profiles of the collected isolates with BglII and PstI allowed identification of six different *H. armigera* single-embedded NPV strains in Spain (referred to as HearSP3, HearSP4, HearSP5, HearSP6, HearSP7, and HearSP8) and two in Portugal (referred to as HearPT1 and HearPT2). No strains were shared by isolates from different geographical regions except HearSP5, which was found in isolates from Olivenza and Toledo. Cluster analysis based on the restriction fragment length polymorphisms of these strains in relation to two previously identified strains from Badajoz (HearSP1) and Cordoba (HearSP2) in Spain, showed no correlation among the strains and their geographical origin. The biological activity of HearSP2, HearSP4, HearSP7, HearSP8, HearPT1, and HearPT2 was compared in terms of pathogenicity (50% lethal concentration, LC50) and virulence (mean time to death). HearPT2 and HearSP7 were significantly more pathogenic than HearSP2, with LC50 values 2.8 and 2.6-fold higher than the latter, respectively, on *H. armigera* second instars. HearSP4 and HearPT2 killed larvae significantly faster than HearSP8, whereas HearSP2, HearSP7, and HearPT1 showed intermediate mean time to death values.

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1. Introduction

*Helicoverpa armigera* (Hübner), the tomato fruitworm, is an important pest of several crops all over the world except on the American continents (Fitt, 1989). In the Iberian Peninsula, it is a key pest on numerous crops including field-grown tomato, pepper, cotton and maize, protected (i.e., glass and plastic houses) vegetable and ornamental crops such as tomato, sweet pepper, lettuce, roses, and carnations, summer cole crops and strawberry (Torres-Vila et al., 2002; Figueiredo et al., 2006).

Environmental and food residue problems associated with chemical control tactics have encouraged the search for more sustainable pest control measures. Baculoviruses are considered as one of the most interesting insect biological control agents and very promising as biopesticides for their high specificity, virulence, ability to persist in the environment and compatibility with other beneficial natural enemies (parasitoids, predators and pathogens) and chemical pesticides (Harper, 1986). Baculovirus-based insecticides, therefore, have the potential to play an important role in integrated pest management (IPM) and organic farming programs. Given the prevalence of *H. armigera* populations showing resistance to a range of chemical insecticides that have already been described worldwide (McCaffery, 1998; Torres-Vila et al., 2002), baculovirus-based products would likely prove useful in Iberia for tomato fruitworm control in several crop systems in rotation with *Bacillus thuringiensis* Berliner (Bt), and other biorational pesticides. Moreover, the use of nucleopolyhedroviruses (NPVs) (Baculoviridae), other biorational and Bt in rotation could be a useful tool to retard the development of Bt resistance in pest populations (Raymond et al., 2006).

Genotypic variants of the same baculovirus, such as those commonly found at different geographical locations and sometimes even in the same host individual, have been reported to differ in their DNA restriction endonuclease (REN) profiles and also in their biological activity (Caballero et al., 1992; Takatsuka et al., 2003; Cory et al., 2005). These differences in pathogenicity and virulence due to natural intraspecific heterogeneity may represent an interesting tool to develop new baculovirus-based bioinsecticides (Moscardi, 1999).

Several NPVs of the single- (SNPV) and multiple-embedded nucleocapsid type (MNPV) have been isolated from *H. armigera* populations all over the world (Gettig and McCarthy, 1982; Figueiredo et al., 1999; Ogembo et al., 2005; Zhang et al., 2005). Some of them...
have been characterized thoroughly, including two genotypes purified from different Chinese isolates, and their genomes have been entirely sequenced (Chen et al., 2001; Zhang et al., 2005). A *H. armigera* NPV (HearNPV) isolated in the Chinese province of Hubei has been produced as a biopesticide and extensively used on cotton (Wang et al., 2001), whereas in Thailand, HearNPV isolates are produced locally for control of *H. armigera* in tomato, cotton, and tangerine (Jones et al., 1998). As the use of foreign NPV isolates can have a negative impact on the biological activity of native strains (Muñoz and Caballero, 2000) and different host biotypes can vary in their susceptibility to geographically distinct isolates (Milks, 1997), the selection of indigenous isolates that are suitable for development as biological control agents requires the characterization of the strains present in each geographical region.

Two NPV isolates from the Iberian Peninsula were identified previously (Figueiredo et al., 1999) and characterized as single-embedded NPVs (SNPVs) (Arrizubieta et al., unpublished data). In our present study, larvae of the tomato fruitworm were surveyed for NPV infection in several locations of the Iberian Peninsula. The collected isolates were compared genetically by restriction fragment length polymorphism (RFLP), and biologically by determining their pathogenicity and mean time to death (MTD). In addition, the RFLP profiles of the different distinct isolates were used for a cluster analysis of the isolates.

2. Materials and methods

2.1. Insect rearing

The *H. armigera* population was maintained at 25 ± 2 °C, 70–80% relative humidity and 16 h:8 h day–night photoperiod on a semi-synthetic diet based on that described by Poitout and Bues (1974).

2.2. Helicoverpa armigera larval sampling and virus isolation

NPV isolates were originally obtained from *H. armigera* larvae with clear signs of patent NPV disease collected in 1999 in two sweet field maize (maize plants and wild host plants) from Olivenza and Toledo (Spain) and in field tomatoes and tomatoes and sweet peppers grown in greenhouses from Oeste (Portugal) (Table 1). Larvae were collected individually into 1.5 ml plastic Eppendorf tubes except some of the samples from Oeste (3–4 larvae per tube), and preserved at −18 or 4 °C in the dark until diagnosis was done. Each sample was macerated in double distilled water and the presence of occlusion bodies (OBs) was verified by phase contrast microscopy of the homogenated tissue at 400×. A total of 49 samples were examined (16 from Spain and 33 from Portugal).

2.3. OB amplification and purification

OB samples were multiplied in groups of 10–20 third instars of *H. armigera*. Only one virus sample was produced at each time to prevent cross-contamination. For this, larvae were starved for at least 12 h and then fed diet plugs that had been surface-contaminated with crude extracts from homogenated NPV-infected larvae. Larvae were reared individually on semi-synthetic diet until death or pupation. Infected larvae were frozen as soon as they died. To purify OBs, dead larvae were macerated in 300 μl distilled water, filtered twice through muslin-like tissue and centrifuged at 1600 g for 5 min at room temperature. Pellets were resuspended in 1 ml water and 0.5 ml of 0.1% sodium dodecyl sulphate (SDS) and centrifuged for 5 min at 1600 g. These pellets were resuspended in 0.3 ml of 0.1% SDS and the suspensions carefully placed on 0.5 ml of a 40% sucrose cushion and centrifuged at 23,000 g during 30 min, resuspended in 1 ml of 0.1% SDS, centrifuged at 4000 g for 5 min and washed twice in distilled water. When necessary, the suspensions were briefly sonicated to break up OB aggregates. Pure OB pellets were finally resuspended in 0.3 ml distilled water and stored at −20 °C for further use. Isolate identity was confirmed after each multiplication by REN digestion and agarose gel electrophoresis (as described in section 2.4 below). Previously identified strains from Spain (HaSP1 and HaSP2, referred as HearSP1 and HearSP2, respectively, in this paper for consistency with ICTV nomenclature) (Figueiredo et al., 1999) and the HearNPV Chinese strain G4 (HearG4 in this paper), whose genome has been completely sequenced (Chen et al., 2001) and was kindly provided by Dr. van Oers (University of Wageningen, The Netherlands), were also multiplied following identical procedures.

2.4. Viral DNA extraction and REN analysis

Purified OB suspensions were incubated with one-third volume of 3× DAS (0.3 M Na2CO3 + 0.5 M NaCl + 0.03 M EDTA, pH 10.5) at 37 °C for 5 min to dissolve the polyhedrin matrix. Heavy particles including undissolved OBs were pelleted by 6000 g centrifugation for 5 min. To rupture virion and nucleocapsid envelopes, virion-containing supernatants were transferred to sterile 1.5 ml vials and incubated at 45 °C with 500 μg/ml proteinase K during 2.5 h and a further 30 min with 1% SDS. Viral DNA was extracted once with an equal volume of TE-buffer-saturated phenol and then at least twice with an equal volume of phenol–chloroform–isoamyl alcohol (25:24:1) or with chloroform until the interface became completely clean. DNA was precipitated with 10% (v/v) 3 M sodium acetate (pH 5.2) and twice the volume of ice-cold 96% ethanol at −20 °C for 15 min; it was washed with 70% cold ethanol and centrifuged for 5 min. DNA pellets were resuspended in 10 mM Tris–HCl pH 8.0 and kept at 4 °C until use.

For REN digestion, 1–2 μg of viral DNA was incubated with 1 U of BglII and PstI according to the supplier’s instructions, at 37 °C for 4–12 h. These RENs were chosen as they allowed proper differentiation of HearNPV strains in previous studies (Figueiredo et al., 1999). Reaction was stopped by the addition of one-sixth volume of loading buffer (0.25% bromophenol blue; 40% sucrose, w/v). Fragments were separated by electrophoresis carried out in 0.7% TAE agarose gels containing 0.25 μg/ml ethidium bromide at 20–30 V for ca. 14 h and visualized on a Gel Doc Imaging System (Bio-Rad, Alcobendas, Spain). DNA fragment sizes were estimated by comparison to a standard molecular weight marker (bacteriophage λ genomic DNA digested with HindIII) with the aid of Quantity One 4.6 (Bio-Rad). HearSP1 fragments were labelled alphabetically (from A to M with BglII and from A to F for PstI) from higher to lower molecular weight using upper case letters.

### Table 1

<table>
<thead>
<tr>
<th>Collection sites</th>
<th>No. diseased larvae</th>
<th>No. isolates</th>
<th>No. HearNPV strains (identified strains)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olivenza (Spain)</td>
<td>9</td>
<td>2</td>
<td>(HearSP1 and HearSP2)</td>
</tr>
<tr>
<td>Toledo (Spain)</td>
<td>10</td>
<td>2</td>
<td>(HearSP1 and HearSP2)</td>
</tr>
<tr>
<td>Oeste (Portugal)</td>
<td>32</td>
<td>8</td>
<td>(HearPT1, HearPT2)</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>
polymorphic fragments appearing in the rest of the isolates were
labelled with lower case letters referring to the closest fragment of
HearSP1 of equal or upper molecular weight followed by a sub-
index that distinguished polymorphic fragments with the same
minor case letter but different molecular weight.

2.5. Cluster analysis

A matrix of presence/absence of RFLP fragments was generated
based on BglII and PstI restriction profiles and this was used to
calculate a similarity matrix based on the Dice similarity coefficient
using SPSS 12.0. This matrix was used in a hierarchical cluster anal-
ysis utilizing the UPGMA (unweighted pair group method analysis)
to construct a dendrogram using NTsys-PC 2.01b (Rohlf, 1997).
The confidence level of this dendrogram was estimated by bootstrap
analysis with Winboot (Yap and Nelson, 1996) with 5000 bootstrap
replications. These analyses included all the observed submolar
bands in the isolates since they usually belong to the most domi-
nant genotype present in the virus population (Muñoz et al.,
1998; Muñoz and Caballero, 2000; Simón et al., 2004).

2.6. Bioassays

A total of eight novel Iberian strains were identified by REN:
HearNPV-SP3 (HearSP3), HearNPV-SP4 (HearSP4), HearNPV-SP5
(HearSP5), HearNPV-SP6 (HearSP6), HearNPV-SP7 (HearSP7), Hear-
NPV-SP8 (HearSP8), HearNPV-PT1 (HearPT1) and HearNPV-PT2
(HearPT2). Viral pathogenicity expressed as 50% lethal concentra-
tion (LC50) and MTD were determined for HearSP4, HearSP7,
HearSP8, HearPT1, and HearPT2. These strains were selected for
testing because they showed the most distinct REN profiles. The
HearSP2 strain was included as a reference.

To determine concentration–mortality responses, five different
viral concentrations which were found to cause 5–95% mortality in
second instars in preliminary assays were used: 1 × 10^3, 1 × 10^4,
1 × 10^5, 1 × 10^6, 1 × 10^7, and 1 × 10^8 OBs/ml. OB concentrations were deter-
mined with an improved Neubauer hemocytometer under a phase
contrast microscope at 400×. Newly molted second instars from
the laboratory colonies were individually separated to prevent can-
nibalism and starved for about 12 h at 20°C. They were then allowed
to drink viral suspensions containing 10% sucrose and 0.05% (w/v)
of the food dye, Fluorella Blue, by using the droplet-feeding method
(Hughes and Wood, 1986). Groups of 25–30 larvae were used per
concentration and viral isolate, and inoculated insects were individu-
ally transferred to 25-well plates containing maize diet plugs. As
controls, larvae were allowed drinking a water solution containing
only the blue dye and sucrose at the same concentrations. Larvae
were incubated at 25 ± 1°C, 70 ± 5% relative humidity and 16.8 h
day–night photoperiod. Mortality was recorded every 24 h for
10 days. The experiment was replicated four times and about 100
larvae were used in total per concentration and viral isolate.

To determine time–mortality responses, similar bioassays were
performed. For these, only the concentration causing the highest
mortality was used (1 × 10^6 OBs/ml). Mortality was recorded at
8 h intervals for 10 days. Larvae that did not die during the assay
were not included in the analysis. Similarly, replicates in which
90% mortality was not achieved after applying Abbott’s correction
for control mortality, were also discarded.

2.7. Statistical analysis

Concentration–mortality data were subjected to Probit analysis
(Finney, 1971) using the POLO-PC program (LeOra Software,
1987). The relative potencies were estimated and compared as described
by Robertson and Preisler (1992). Time–mortality results of indi-
viduals that died due to NPV infection by different isolates were
subjected to Weibull analysis (Aitkin et al., 1989). The validity of
the Weibull model was determined using the Kaplan macro pre-
sent in the GLIM program.

3. Results

3.1. Identification of NPV strains present in Iberian isolates

The number of field-collected larvae from the Iberian Peninsula
with baculovirus infection symptoms and the number of larvae in
which the presence of OBs was detected by microscopic inspection is
presented in Table 1.

From the seven field-collected isolates from Olivenza, Spain
(Table 1), five different REN profiles were obtained with both BglII
and PstI. Similarly, the three isolates from Toledo, Spain and the
ten isolates from Oeste, Portugal, each exhibited two distinct REN
profiles. In total, eight distinctive strains were obtained by combing
the isolates BglII and PstI profiles from all locations (Fig. 1).
Seven of these strains (HearSP3, HearSP4, HearSP5, HearSP7,
HearSP8, HearPT1, and HearPT2) could be differentiated based on
their BglII profiles alone (Fig. 1b). An additional isolate, HearSP6,
which showed a BglII profile identical to HearSP5 could be distin-
guished from all the others with PstI (Fig. 1c). All the different iso-
lates sharing the same HearNPV strain came from the same origin
(two isolates from Olivenza consisting of HearSP7; two from Tole-
do of HearSP8, two from Oeste of HearPT1 and eight from Oeste of
HearPT2) except HearSP5, which was found in two isolates from
Olivenza and one isolate from Toledo.

All Iberian strains exhibited the BglII fragments A, I, J, K, and M
as well as the PstI fragments A, B, C, E, F, G, H and L and the PstI fragment D
varied among the strains (Fig. 1b, c, and d). The BglII fragments b1,
f1, g1, h1, i1, i2, j1, and PstI fragment d1 have strain recognition va-

The Chinese HearG4 genotype displays a BglII profile in which only four out of 13 fragments show the same molecular weight to those of HearSP1 (Fig. 1a) but its PstI profile is identical to that of the Iberian strains (not shown).

3.2. Cluster analysis of the Iberian HearNPV strains

The strains presented some polymorphism (Dice coefficient
≥0.69). The cophenetic correlation (correlation between the
cophenetic value matrix and the similarity matrix upon which the
clustering was based) was equal to 0.90 (Mantel t-test = 6.37),
revealing a good fit for the cluster analysis. Some of the strains
were more closely related to geographically distant strains than
to those collected in the same location, as observed by the cluster
dendrogram (Fig. 2). In fact, taking into account a similarity thresh-
old of 87%, four groups can be identified: (1) HearSP1, HearSP2,
HearPT1, and HearPT2, (2) HearSP3, HearSP5, HearSP6 and
HearSP7, (3) HearSP8, and (4) HearSP4. The HearSP4 and HearSP8
strains were the most different and, even at lower estimated sim-
ilarities (77%), they continued to be separated from all the others.

The Chinese HearG4 genotype displayed a BglII profile identical to
that of the Iberian strains (not shown).
and HearSP7 at the same Dice value (Fig. 2). This is also the position at which the UPGMA cluster analysis places HearPT1 when submolar bands are not considered (dendrogram not shown).

3.3. Biological activity of six Iberian HearNPV strains

Regressions lines for all six virus strains fitted the Probit model well ($\chi^2 = 29.21, df = 10; p = 0.001$) but they could not be fitted in parallel ($\chi^2 = 15.64; df = 5; p = 0.008$). The LC50 ratios indicated that HearSP7 and HearPT2 were 2.6- and 2.8-fold more pathogenic, respectively, than HearSP2 which showed the highest LC50 values. The LC50 values of HearSP4, HearSP8, and HearPT1 were statistically similar to that of HearSP2 (Table 2).

The different strains killed between 95.5 and 97.7% of the treated second instars and in a time range of 64-160 hpi (hours post-infection), with the exception of four larvae that died later (176-232 hpi). The MTD values for the different strains ranged from 98.4 hpi (HearPT2) to 115.2 hpi (HearSP8). HearSP4 and HearPT2 both killed larvae significantly faster than HearSP8, whereas HearSP2, HearSP7 and HearPT1 showed intermediate MTD values that were not significantly different from those of HearSP4, HearPT2 or HearSP8 (Fig. 3). The instantaneous risk of death increased over time (shape parameter of the Weibull distribution $\alpha = 3.87$). Time-mortality distribution curves peaked twice: once at 72-80 hpi and again at 112-120 hpi (data not shown).

4. Discussion

The REN profiles of all the strains analyzed in this work were very similar but not identical to those of the HearNPV isolates described by Figueiredo et al. (1999), indicating that they constitute novel HearNPV strains. The novel strains represented 40% of the to-

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**Fig. 1.** REN profiles of *Helicoverpa armigera* nucleopolyhedrovirus [HearNPV-G4 (G4)] and of the ten distinct genotypic Iberian strains of HearNPV [HearSP1 (SP1), HearSP2 (SP2), HearSP3 (SP3), HearSP4 (SP4), HearSP5 (SP5), HearSP6 (SP6), HearSP7 (SP7), HearSP8 (SP8), HearPT1 (PT1) and HearPT2 (PT2)] obtained with BglII (a and b) and PstI (c). DNA digested with HindIII was used as a molecular weight marker and fragment sizes in base pairs (bp) are displayed above the marker fragments or to the right of the HearG4 fragments. A line diagram of the REN profiles is displayed (d) to show more clearly hypermolar (thicker lines), equimolar or submolar (dotted lines) fragments and to include fragment nomenclature to the left of each profile: capital letters were designated for the Iberian type strain (HearSP1) profile; and small letters followed by a number for the polymorphic fragments appearing in different strains.
tal number of isolates collected (larval cadavers where OBs were observed), but this figure may not be a realistic representation of field diversity, as no systematic effort at sampling was performed. Two different strains were identified in each of the three geographical regions surveyed, indicating a high variability of variants within each collection site. This is probably because the analysis was performed from individual cadavers, which increases the chances of quantifying the diversity present compared to analyses based on isolates composed of pooled groups of larval cadavers. Also, all isolates sharing the same HearNPV strain came from the same origin, except HearSP5, which was found in two isolates from Olivencia and one isolate from Toledo. It is fairly common to find the same strain in isolates from different origins, especially from sites geographically so close (Laitinen et al., 1996; Cooper et al., 2003; Graham et al., 2004).

Interestingly, intraspecific diversity was detected among the eight HearNPV strains identified. Within strains HearSP3, HearSP4 and HearPT1, a mixture of different genotypic variants was detected, as indicated by the presence of submolar bands in their corresponding REN profiles. These results suggest that insects were not infected by single variants but rather a mixture of them in a single isolate. This is a common phenomenon in baculovirus wild-type isolates, which are usually formed by a mixture of genotypes in distinct proportions (Muñoz et al., 1998; Murillo et al., 2001; Kamiya et al., 2004) even when obtained from single host individuals (Cherry and Summers, 1985; Graham et al., 2004; Cory et al., 2005), as occurred in our study. Furthermore, the absence of submolar bands is not a guarantee of the presence of a single genotype within the isolate (Vickers et al., 1991).

Some strain groups were highly similar with a relatively high bootstrap value. These strains were also placed together using other methods of classification (nearest neighbor, average linkage between groups or within groups – data not shown). It should be noted that the cluster analysis presented here was based on

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**Table 2**

Concentration–mortality responses for six *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) strains on second instar *H. armigera* larvae.

<table>
<thead>
<tr>
<th>Straina</th>
<th>Intercept ± SE</th>
<th>Slope ± SEb</th>
<th>( \chi^2 )</th>
<th>( p )</th>
<th>( \text{LC}_{50} ) (OBS/ml) c</th>
<th>Potencyd</th>
<th>95% Fiducial limits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>HearSP2</td>
<td>-4.87 ± 0.65</td>
<td>1.07 ± 0.13 b</td>
<td>2.32</td>
<td>0.51</td>
<td>34897</td>
<td>1.00 a</td>
<td>–</td>
</tr>
<tr>
<td>HearSP4</td>
<td>-3.85 ± 0.49</td>
<td>0.87 ± 0.10 ab</td>
<td>2.74</td>
<td>0.43</td>
<td>26149</td>
<td>1.34 a</td>
<td>0.78</td>
</tr>
<tr>
<td>HearSP7</td>
<td>-3.40 ± 0.43</td>
<td>0.82 ± 0.09 ab</td>
<td>2.06</td>
<td>0.56</td>
<td>13399</td>
<td>2.59 b</td>
<td>1.55</td>
</tr>
<tr>
<td>HearSP8</td>
<td>-3.08 ± 0.39</td>
<td>0.71 ± 0.08 a</td>
<td>2.25</td>
<td>0.52</td>
<td>20043</td>
<td>1.74 ab</td>
<td>0.69</td>
</tr>
<tr>
<td>HearPT1</td>
<td>-4.66 ± 0.57</td>
<td>1.09 ± 0.12 b</td>
<td>1.56</td>
<td>0.67</td>
<td>18687</td>
<td>1.86 ab</td>
<td>0.96</td>
</tr>
<tr>
<td>HearPT2</td>
<td>-3.13 ± 0.39</td>
<td>0.77 ± 0.08 ab</td>
<td>2.35</td>
<td>0.53</td>
<td>12327</td>
<td>2.83 b</td>
<td>1.44</td>
</tr>
</tbody>
</table>

Probit regression (pooled data from four replications).

a HearNPV isolates from Spain and Portugal. See text and Table 1 for origin of isolates.

b SE, Standard error; slopes followed by the same letter were not different at \( p = 0.05 \).

c OBs, Occlusion bodies.

d Potency (i.e., lethal concentration ratios with respect to that of HearSP2) followed by the same letter were not different at \( p = 0.05 \).
presence/absence of fragments with similar electrophoretic mobility and not in the similarity/divergence of the genome. Therefore, the strain clustering data can only be considered as an approximation of phylogenetic relatedness.

Genotype phylogeny was not found to be associated with geographical origin; the most related genotypes were not always the ones from the same or closely located sites. REN analysis of several Heliothinae NPVs verified that some strains were more closely related to a geographically distant strain than to those originating from a closer location (Gettig and McCarthy, 1982; Figueiredo et al., 1999), which is not unexpected due to the high migration abilities of Heliothinae species (Fitt, 1989). Migrations allow the transport of associated NPVs over long distances and those originating from a closer location (Gettig and McCarthy, 1982; Figueiredo et al., 1999), which is not unexpected due to the high migration abilities of Heliothinae species (Fitt, 1989). Migrations allow the transport of associated NPVs over long distances and those originating from a closer location (Gettig and McCarthy, 1982; Figueiredo et al., 1999), which is not unexpected due to the high migration abilities of Heliothinae species (Fitt, 1989). However, HearNPV populations may be dispersed by insectivorous birds (Entwistle et al., 1993) and larval parasitoids (Young and Yearian, 1989). Such organisms are very frequent in the Portuguese and Spanish fields and greenhouses as natural enemies of noctuids (Meierrose et al., 1989; Figueiredo and Mexia, 2000). On the other hand, the well known variability of NPV genomes contributes to differentiate closely located genotypes. The high number of common per site and the near absence of common strains among sites reveal the high genomic plasticity of HearNPV (Meierrose et al., 1989; Figueiredo and Mexia, 2000). On the other hand, the well known variability of NPV genomes contributes to differentiate closely located genotypes. The high number of common per site and the near absence of common strains among sites reveal the high genomic plasticity of HearNPV (Meierrose et al., 1989; Figueiredo and Mexia, 2000). Without a doubt, intrapopulation diversity within a single host is ecologically relevant as the larva represents the basic unit of virus inoculum (Cooper et al., 2003). In addition, the maintenance of diversity may be an important mechanism for virus adaptation and survival under different conditions (e.g., host density, host resistance, host plant), and therefore, represent an evolutionary advantage for the virus (Cory and Myers, 2004).

Concentration–mortality assays resulted in significant differences among the Iberian HearNPV strains; LC50 values differed by 2.5-fold among strains. This level of heterogeneity in HearNPV and suggest that, along with NPV strain dispersion, genotypic selection also occurs within individual larvae, as suggested by Graham et al. (2004). Without a doubt, intrapopulation diversity within a single host is ecologically relevant as the larva represents the basic unit of virus inoculum (Cooper et al., 2003). In addition, the maintenance of diversity may be an important mechanism for virus adaptation and survival under different conditions (e.g., host density, host resistance, host plant), and therefore, represent an evolutionary advantage for the virus (Cory and Myers, 2004).

Differences among strains in the mean speed to kill varied only moderately since the difference between the slowest and fastest virus was only of about 16 h. The MTD values obtained for the Iberian isolates were similar to those reported for the fastest-killing Heliothinae SNPV isolates studied by Hughes et al. (1983), which included HearNPV strains from South Africa and Australia, a Helicoverpa zea SNPV from the US (Elcar®), a Helicoverpa punctigera SNPV from Australia, and a Heliotris virescens SNPV from the US. In fact, Hughes et al. (1983) verified that the most virulent isolates had lethal time (LT50) values ranging from 72.8 to 79.6 h in neonates. In contrast, the MTD values obtained in our study seemed to be lower than those obtained by Ogembo et al. (2005), who reported MTD values of 6.5 days for second instars inoculated with similar concentrations. MTD values were also lower than those obtained by Teakle et al. (1985), who reported a LT50 value of 4.1–4.5 days for second instars. In contrast, our results were more similar to those of Williams and Payne (1984) with neonates and third-instar larvae.

The strategy to control H. armigera will probably be based on inundative applications of a microbial insecticide using a highly virulent strain/variant. Therefore, strains HearPT2, HearPT1, and HearSP7 seem to be the best candidates for potential insecticidal formulations, although extensive field evaluation is required.

Finally, these results can contribute to understand how HearNPV varies spatially in natural insect populations. This information is important in understanding baculovirus–insect evolution, design pest control programs, and in assessing the environmental impact of viral releases (Cory et al., 2005). Further analysis of the genotypic composition of these strains and their individual biological activities as well as comparison with strains from other regions of Iberia, North Africa and France, the immediate migration areas for the Iberian H. armigera populations, would give a broader picture of the ecology and evolution of this virus.

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