Selection of a Nucleopolyhedrovirus for Control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae): Structural, Genetic, and Biological Comparison of Four Isolates from the Americas

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ABSTRACT *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is the principal pest of maize in tropical and subtropical regions of the Americas. Larvae of this species are susceptible to a nucleopolyhedrovirus (NPV) which has attracted interest as a potential biocontrol agent. Four strains of NPV isolated from infected *S. frugiperda* larvae in the United States, Nicaragua, and Argentina were subjected to a structural, genetic, and biological comparison to select a candidate isolate for use in biocontrol experiments in Mexico and Honduras. All isolates had an occlusion body polyhedrin protein of 32 kDa, but the virions of each isolate differed subtly in the pattern and abundance of certain structural polypeptides revealed by SDS-PAGE analysis. Restriction endonuclease analysis of viral DNA confirmed that these isolates were strains of a single virus species but showed that they were not genetically homogeneous; each isolate could be differentiated from the others using common restriction enzymes. Droplet feeding bioassays indicated that an isolate from Nicaragua (Sf-NIC) and an isolate from the United States (Sf-US) had the highest infectivity when tested against 2nd instars originating from a Honduran *S. frugiperda* colony. No significant differences were detected in the speed of kill of Sf-NIC (102.7 h), Sf-US (102.3 h), and Sf-AR (103.4 h), whereas that of Sf-2 (97.3 h) was significantly shorter. Additional bioassays of the Sf-NIC isolate against 2nd to 6th instars demonstrated that LC_{50} values increased with larval stage from 2.03×10^5 OBs/ml for 2nd instars to 1.84×10^8 OBs/ml for 5th instars. The concentration required to elicit a lethal infection of 6th instars was so high that a reliable estimate of LC_{50} could not be obtained. The mean time to death for each stage challenged with the Sf-NIC isolate increased with instar from an average of 102.7 h in 2nd instars to 136.9 h in 5th instars.

KEY WORDS *Spodoptera frugiperda*, nucleopolyhedrovirus, baculovirus, geographic isolates, bioinsecticide

THE FALL ARMYWORM, *Spodoptera frugiperda* (J. E. Smith), is widely distributed in the tropical and subtropical regions of the Western Hemisphere (Sparks 1979, Andrews 1980). This insect occurs as a pest throughout much of Latin America, inflicting substantial losses to many agricultural crops of the family Graminae, particularly maize (Sparks 1979). Control of the pest typically requires 2–4 applications of chemical insecticides during the crop cycle and represents a major economic input involved in maize production (Hruska and Gladstone 1987). An attractive alternative for control of *S. frugiperda* is the integration of common control practices with the management of natural enemies, especially parasitoids and entomopathogens that attack the juvenile stages (Gardner and Fuxa 1980, Ashley 1986).

S. frugiperda is susceptible to a nucleopolyhedrovirus (NPV) that has been reported to be one of the most prevalent entomopathogens in natural populations (Gardner and Fuxa 1980) and is widely distributed throughout the Americas (Shapiro et al. 1991). The potential role of this virus as an alternative for the management of *S. frugiperda* has been recognized (Fuxa 1991).

Restriction endonuclease (REN) analysis is an efficient and relatively simple method for identification of baculoviruses and is considered as an important tool to distinguish NPV isolates (Lee and Miller 1978). This method has demonstrated that genetic differences can be detected among *S. frugiperda* MNPV (SfMNPV) isolates from different regions or countries (Loh et al. 1982), among isolates within a single locality (Shapiro et al. 1991), and even among strains within a single host (Maruniak et al. 1984, Garcia-Maruniak et al. 1996). Phenotypic variation among different isolates also has been reported for many NPVs in terms of

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survival time (Hughes et al. 1983), pathogenicity (Williams and Payne 1984, Caballero et al. 1992), and host range (Bilimoria 1983). Information on susceptibility of *S. frugiperda* to NPV with reference to insect migration also is available (Fuxa 1987).

In the study of host–baculovirus interactions, it is well recognized that factors such as host stage and the geographic origins of both virus and host can affect the characteristics of the dose–response curve and the period of survival of infected hosts (Smits and Vlak 1988, Milks 1997). There is little or no information on the effect of host stage on susceptibility of *S. frugiperda* to SfMNPV. Moreover, there are relatively few studies that have examined virulence in terms of both host susceptibility and lethal time responses for a range of larval stages (Smits and Vlak 1988). Most of the larval instars of *S. frugiperda* may be present at any one time in the field because of overlapping generations, making data on stage-related virulence of practical importance.

The aim of our study is to select an NPV isolate for use against *S. frugiperda* in a control program designed for small-scale maize growers in Mexico and Central America. The specific objectives of this work were to determine the biochemical identity and relationship between 4 geographical SfMNPV isolates, to compare the responses of *S. frugiperda* 2nd instars to these 4 MNPVs, and to determine the virulence of one highly infective isolate from Central America in each larval instar.

Materials and Methods

Insect Source and Rearing. *S. frugiperda* pupae were obtained from the 2nd generation of a culture which was started with larvae collected in a corn field at El Zamorano, Honduras, in 1997, and maintained on artificial medium (Greene et al. 1976) at ambient laboratory temperatures in the Escuela Agricola Panamericana, El Zamorano. In Spain, the colony was continuously maintained in a growth chamber at 26 ± 1°C, 85% RH, and a photoperiod of 16:8 (L:D) h using a semisynthetic diet (Poitout and Bues 1974). From this regular source of hosts, we selected newly molted 2nd, 3rd, 4th, or 5th instars for use in bioassays. Insects used in the bioassays had been reared in our laboratory for 5 mo.

Virus Isolates and Amplification. NPV isolates were obtained from 4 locations. The plaque-purified SfMNPV-2 isolate (hereafter referred to as Sf-2) was kindly provided by J. E. Maruniak, University of Florida (Maruniak et al. 1984), and a wild isolate from Louisiana (hereafter named Sf-US) was received as a polyhedra suspension from J. R. Fuxa, Louisiana State University Agricultural Center (Fuxa 1987). An Argentinean isolate (hereafter named Sf-AR) was obtained from M. L. Vera, San Miguel de Tucumán, Argentina (Vera et al. 1995), and a Nicaraguan isolate (named Sf-NIC) was obtained from P. Castillo, Universidad Nacional Autónoma de Nicaragua, León, Nicaragua. All of these isolates represented viruses originally collected from infected *S. frugiperda* larvae in the field, except Sf-2 which was a plaque-purified variant derived from a wild-type stock.

Each virus isolate was amplified by feeding *S. frugiperda* early 4th instars with diet contaminated on the surface with the appropriate virus. Only 1 virus isolate was produced at a time to prevent cross-contamination, and an aliquot of each inoculum was kept to confirm the fidelity of the amplification by restriction endonuclease analysis (described later).

Virus Purification and Virion Extraction. Viral occlusion bodies (OBs) were extracted from dead diseased larvae by trituration in water and purified by filtration and differential centrifugation as described previously (Caballero et al. 1992). The OBs were resuspended in double-distilled water, and the concentration was determined using a Thoma counting chamber (Hawksley, Lancing, United Kingdom) under phase-contrast microscopy. Virions were released from OBs by incubation with DAS buffer (0.3 M Na_2CO_3, 0.5 M NaCl, 0.03 M EDTA, pH 10.5) at room temperature for 5 min. Undissolved OBs were removed by low-speed centrifugation (1,480 x *g*) for 3 min at 4°C. The supernatant was centrifuged through a 36:56% (wt:wt) continuous sucrose gradient at 40,320 × *g* at 4°C for 120 min. The multiple bands containing polyhedra-derived virions were collected, washed twice with double-distilled water, and finally resuspended in TE (10 mM Tris/HCl, 1 mM EDTA, pH 8) buffer. Virions were stored at 4°C until used for DNA isolation or protein analysis.

Isolation and Analysis of Viral DNA. Gradient-purified enveloped virions in TE buffer were incubated at 45–50°C with 200 [mg]g/ml of proteinase K for 2.5 h and then with 1% (wt:vol) sodium dodecyl sulfate (SDS) for an additional 0.5 h at the same temperature. Viral DNA was extracted once with TE buffer-saturated phenol and then several times with TE buffer-saturated phenol-chloroform-isoamyl alcohol (25:24:1) until the interface was completely clean. The aqueous phase containing the viral DNA was dialyzed at 4°C for 48 h against 3 or 4 changes of 10 mM TE buffer. The DNA concentration was determined by reading the optical density at 260 nm. For REN analysis, 1 μg of DNA was incubated with 10 U of one of the restriction enzymes *Hin*dIII, *Pst*I, *Bgl*II, or *Bam*HI (Amersham, Cardiff, United Kingdom). These enzymes were chosen because they allowed the best comparison with DNA profiles of previously characterized SfMNPV isolates. Reactions were stopped by the addition of loading buffer (0.25% [wt:vol] bromophenol blue, 40% [wt:vol] sucrose in water). Electrophoresis was performed using horizontal 0.8% agarose gels in TAE buffer (0.04 M Tris-Acetate, 0.001 M EDTA, pH 8.0). DNA fragments were visualized by staining with ethidium bromide.

Viral Polypeptide Analysis. Polyhedra and virions of the 4 SfMNPV isolates were solubilized in sample buffer (125 mM-Tris/HCl, pH 6.8, 2% (wt:vol) glycerol, 0.0001% (wt:vol) bromophenol blue, and 5% (vol:vol) 2-mercaptoethanol) by boiling for 3 min. Electrophoresis was carried out in a 12.5% (wt:vol) polyacrylamide gel at 20 mA for ≈2 h (Laemmli 1970). After electrophoresis, gels were stained for 40–50 min

in a solution containing 25% (vol:vol) methanol, 10% (vol:vol) acetic acid, and 0.12% (wt:vol) Brilliant Blue R250, and destained in 7.5% (vol:vol) methanol and 5% (vol:vol) glacial acetic acid. Mark 12 wide-range protein standards (Novex, San Diego, CA) were used as molecular weight markers.

Bioassay Procedures. Bioassays for all instars were carried out using the droplet-feeding method of Hughes and Wood (1981). First, the insecticidal activity of each SfMNPV isolate was determined for 2nd instars in terms of LC_{50} and LT_{50}. A 2nd experiment involved determining the responses of 2nd, 3rd, 4th, and 5th instars of *S. frugiperda* to the isolate from Nicaragua. This isolate was selected for further study because of its high infectivity and the fact that this study forms part of a program targeted at maize growers in Mesoamerica for which a native isolate may be more desirable in terms of environmental impact.

Larvae used in bioassays were selected when they were beginning to molt, as determined by head capsule slippage, and all insects comprising 1 repetition came from a single cohort of the culture. Larvae were starved for 12–16 h at 25°C to encourage them to take up virus droplets and were then allowed to drink from an aqueous suspension containing 10% (wt:vol) sucrose, 0.001% (wt:vol) Fluorella blue, and OBs at 5 different concentrations. The concentration range used for each virus isolate and larval instar was determined in a preliminary bioassay. Larvae that ingested the solution within 10 min were transferred to individual cells of a 25-compartment petri dish with a formaldehyde-free diet plug. Bioassays with 25 larvae per virus concentration plus 25 larvae as a control were replicated 6 times in the 1st experiment and 3 times in the 2nd.. Bioassays were conducted at a constant temperature of 25 ± 2°C, and larval mortality was recorded at 12-h intervals (12, 24, 36 h, and so on) until larvae had either died or pupated. The dose–mortality data were analyzed using POLO-PC (LeOra Software 1987), which is based on the probit analysis method (Finney 1971). The time–mortality data were analyzed using analysis of variance (ANOVA) models in the generalized linear interactive modeling (GLIM) program (McCullagh and Nelder 1989). Data of time to death were obtained as one piece of the information from each larva and, therefore, each data point was independent of the others. These data were found not to have a normally distributed error structure. To account for this, a reciprocal transformation of time to death was performed. Model-checking showed that this transformation satisfied the assumptions of normality required by ANOVA. Differences between individual treatments were assessed using independent contrasts. To perform these contrasts, data in different treatments were grouped together, and the old treatment variable was replaced in the ANOVA models by the new grouping. If this replacement caused a nonsignificant change in the variation explained by the model, there was no significant difference between the grouped treatments.

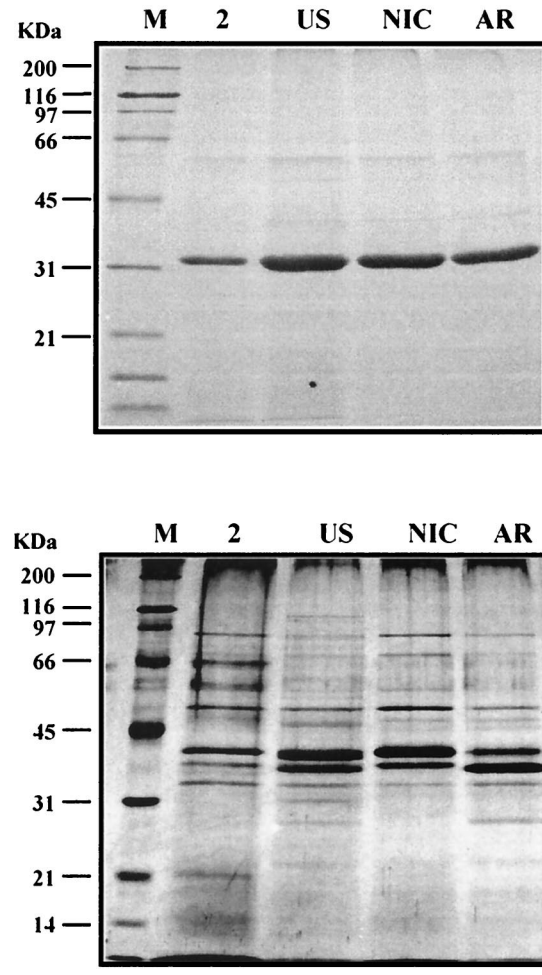

Fig. 1. Electrophoresis of polyhedrins (upper) and virion proteins (lower) of the 4 SfNPV isolates in 12.5% SDS-polyacrylamide gel. M (Markers), 2 (SfMNPV-2), US((SfMNPV-US), NIC (SfMNPV-NIC), and AR (SfMNPV-AR). Mark 12 wide-range protein standards (Novex, San Diego, CA) were used as molecular weight markers.

Results

Identification of SfMNPV Isolates. Polyhedrins of the 4 virus isolates gave a single polypeptide band with an estimated molecular weight of 32 kDa (Fig. 1A). The virion structural polypeptide profiles appeared similar by SDS-PAGE analysis (Fig. 1B). However, there were clear differences in the relative abundance of specific polypeptides, their electrophoretic mobilities in SDS-PAGE, and the presence or absence of certain entities. For example, all the isolates had 2 major polypeptides in the molecular weight band at 38–41 kDa that showed differences in the intensity of staining as well as slight differences in molecular weight. The Sf-NIC isolate polypeptide profile had other differences, including the absence of a few polypeptides such as 1 band at 37 kDa, present only in the Sf-2 isolate, 1 band at 31 kDa, present only in the Sf-US isolate, and 1 band at 29 kDa present in the Sf-US

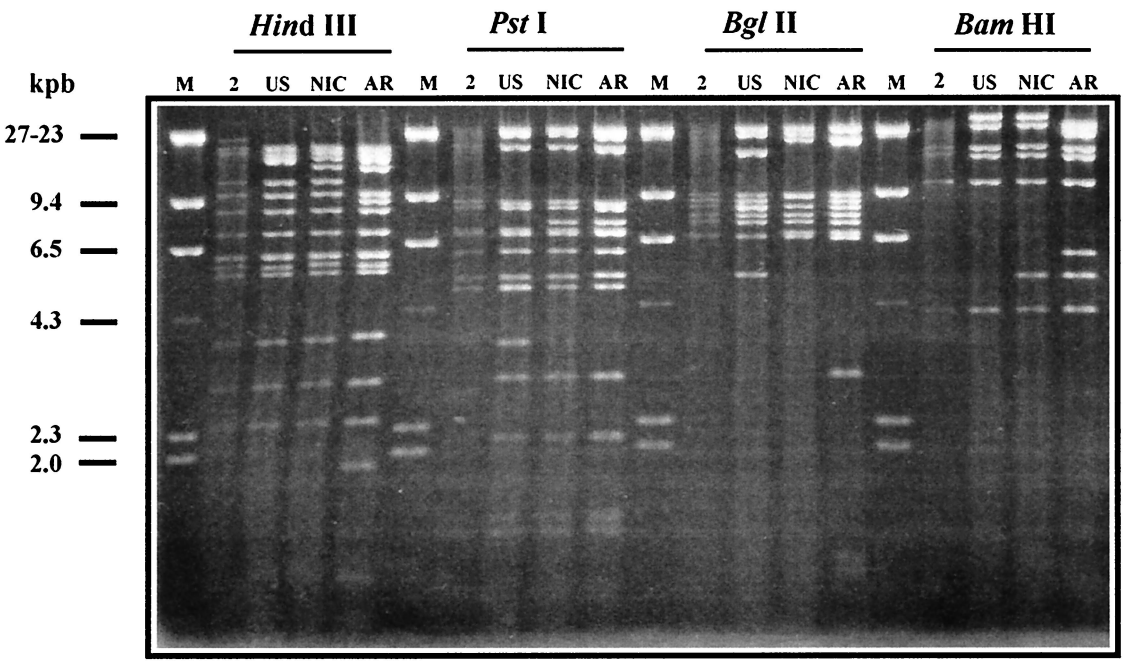

Fig. 2. Restriction endonuclease profiles of DNA of the 4 SfMNPV isolates following treatment with *Hin*dIII, *Pst*I, *Bgl*II, or *Bam*HI and electrophoresis in a 0.8% agarose gel. M (Marker), 2 (SfMNPV-2), US((SfMNPV-US), NIC (SfMNPV-NIC), and AR (SfMNPV-AR). A *Hin*dIII digest of γ DNA was used as molecular marker (fragment molecular weights of 27,491, 23,130, 9,416, 6,557, 4,361, 2,322, and 2,027 bp).

and Sf-AR isolates. Other minor differences among the 4 SfMNPV isolates can be seen in Fig. 1B.

Restriction endonuclease profiles indicated that the 4 MNPV isolates were genetically similar but they could be distinguished using ≥1 enzymes (Fig. 2). For example, following treatment with *Hin*dIII, the Sf-2 19.5-kb fragment, the Sf-US 14.4-kb double fragment, and the Sf-AR 9.4-kb fragment were unique to each of these isolates. The *Hin*dIII digests of Sf-NIC DNA had a 13.1-kb fragment, absent in Sf-2 and Sf-US, and 2 fragments at 15.9 kb and 14.9 kb, whereas in this region, Sf-US and Sf-AR both had corresponding fragments of 15.4 kb and 14.4 kb. The *Pst*I digestion patterns were identical for Sf-NIC and Sf-AR, both of them containing a 7.4-kb fragment absent in Sf-2 and Sf-US, whereas Sf-2 and Sf-US had unique fragments at 2.7 kb and at 3.4 kb, respectively. The *Bgl*II digestion patterns differed in that Sf-NIC had a double fragment at 9.2 kb whereas, in the same region, the other 3 SfMNPV isolates had 2 single fragments of 9.2 and 8.6 kb. Additionally, Sf-US showed unique *Bgl*II fragments at 14.3 kb, 8.2 kb (doublet), and 5.1 kb, whereas Sf-AR had a fragment at 2.7 kb. The *Bam*HI digestion patterns were identical for Sf-2 and Sf-US isolates, whereas the isolate Sf-NIC had an additional fragment at 5.1 kb. Sf-AR also had this fragment at 5.1 kb, but had additional unique *Bam*HI fragments at 21.5 kb (doublet), a single fragment at 6.5 kb, but lacked the largest fragment of 49.8 kb common to the other isolates. The estimates of the genome sizes based on the *Bam*HI fragments were 121.8 kbp for Sf-2 and Sf-US, compared with 126.9 kbp for Sf-NIC and Sf-AR.

Biological Activity of the SfMNPV Isolates. Mortality from viral disease was related to dosage for all of the 4 virus isolates (Fig. 3A) and there were no significant differences ($\chi^2 = 7.2$, df = 3, $P = 0.07$) among the slope of the fitted probit regression lines (Table 1). Their similar slope values allowed us to fit all the regression lines in parallel with a common slope of 0.81, and it was possible to determine the relative potencies of all virus isolates. Sf-NIC had the lowest LC_{50} value, but it did not differ significantly from the LC_{50} value of Sf-US as indicated by the 95% confidence interval (0.58–1.4) of their relative potency (RP: 0.90). The Sf-AR isolate was 4 times (RP: 1.7–7.2) more active that the cloned Sf-2 isolate. The mean times to death were 102.7 h (CL: 100.5 and 105.1), 97.3 h (CL: 94.6 and 99.7), 102.3 h (CL: 100.1 and 104.7), and 103.4 h (CL: 100.1 and 107.3) for Sf-NIC, Sf-2, Sf-US, and Sf-AR, respectively. These differences were small but significant ($F = 2.97$; df = 3, 394; $P = 0.03$). Viruses Sf-NIC, Sf-US, and Sf-AR appeared to kill the larvae at a similar rate. This was confirmed by grouping these viruses together in the ANOVA model, which resulted in a nonsignificant change in the amount of variation explained by virus treatment ($F = 0.34$; df = 1, 395; $P = 0.56$).

Susceptibility of *S. frugiperda* Instars to Sf-NIC. Susceptibility to Sf-NIC increased with dosage for 2nd to 5th instars (Fig. 3B). The 6th instars could only be infected by extremely large concentrations of OBs and this prevented calculation of an accurate LC_{50} value. There were significant differences ($\chi^2 = 1.2$, df = 3, $P = 0.75$) among the slopes of the fitted probit regression lines, and it was possible to fit a common slope of 0.79 ($\chi^2 = 11.94$, df =

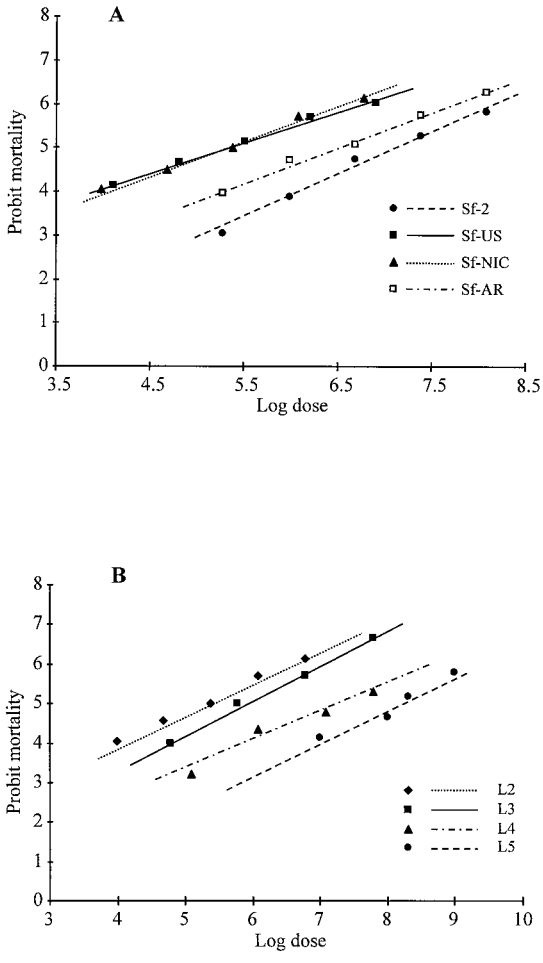

Fig. 3. Observed mortality (points) and log-dose-probit regression lines fitted for the 4 SfMNPV isolates in 2nd instar *Spodoptera frugiperda* larvae (A) and for SfMNPV-NIC isolate in 2nd to 5th instar *S. frugiperda* larvae (B).

12, $P = 0.45$) (Table 2). Relative potencies (RP) and 95% CL of the line for L2 with respect to L3 (RP = 3.96, CL = 1.94–8.07), L3 with respect to L4 (RP = 20.58, CL = 4.69–120.41), and L4 with respect to L5 (RP = 10.63, CL = 1.49–54.90) showed a clear decrease in susceptibility between successive instars, which was statistically significant in all cases. The duration of the interval between infection and death was also significantly correlated with insect stage, with mean times to death of 102.7 h (CL: 100.5 and 105.1), 118.3 h (CL: 114.6 and 122.1), 122.2 h (CL: 115.49 and 129.3), and 136.9 h (CL: 130.2 and 144.3) for 2nd, 3rd, 4th, and 5th instars, respectively. These differences were significantly different ($F = 44.7$; df = 3, 204; $P < 0.01$), but grouping instars 3 and 4 together caused a nonsignificant change in the amount of variation explained ($F = 0.34$; df = 1, 205; $P = 0.39$).

Discussion

Analysis of 4 geographically diverse SfMNPV isolates indicated that they shared a number of structural and genetic similarities. Polyhedrins of the 4 isolates had indistinguishable molecular weights, but differences were detected in the polypeptide composition of virions using SDS-PAGE. Restriction endonuclease analysis of viral DNAs indicated a similar pattern of inter-relationships among these isolates. DNA fragment profiles of Sf-2 with enzymes *Hin*dIII, *Pst*I, *Bgl*II, and *Bam*HI were identical to those described by Maruniak et al. (1984). Likewise, the DNA profiles of Sf-US with *Hin*dIII and *Bam*HI were consistent with those previously reported for certain isolates from Louisiana (Shapiro et al. 1991). However, the restriction fragment profiles of Sf-NIC and Sf-AR indicate them to be distinct variants of this virus species. These 2 isolates can be distinguished by the 9.2 kb *Bgl*II double fragment in Sf-NIC and the 2.7 kb *Bgl*II fragment of Sf-AR. The genome sizes of Sf-NIC and Sf-AR are consistent with those previously reported for these viruses (Maruniak et al. 1984, Shapiro et al. 1991, Fuxa et al. 1994).

The occurrence of geographic variants has been demonstrated for several other NPVs (Maeda et al. 1990, Caballero et al. 1992, Laitinen et al. 1996) and granulosis viruses (Vickers et al. 1991). Such studies generally have indicated that geographical variants are related strains of the same virus that show limited differences in the presence and distribution of restriction enzyme cleavage sites. However, minor genetic differences can have biologically significant consequences in terms of the phenotypic characteristics of a virus (Possee and Rorhmann 1997).

Table 1. LC_{50} values and relative potencies for the 4 SfMNV isolates in second instars of *S. frugiperda*

Virus isolate	n^a	Slope ± SE	Intercept ± SE	LC_{50} (OBs/ml)	95% CL	χ^{2b}	df[c]	P^d	RP	95% CL
Sf-2	5	0.95 ± 0.072	−1.73 ± 0.52	1.23×10^7	7.39×10^6–2.07×10^7	3.24	3	0.36	1	—
Sf-AR	5	0.82 ± 0.062	−0.30 ± 0.42	3.05×10^6	2.17×10^6–4.20×10^6	1.75	3	0.62	4.3	2.8–6.8
Sf-US	5	0.71 ± 0.061	1.23 ± 0.34	2.21×10^5	1.49×10^5–3.22×10^5	1.09	3	0.78	58.1	37.3–91.4
Sf-NIC	5	0.79 ± 0.063	0.79 ± 0.35	2.04×10^5	1.44×10^5–2.85×10^5	1.53	3	0.68	64.5	41.7–100.5

Parameters obtained from the POLO-PC program (LeOra Software 1987). The relative potencies (RP) of the lines Sf-AR, Sf-US, and Sf-NIC relative to the line Sf-2 were estimated after fitting all the regression lines with a common slope ($\chi^2 = 14.61$, df = 15, $P = 0.48$).

[a] Number of virus dosages used.

[b] Goodness-of-fit chi-square.

[c] Degrees of freedom for chi-square.

[d] Probability of a greater chi-square.

Table 2. LC_{50} values and relative potencies of Sf-NIC virus isolates for 2nd–5th instars of *S. frugiperda*

Host instar	n^a	Slope ± SE	Intercept ± SE	LC_{50} (OBs/ml)	95% CL	X^{2b}	df[c]	P^d	RP	95% CL
L_5	4	0.80 ± 0.166	−1.62 ± 1.38	1.84×10^8	9.63×10^7–3.54×10^8	3.38	2	0.18	1	—
L_4	4	0.70 ± 0.116	−0.08 ± 0.81	1.66×10^7	8.31×10^6–3.4×10^7	5.91	2	0.05	11.1	4.2–28.6
L_3	4	0.88 ± 0.121	−0.22 ± 0.74	8.05×10^5	3.75×10^5–1.71×10^6	0.44	2	0.80	228.6	84.8–627.8
L_2	5	0.79 ± 0.063	0.79 ± 0.35	2.04×10^5	1.44×10^5–2.85×10^5	1.53	3	0.68	902.5	430.6–1913.8

Parameters obtained from the POLO-PC program (LeOra Software 1987). The relative potencies (RP) of the lines for L_4, L_3, and L_2 relative to the line for L_5 were estimated after fitting all the regression lines with a common slope ($X^2 = 11.94$, df = 12, $P = 0.45$).

[a] Number of virus dosages used.

[b] Goodness-of-fit chi-square.

[c] Degrees of freedom for chi-square.

[d] Probability of a greater chi-square.

Differences in biological activity are not unusual among virus isolates from distinct geographical regions (Hughes et al. 1983, Shapiro and Robertson 1991, Caballero et al. 1992) or among clonal variants derived from a single wild-type virus (Lynn et al. 1993). Very large differences in virus infectivity can be associated with small changes in virus structure (Crook 1981) or genetic composition, particularly the presence or absence of key genes, such as those involved in blocking host cell apoptosis (programmed cell suicide) which are now being reported in increasing numbers from baculoviruses (Clem 1997) and other insect viruses (Birnbaum et al. 1994). Insect diet also has been shown to be influential in affecting the susceptibility of insects to baculoviruses (Duffey et al. 1995) which is why, for the sake of uniformity, all tests reported here consistently used a standard semisynthetic diet.

S. frugiperda larvae were markedly less susceptible to NPV infection with increasing host stage as reported for many other insect host–baculovirus systems (Briese 1986, Smits and Vlak 1988, Ali and Young 1991). This stage-related increase in resistance has been found to increase more or less constantly with larval weight in some species (Payne et al. 1981), whereas in others the degree of resistance increases more rapidly (Briese 1986, Sait et al. 1994). Although in many cases the physiological basis of this stage-dependent resistance remains uncertain, it appears that *Trichoplusia ni* larvae are able to rid themselves of infection by *Autographa californica* NPV by sloughing off infected gut cells during the molt from the 4th to the 5th instar (Engelhard and Volkman 1995).

Clearly the selection of an isolate as a biocontrol agent requires that geographical variants be tested against an insect population from the locality in which the program is to be run. The theory of new associations applied to the selection of natural enemies for biological control proposes that hosts (pests) that have been long associated with their natural enemies are more likely to have developed resistance or strategies for evading predation or parasitism by the native enemy, whereas no such barriers should exist between a host and a natural enemy that have no evolutionary history of coassociation (Hokkanen and Pimentel 1984). As such, the theory argues that old (established) associations will have a lower probability of being successful in a biocontrol program than new enemy–victim associations. However, the validity of the new associations theory has been questioned (Waage and Mills 1992). The finding of the current study (i.e., that insects from a Honduran population were most susceptible to a virus variant from neighboring Nicaragua) goes against the new associations theory and, not surprisingly, suggests that there is strong selective pressure for the virus to retain high infectivity toward the local host population, presumably via the process of host–pathogen coevolution. As a result of the findings of this study, the Sf-NIC isolate was selected for formulation and field trails currently in progress in Honduras and Mexico (Williams et al. 1999).

Acknowledgments

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