Comparative Infectivity of Homologous and Heterologous Nucleopolyhedroviruses against Beet Armyworm Larvae[1]

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J. Agric. Urban Entomol. 32: 13–24 (2016)

ABSTRACT Homologous and heterologous nucleopolyhedroviruses (NPVs) were assayed to determine their effectiveness against beet armyworm larvae, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Included were three NPV isolates from *S. exigua*, one isolate each from *S. littoralis* Boisduval, *S. litura* (F.), and *S. ornithogalli* (Guenée), two isolates from other noctuids, *Helicoverpa armigera* (Hübner) and *Rachiplusia ou* (Guenée), and one isolate from *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Before bioassays, all of the NPVs were serially passed four times through *S. exigua* larvae to increase their concentrations. The narrow host-range homologous SeMNPVs from South Carolina (SeMNPV-SC), Maryland (SeMNPV-MD), and Florida (SeMNPV-FL = Spod-X®) were three of the most effective NPVs against the homologous host, *S. exigua*, based on median lethal concentration values (LC_{50}). Three heterologous isolates (HearNPV from *H. armigera*, SpliNPV from *S. littoralis*, and RoMNPV from *R. ou*) also had low LC_{50} values. According to their LC_{50} values, the least active NPVs were from *P. xylostella* (PlxyMNPV), *S. ornithogalli* (SoNPV), and *S. litura* (SpltNPV). Based on median time to kill (LT_{50}) at LC_{90} concentrations, SeMNPV-SC and Spod-X® were the most effective isolates. The slowest acting NPVs were from *S. litura*, *S. littoralis*, and *S. ornithogalli*. The virulence of homologous and heterologous NPVs did not appear to be correlated with their genomic relatedness to SeMNPV or to the relatedness of the original NPV insect hosts to *S. exigua*.

KEY WORDS *Spodoptera exigua,* insect viruses, NPV, Spod-X

The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), is polyphagous and feeds on many field and greenhouse crops, such as asparagus, celery, citrus, lettuce, pepper, tomato (Atkins 1960, Vlak et al. 1982, Capinera 2011). As a result of the widespread use of pesticides, some populations of *S. exigua* have developed resistance to different classes of insecticides (Ahmad & Arif 2010, Lai & Su 2011), as well as to the biorational materials azadirachtin (Abdullah et al. 2000), spinosad (Wang et al. 2006), and *Bacillus thuringiensis*

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Berliner (Moar et al. 1995, Hernández-Martinez et al. 2009). Thus, there is a need for additional materials to control this pest.

For more than 30 years, a multiple nucleopolyhedrovirus (SeMNPV) from *S. exigua* has been used for control of *S. exigua* larvae on chrysanthemum and pepper in European greenhouses with excellent results (Vlak et al. 1982, Smits et al. 1987, Smits & Vlak 1988, Bianchi et al. 2002, Lasa et al. 2007). SeMNPV also has been an effective microbial control agent against *S. exigua* on cabbage, tomatoes, peppers, and chick peas in the field (East et al. 1986, Kolodny-Hirsch et al. 1993). In 1993, the U.S. Environmental Protection Agency granted a tolerance exemption to a registered SeMNPV isolate, marketed as Spod-X® (Certis USA, Columbia, MD) (U.S. EPA 1993).

In the present study, the activities of homologous SeMNPV isolates, heterologous spodopteran NPVs, and heterologous non-spodopteran NPVs were evaluated against *S. exigua* larvae. The primary goal of this study was to determine the efficacy of these isolates on *S. exigua* larvae using both pathogenicity (LC_{50}) and speed of kill (LT_{50}). We also examined the importance of taxonomic relationships of NPVs on their relative efficacy against *S. exigua* larvae.

Materials and Methods

Insects. Larvae of the beet armyworm were collected in 2000 from a field of maize, *Zea mays* L., at the Coastal Research and Education Center, Clemson University, Charleston, SC. A laboratory colony was established from this collection, and it was maintained at Clemson University, Clemson, SC. Larvae were reared on a sterilized bean-based artificial diet (Burton 1969).

Virus inocula. The homologous SeMNPV isolates SeMNPV-FL (Spod-X®), SeMNPV-SC, and SeMNPV-MD, and the heterologous spodopteran NPVs from the Egyptian cotton leafworm, *Spodoptera littoralis* (Bois.) (SpliNPV), the tobacco cutworm, *S. litura* (F.) (SpltNPV), and the yellowstriped armyworm, *S. ornithogalli* (Guenée) (SoNPV) were evaluated. Also included in this study were the heterologous non-spodopteran NPVs from the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (HearNPV), the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (PlxyMNPV), and the gray looper moth, *Rachiplusia ou* (Guenée) (Lepidoptera: Noctuidae) (RoMNPV). SeMNPV-FL (Spod-X®) was originally isolated in Florida, SeMNPV-SC was obtained from Clemson University, Clemson, SC, and SeMNPV-MD was obtained from USDA-ARS, Beltsville, MD. Isolates of SpltNPV, SpliMNPV, SoMNPV, and RoMNPV were obtained from USDA-ARS, Beltsville, MD, and PlxyMNPV was obtained from USDA-ARS, Columbia, MO. The single multicapsid HearNPV was obtained from the Chinese Academy of Forestry, Beijing, China.

The NPVs were serially passed through *S. exigua* larvae from the laboratory colony four times to increase the virus concentration. Infected and virus-killed insects were collected and frozen (−20°C) until used. Viral occlusion bodies (OBs) were collected by blending the larvae in 0.1% sodium dodecyl sulfate, followed by centrifugation at 1000 rpm for one minute to remove debris. The aqueous suspension was then centrifuged at 5000 rpm for 20 min to collect the sedimented OBs. The OBs were held at 4°C until used, and concentrations (OBs/ml) were determined using a double-line haemocytometer with improved Neubauer ruling

and phase microscopy (430× magnification). A limitation to our procedure is that latent viruses within the host species could have been activated and amplified through the serial passage procedure, thus acting as a contaminant in our virus preparations. Although we had no direct evidence of contamination by these latent, sublethal infections in our laboratory colony, we recognize that if present they could lead to increased susceptibility to the other viruses we tested (Cabodevilla et al. 2011).

Bioassays. NPVs were tested at the Insect Pathology Laboratory, Clemson University, Clemson, SC during 2000–2002 (Pudjianto 2003). Six serial dilutions of OB suspensions from 1×10^1 to 1×10^6 OBs/ml were made from each isolate. Virus suspensions (0.1 ml) were applied to the diet surface (1338 mm^2 surface area) in a 30-ml plastic container (Jet Plastica Industries, Inc., Hatfield, PA). For untreated controls, 0.1 ml of sterile distilled water was pipetted on to the diet surface. One second-instar *S. exigua* (4-day-old) was placed into each container for 14 d in the laboratory at 28–31°C, 60–70% RH, and a photoperiod of 14:10 L:D. Tests were repeated five times with 10 larvae per virus dilution per isolate. Therefore, a total of 300 larvae were evaluated for each NPV with 50 larvae being tested at each dilution. The untreated control also had 10 larvae per replicate. Mortality was assessed initially three days after treatment, then daily for 14 d. Effectiveness of each virus was measured using both concentration-mortality (median lethal concentration = LC_{50}) and time-mortality (median lethal time = LT_{50}) (or speed of kill) (Ma et al. 2006). LC_{50} values were based on the concentration of OB/mm of the treated surface. LT_{50} values were determined using LC_{90} concentrations for each NPV.

Statistical methods. Mortality results were analyzed using probit analysis (PROC PROBIT) (SAS Institute 1985) to calculate LC_{50} values for each isolate by plotting Log_{10} OB concentration (x) vs larval mortality (y). Data were corrected using Abbott's formula (Abbott 1925) to account for natural mortality in the untreated controls. Following analyses of variance of LC_{50} and LT_{50} values, means were separated according to Fisher's Protected Least Significant Difference (LSD) test ($\alpha = 0.01$) (Steel & Torrie 1960).

Results

All of the NPVs evaluated had a significant effect on *S. exigua* mortality (Table 1), and mortalities in the untreated controls were negligible. Individual probit regression lines for calculations of LC_{50} values were: $y = 1.55x + 4.43$ (SeMNPV-SC), $y = 1.22x + 3.30$ (SeMNPV-MD), $y = 1.49x + 2.12$ (SeMNPV-FL = Spod-X), $y = 0.94x + 3.19$ (SpliNPV), $y = 1.21x + 2.83$ (RoMNPV), $y = 0.94x + 2.75$ (HearNPV), $y = 1.12x + 1.53$ (PlxyMNPV), $y = 1.14x + 1.46$ (SoNPV), and $y = 1.23x + 1.05$ (SpltNPV).

The homologous SeMNPV-SC (LC_{50} = 0.03 OBs/mm^2), SeMNPV-MD (LC_{50} = 0.26 OBs/mm^2), and Spod-X®, (LC_{50} = 0.90 OBs/mm^2) had lower LC_{50} than the other NPVs ($P < 0.01$), except for the heterologous SpliMNPV (LC_{50} = 0.74 OBs/mm^2) and RoMNPV (LC_{50} = 0.85 OBs/mm^2) (Table 1). The LC_{50} for SeMNPV-SC was 8.7- and 30-fold lower than those of SeMNPV-MD and Spod-X®, respectively. In contrast, spodopteran NPVs from *S. litura* (SpltMNPV, LC_{50} = 16.2 OB/mm^2) and *S. ornithogalli* (SoMNPV, LC_{50} = 13.4 OB/mm^2) required significantly more infectious virus than the homologous NPVs to produce 50%

Table 1. Virulence of homologous and heterologous nucleopolyhedroviruses against second instar beet armyworms, *Spodoptera exigua*.

Source of NPV	NPV[a]	LC_{50} (95% CL)[b]	LT_{50} (95% CL)[c]
Spodoptera exigua-SC	SeMNPV-SC	0.03 (0.02–0.05) a[e]	4.3 (3.6–4.8) a
S. exigua-MD	SeMNPV-MD	0.26 (0.18–0.39) b	6.3 (5.8–6.8) b
S. exigua-FL	SeMNPV-FL[d]	0.90 (0.63–1.22) c	4.2 (4.0–4.3) a
S. littoralis	SpliNPV	0.74 (0.52–1.06) c	7.8 (7.5–8.1) d
Rachiplusia ou	RoMNPV	0.85 (0.54–1.49) c	5.9 (5.8–6.1) b
Helicoverpa armigera	HearNPV	2.41 (0.97–8.75) c	5.9 (4.6–6.8) b
Plutella xylostella	PlxyMNPV	12.61 (8.65–19.4) d	5.5 (5.1–5.8) b
S. ornithogalli	SoNPV	13.34 (9.15–19.0) d	10.0 (9.6–10.4) e
S. litura	SpltNPV	16.20 (11.5–22.6) e	6.8 (5.9–7.5) c

[a]NPVs were diluted in distilled water; five replicates; six virus concentrations per virus per replicate; 10 larvae per virus concentration per replicate; 10 untreated larvae per replicate.
[b]LC_{50s} were expressed as OBs/mm^2 of diet surface area.
[c]LT_{50s} were expressed as days post-virus exposure. LT_{50} values were determined using LC_{90} concentrations for each NPV.
[d]Spod-X® (Certis USA, Columbia, MD).
[e]Means in the same column followed by the same letter are not significantly different ($P < 0.01$) according to Fisher's protected Least Significant Difference (LSD) test (Steel & Torrie 1960).

mortality ($P < 0.01$). The single nucleocapsid HearNPV (LC_{50} = 2.4 OB/mm^2) from *H. armigera* was significantly less active than the NPV isolates from SeMNPV, but was significantly more active than the NPVs from *S. litura, S. ornithogalli*, and *P. xylostella* (LC_{50} = 12.6 OB/mm^2) (Table 1).

In terms of speed of kill, Spod-X® (LT_{50} = 4.2 d) and SeMNPV-SC (LT_{50} = 4.3 d) were the fastest acting isolates, and they had a significantly lower LT_{50} values than the other isolates ($P < 0.01$), including the other homologous isolate, SeMNPV-MD (LT_{50} = 6.3 d) (Table 1). SpltNPV (LT_{50} = 6.8 d), SpliNPV (LT_{50} = 7.8 d), and SoNPV (LT_{50} = 10.0 d) were the slowest acting NPVs. The LT_{50} values of PlxyMNPV (LT_{50} = 5.5 d), HearNPV (LT_{50} = 5.9 d), RoMNPV (LT_{50} = 5.9 d), and SeMNPV-MD were similar (Table 1).

For combined activities (LC_{50} and LT_{50}), the *S. exigua* NPV isolates from South Carolina was the most effective (Table 1). SeMNPV-MD was significantly more active than Spod-X® in terms of LC_{50}, but it was significantly slower acting than Spod-X®. Although SeMNPV-MD required only 0.26 OB/mm^2 to cause 50% mortality, it needed two more days than the other SeMNPV isolates to achieve it. On the other hand, Spod-X® was the least active of the three SeMNPV isolates in terms of LC_{50}, but it was as fast acting as SeMNPV-SC (Table 1).

In a similar manner, SpliMNPV required low levels of inocula (LC_{50} = 0.74 OB/mm^2) to cause 50% mortality, but it was slow acting (LT_{50} = 7.8 d). Among the heterologous NPVs, RoMNPV was most active (LC_{50} = 0.85 OB/mm^2, LT_{50} = 5.9 d), while SoMNPV (LC_{50} = 13.3 OB/mm^2, LT_{50} = 10.0 d) and SpltMNPV (LC_{50} = 16.2 OB/mm^2, LT_{50} = 7.8 d) were the least active (Table 1). Overall, there appeared to be little relationship between pathogenicity and speed of kill for both the homologous and heterologous isolates.

Discussion

Homologous NPVs. It is known that homologous NPVs are generally more effective against their host species than are heterologous NPVs (Shapiro & Vaughn 1995, Shapiro & Hamm 1999, Shapiro 2000). Because baculoviruses have co-evolved with their lepidopteran hosts (Zanotto et al. 1993, Herniou et al. 2004), viruses retain high infectivity toward local host populations, probably as a results of continuous host-pathogen coevolution (Elvira et al. 2013). For the present study, the homologous SeMNPV isolates were, in general, more effective against *S. exigua* larvae than were heterologous NPVs from insects of the same genus (*Spodoptera*), the same family (Noctuidae), or from other lepidopteran families. However, the effectiveness of a homologous NPV may vary according to several factors, and differences in virulence occur in virus genotypes from a single insect (Shapiro et al. 1992, Hodgson et al. 2001, Cory et al. 2005), from plaque-purified clones (Lynn et al. 1989, 1993, Simón et al. 2004, Harrison et al. 2008), and from different geographical isolates (Shapiro et al. 1984, Shapiro & Dougherty 1985, Rios-Velasco et al. 2012). In some cases, multiple genotypes have been found within isolates, which vary in both pathogenicity and speed of kill (Simón et al. 2004, Cory et al. 2005). In addition, serial passage of an NPV through a host insect may cause replication of sublethal viruses or activate latent ones already in the host, which could lead to contaminated isolates and inconsistent results (Pavan et al. 1981, Burden et al. 2003, Cabodevilla et al. 2011).

Heterologous spodopteran NPVs. We hypothesized that the heterologous NPVs from closely-related *Spodoptera* species would infect *S. exigua* larvae and have activity comparable to the homologous SeMNPV isolates. In some respects this postulate was correct, as the three heterologous spodopteran NPVs (SpliMNPV, SpltMNPV, SoMNPV) were infectious to *S. exigua* larvae, and the LC_{50} for SpliMNPV was comparable to the LC_{50} for Spod-X®. However, LC_{50} values for SeMNPV-SC and SeMNPV-MD were significantly lower than the LC_{50} for SpliMNPV. Surprisingly, the other two spodopteran NPVs (SoMNPV, SpltMNPV) had the highest LC_{50} values of any NPV tested. Furthermore, LT_{50} values for SpliMNPV, SpltMNPV, and SoMNPV were significantly higher than LT_{50} values for the SeMNPV isolates and for the heterologous non-spodopteran NPVs, PlxyMNPV and RoMNPV (Table 1).

Despite the close phylogenetic relatedness of SeMNPV, SpliNPV, and SpltNPV, significant differences in pathogenicity of these NPVs were seen in this study, and similar results have been reported (Bulach et al. 1999, Simón et al. 2004). For example, SpliNPV replicated in the heterologous hosts *S. exigua*, *S. litura*, and *S. frugiperda* (J. E. Smith), and caused lethal infections with typical wilt symptoms ("melting" of the cadaver as it degenerates), but it did not infect species from other noctuid genera (Harpaz & Raccah 1978, Simón et al. 2004). The NPV from *S. frugiperda* (SfMNPV) infected homologous and heterologous hosts (*S. exigua* and *S. littoralis*), but heterologous hosts did not wilt as there was limited replication with no hypodermal involvement, and no virus progeny were produced (Simón et al. 2004). SeMNPV replicated in all hosts, but gene expression was significantly lower at the haemocoel transmission stage (Simón et al. 2004). Feng et al. (2007) determined that SpltNPV caused lethal infection in second and third stage *S. exigua* larvae at high concentrations, but the insects did not wilt. In fourth-instar larvae, however, replication was limited

as apoptosis occurred in infected haemocytes and fat bodies. SpliMNPV and related SpltNPV (Huang & Levin 2001) caused lethal infection in *S. litura* larvae, but SpliMNPV was more virulent (Takatsuka et al. 2007).

Our results show that the pathogenicity of heterologous spodopteran NPVs were, in general, lower than the activities of homologous SeMNPV isolates. In addition, activities of NPVs against a heterologous host, *S. exigua*, could not be predicted on the basis of genomic relatedness of the host species of the NPVs or on the relatedness of the infected host insects. These results agree with Goulson (2003) who concluded that host susceptibility was poorly predicted by taxonomic relationships of NPVs.

Heterologous non-spodopteran NPVs. In the present study, we found that the non-spodopteran heterologous RoMNPV was as effective as Spod-X®. However, HearNPV and PlxyMNPV were significantly less active than any of the SeMNPV isolates. The activities of some heterologous NPVs against *S. exigua* larvae have been tested previously. Shapiro (2000) compared the activity of SeMNPV-MD with NPVs from the greater wax moth, *Galleria mellonella* (L) (Lepidoptera: Pyralidae) (GmMNPV) (Harrison et al. 2012), and from the noctuids *Helicoverpa armigera* (Lepidoptera: Noctuidae) (HearNPV), *Autographa californica* (Speyer) (AcMNPV), and a variant of AcMNPV from the celery looper, *Anagrapha falcifera* (Kirby) (AfMNPV) (Harrison & Bonning 1999). In Shapiro's (2000) study, SeMNPV-MD was 23- and 48-fold more effective (=LC_{50}) against *S. exigua* larvae than AcMNPV and AfMNPV, respectively; while efficacy was so low that LC_{50} values could not be calculated for HearNPV and GmMNPV (Shapiro 2000). In similar experiments, Shapiro & Shepard (2006) compared the activities of SeMNPV-MD, SfMNPV, HearNPV, AcMNPV, and its variants, AfMNPV, PlxyMNPV, and RoMNPV (Harrison & Bonning 1999, Harrison et al. 2012), against *S. exigua* larvae. AcMNPV and RoMNPV had similar LC_{50} values to the homologous SeMNPV-MD, but SeMNPV-MD was 60- and 79- fold more active than PlxyMNPV and GmMNPV, respectively. However, in contrast to Shapiro (2000), Shapiro & Shepard (2006) reported that the heterologous AfMNPV and HearNPV were significantly more active than the homologous SeMNPV-MD. Therefore, it appears that some non-spodopteran heterologous are inactive against *S. exigua* larvae, while other NPVs have significant activity, but this activity can vary considerably from test to test.

Measures of NPV effectiveness. Some studies on the pathogenicity of baculoviruses used median lethal concentration (LC_{50}) (Fuxa & Richter 1990, Shapiro & Shepard 2006) or median lethal dose (LD_{50}) (Caballero et al. 2009, Jakubowska et al. 2010) as a measure of effectiveness, while other studies used speed of kill (LT_{50}) (Gramkow et al. 2010, Behle & Popham 2012) or survival time (ST_{50}) (Farrar et al. 2007) as the primary criterion for determining efficacy. In the present study, if lethal concentration were the lone criterion, the SeMNPV isolates, SpliMNPV, and RoMNPV would be appear the most effective. On the other hand, if speed of kill were the prime criterion, SeMNPV-SC, SeMNPV-FL, PlxyMNPV, RoMNPV, and HearNPV would appear the most effective. However, we contend that it is preferable to consider more than one measure of NPV effectiveness. In that regard, both lethal concentration (or lethal dose) and speed of kill (or survival time) have been used in some studies, and this has led to a better understanding of the effectiveness of these pathogens, although results have been variable (Ma et al. 2006). For example, Muñoz et al. (2004) evaluated

three cloned variants of a wild-type isolate of SeMNPV-FL. Although pathogenicities (LD_{50}) of the isolates were similar, speed of kill (mean time to death) varied from 28 to 114 h.

Therefore, we considered both pathogenicity (LC_{50}) and speed of kill (LT_{50}) to obtain a clearer understanding of the effectiveness of these NPVs. All of the SeMNPV isolates, RoMNPV (subfamily Plusiinae), and HearNPV (subfamily Heliothinae) had both low LT_{50} and low LC_{50} values in the present study. However, SpliNPV had a low LC_{50} value, but an LT_{50} value that was significantly higher than most of the other isolates. In contrast, PlxyMNPV from *P. xylostella*, which is not closely related to the noctuids, had a high LC_{50} value against *S. exigua* larvae, but it had an LT_{50} value that was comparable to the LT_{50} values of NPVs from some of the noctuids (SeMNPV-MD, HearNPV, and RoMNPV). In addition, the LT_{50} value of PlxyMNPV was significantly lower than the LT_{50} values for the three heterologous spodopteran NPVs (SpliNPV, SoNPV, and SpltNPV) (Table 1).

In the present study, we saw poor relationship between LC_{50} values and the corresponding LT_{50} values for homologous and heterologous NPVs. Low LC_{50} values did not necessarily translate into slow speed of kill, and low LT_{50} values did not always predict low pathogenicity. Similar results have been reported for wild-type, heterogeneous viruses (Shapiro & Dougherty 1985, Cory et al. 2005). However, there also have been published reports of significant relationships between these measures of NPV effectiveness. For example, Murillo et al. (2006) compared LD_{50} and LT_{50} values of single and mixed SeMNPV genotypes, and they determined that mixed genotypes were more virulent and that an increase in one measure of effectiveness was accompanied by a decrease in another. An inverse relationship between pathogenicity and speed of kill also has been shown for some non-spodopteran NPVs (Shapiro et al. 1992, Escribano et al. 1999, Hodgson et al. 2001). Conversely, other studies with non-spodopteran NPVs reported significant positive correlations between pathogenicity and speed of kill (Gupta et al. 2007, Mehrvar et al. 2008). Also, Shapiro et al. (1992) showed that serial passage of LdMNPV through its host increased the positive correlation between speed of kill and pathogenicity. Apparently, the relationship between pathogenicity and speed of kill is not only dependent on the host-pathogen relationship, but also on changes in the proportion of virus biotypes during *in vivo* selection (Veber 1962, Kolodny-Hirsch et al. 1997).

Pest management considerations. Because the host-specific SeMNPV isolates were the most virulent, they probably would be preferred to other NPVs for control of *S. exigua*. RoMNPV and HearNPV also were effective against *S. exigua* larvae, and they should be considered for further testing, because they have been shown to be pathogenic against several agriculturally important pests (Moscardi 1999, Anonymous 2013, Shapiro & Shepard 2006). In prior studies, however, the activity of HearNPV against *S. exigua* varied from poor (Shapiro 2000) to excellent (Shapiro & Shepard 2006). Furthermore, Herz et al. (2003) determined that HearNPV activity (LD_{50}, ST_{50}) in the homologous host, *H. armigera*, was significantly greater than activity in the heterologous host, *S exigua*, and that replication in *S. exigua* was restricted to nervous tissue.

As expected, the narrow host-range homologous SeMNPVs were the most effective NPVs against the homologous host, *S. exigua*. Baculoviruses co-evolve with their lepidopteran insect hosts and retain high infectivity toward local host

populations (Zanotto et al. 1993, Herniou et al. 2004, Elvira et al. 2013). In addition, we conclude that both pathogenicity (LC_{50}) and speed of kill (LT_{50}) are key parameters for selection of virulent NPVs, but these parameters can vary from isolate to isolate. Moreover, the virulence of homologous and heterologous NPVs does not appear to be correlated with their genomic relatedness to SeMNPV, or the relatedness of the original NPV hosts to *S. exigua*. Our results also illustrate that erroneous conclusions could be reached if only LC_{50} or only LT_{50} were the sole criterion for virulence. In addition, this study supports the conclusion that broad-spectrum NPVs depend on the pathogen-host interrelationship (Bos & Parlevliet 1995, Francl 2001) and are not uniform from host to host.

Acknowledgments

This paper is Technical Contribution No. 6193 of the Clemson University Experiment Station. We thank Mark Schaffer and other staff members at Clemson University and the Coastal Research and Education Center, Charleston, SC for their excellent technical, clerical, and administrative assistance.

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