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Biological activity of recombinant *Spodoptera exigua* multicapsid nucleopolyhedrovirus against *Spodoptera exigua* larvae

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ABSTRACT

In this study the potency and efficacy of recombinant *Spodoptera exigua* multicapsid nucleopolyhedrovirus (SeXD1) were compared with that of the wild-type *S. exigua* virus (wt-SeMNPV). Time–dose–mortality bioassays of SeXD1 and wt-SeMNPV were conducted on 2nd to 5th instars of *S. exiuga* larvae. The results show that the recombinant SeXD1 killed insects significantly faster than wt-SeMNPV. At high dosages, the LT_{50} values for SeXD1-infected 2nd to 5th instars were 1.3, 1.3, 1.0 and 0.4 days shorter than those for wt-SeMNPV, respectively. LD_{50} values also decreased with increases in infection time. LD_{50} of SeXD1 occurred 1 day earlier than that of wt-SeMNPV at the same instar, but no significant difference between those for SeXD1 and wt-SeMNPV was observed. In addition, the effect of SeXD1 on feeding was evaluated in 5th instar *S. exigua* larvae. While nutritional indices, such as consumption index (CI) and relative growth rate (RGR), were significantly reduced by SeXD1 infection, digestibility and food conversion efficiency showed a slight increase. As a 50% overall reduction in consumption and lower CI and RGR values were found for larvae infected with the recombinant SeXD1 virus, these findings show that SeXD1 may provide better protection than wt-SeMNPV.

1. Introduction

The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), is a major pest of agricultural crops in many tropical and subtropical regions. This insect is a widely distributed polyphagous pest of numerous cultivated crops including cotton, tomato, celery, lettuce, cabbage, and alfalfa. This pest has caused considerable economic losses in more than 20 provinces of China over the last decade. Moreover, *S. exigua* has developed resistance to many chemical insecticides (Brewer and Trumble 1989; Van Laecke and Degheele 1991), and is not highly susceptible to spinosad (Moulton et al., 2000), a naturally occurring insecticide derived from a soil bacterium.

S. exigua wild-type multicapsid nucleopolyhedrovirus (wt-SeMNPV) shows a high degree of specificity and infectivity (Smits and Vlak, 1988; Bianchi et al., 2000) against its host, which has permitted its use as a biological control agent (Gelernter et al., 1986; Kolodny-Hirsch et al., 1997). However, its slow action against larvae often allows the pest to feed and damage the crop over considerable period of time; which is one of the major drawbacks on the use of wt-SeMNPV as a microbial insecticide. To overcome this problem, research has focused on identifying highly virulent strains (Muñoz et al., 1997, 1999; Murillo et al., 2006). Unfortunately, these efforts have been hampered by the tendency of wt-SeMNPV to quickly generate defective mutations in cell cultures, and these deletions are no longer infectious for *S. exigua* larvae *per os* (Heldens et al., 1996). A recent study successfully generated a recombinant variant of SeMNPV (SeXD1) with 10.6 Kbp of sequence information deleted that was located between 13.7 and 21.6 map units of SeMNPV and includes *egt, gp37*, *chitinase* and *cathepsin* genes, as well as several genes unique to SeMNPV (Dai et al., 2000). This recombinant variant replicates both *in vitro* and *in vivo* and exhibits a superior ability to kill larvae compared to the wild-type virus.

To date, a time-mortality assay for the recombinant SeXD1 has only been performed with 3rd instar larvae (Dai et al., 2000) and the effects of SeXD1 on other larval instars, as well as its impact on food utilization efficiency, have not been determined. Additionally, data from baculovirus–insect time–dose–response experiments have only been analyzed by separate models examining time trends for each dose or dose trends for each time treatment. Such methods are limited by the exclusion of the entire dataset in the estimation. A complementary log–log (CLL) model for time–dose–mortality would best be performed by eliminating problems arising from the separate description of dose and time trends in relation to mortality in an experimental population. This approach has already been developed for modeling chemical

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insecticides (Preisler and Robertson, 1989) and fungal agents (Shi et al., 2008). In our study, we used a CLL model to quantify the dosage, time and mortality relationships of *S. exigua* 2nd to 5th instars after ingesting SeXD1 to assess whether the effects of SeXD1 in the SeXD1/*S. exigua* system are age-specific. We also explored the effect of SeXD1 on the food consumption and utilization by 5th instar larvae.

2. Materials and methods

2.1. Insect and virus cultivation/production

Larvae of *S. exigua* were reared on an artificial diet (Li et al., 2002) at 27 ± 1 °C, 75% relative humidity and a 14 h light/10 h dark photoperiod. The wt-SeMNPV and the recombinant virus SeXD1 (Dai et al., 2000) were maintained in our laboratory. Occlusion bodies (OBs) were propagated in early 4th instar *S. exigua* larvae. The larvae were fed artificial diet contaminated with the appropriate virus and then reared on virus-free diet. The infected larvae were collected after death. OBs were purified by filtration and centrifugation as described previously (Muñoz et al., 1997), quantified by counting in a Neubauer chamber, and stored at −20 °C until used.

2.2. Bioassays

Laboratory bioassays were conducted to investigate the effect of time and dose on the ability of the two types of virus to kill 2nd to 5th instar larvae. *S. exigua* were separated into 1st to 4th pharate instars and only those larvae that molted overnight were selected for use in the experiments. These larvae were deprived of food for up to 8 h before each experiment. Bioassays were performed using the diet plug method as described by Doyle et al. (1990). Briefly, infections were carried out by the inoculation of a small cube of artificial diet with the virus and water (control). Larvae were fed on the diet for 24 h, and data were recorded only from larvae that had consumed all of the food within this time frame. They were subsequently transferred to uncontaminated diet and reared in environmental chambers. The dosages ranged from 1 to 10^4 OBs/larva for 2nd instars, from 5 to 5×10^4 OBs/larva for 3rd instars, and from 10^2 to 10^6 OBs/larva for 4th and 5th instars. Thirty larvae were inoculated per instar/virus treatment. Each larva was examined daily. Experiments ended on the 9th day post-infection and three replicates were used for each trial in each instar.

2.3. Measurement of food utilization efficiency

To compare the food utilization efficiency between wt-SeMNPV and SeXD1, a gravimetric technique (Kumar et al., 2007) was used to determine weight gain, food consumption and the weight of feces produced. Fifth instars that had molted in the previous 6–8 h were selected for this part of our study. The larvae were weighed and placed in individual treatment or control cups. Larvae were inoculated with pre-determined quantities of wt-SeMNPV and SeXD1 (ranging from 10^2 to 10^6 OBs/larvae) or reared on an untreated artificial diet (control) for 24 h. Ten larvae from each treatment were then selected for re-weighing and were reared thereafter on a virus-free diet. The sample diet and larvae were weighed, dried at 6 °C for 48 h, and reweighed to establish a dry-to-fresh weight ratio. The daily quantities of remaining food and feces were dried at 60 °C for 48 h, and reweighed. The experiment continued for 4 days and observations were made every 24 h, with three replicates with three different cohorts of insects. All indices were calculated using measured or estimated dry weights.

The nutritional indices that we calculated, using Eqs. (1)–(5), respectively (Kumar et al., 2007), included: a Consumption Index (CI), Relative Growth Rate (RGR), Approximate Digestibility (AD), Efficiency of Conversion for Ingested Food (ECI), and Efficiency of Conversion of Digested food (ECD):

$$\text{consumption index (CI)} = \frac{E}{T \times A} \tag{1}$$

$$\text{relative growth rate (RGR)} = \frac{P}{T \times A} \tag{2}$$

$$\text{approximate digestibility (AD)} = \frac{E-F}{E} \times 100 \tag{3}$$

$$\text{efficiency of conversion for ingested food (ECI)} = \frac{P}{E} \times 100 \tag{4}$$

$$\text{efficiency of conversion of digested food (ECD)} = \frac{P}{E-F} \times 100 \tag{5}$$

where A is the mean dry weight of the larva, E is the dry weight of food consumed, F is the dry weight of the feces, P is the dry weight gain of the insect, and T is the duration of the experimental period.

2.4. Statistical analysis

We used a previously described time–dose–mortality model to analyze our data (see Preisler and Robertson, 1989; Feng et al., 1998). Considering a bioassay that includes i dosages and j observation times, the cumulative mortality probability is calculated as:

$$P_{ij} = 1 - \exp[-\exp(\tau_j - \beta 1 g d_i)] \tag{6}$$

$$\tau_j = \ln\left[\sum_{k=1}^{j} \exp(\gamma_k)\right] \tag{7}$$

where β is the slope that describes the dose effect, t is the parameter for the time effect of d_i during the period from the start of the experiment to the j_{th} observation, τ_j is the categorical variable that corresponds to time t_j , and γ_j is the parameter that corresponds to the time interval (t_{j-1}, t_j). The linear portion of Eq. (6) is the complementary log–log line, or the linear predictor of the CLL model. The model assumes that lg[-lg(1-P_{ij})] is linear in the covariates. The conditional mortality probability, which is the probability of an insect dying in a given time interval, is given as follows:

$$Q_{ij} = 1 - \exp[-\exp(\gamma_j - \beta 1 g d_i) \tag{8}$$

where the parameters β and γ_j are the same as in Eqs. (6) and (7). The values of β and γ_j were calculated by applying the binomial response variable to the maximum likelihood equation (Nowierski et al., 1996), which enabled the determination of τ_j. LD_{50} values and their confidence limits were estimated using the formula described by Preisler and Robertson (1989):

$$LD_{50} = (-0.3665 - \tau_j)/\beta \tag{9}$$

An estimate of LT_{50} for any dosage can be obtained by linear interpolation (Feng et al., 1998; Nowierski et al., 1996) using the following equation:

$$LT_{50} = \frac{t_j + (t_{j+1} - t_j)(0.5 - p_{ij})}{p_{ij+1} - p_{ij})} \tag{10}$$

The goodness-of-fit for the binomial variable was determined using a modification of Pearson's χ^2 method or the Hosmer and Lemeshow Test.

The procedures, including modeling, goodness-of-fit tests and the virulence indices estimations (LD_{50} and LT_{50}), were conducted using the DPS data processing software (Tang and Feng, 1997). The model was calibrated by adding small positive doses at all levels.

Data from the nutritional indices were compared by analysis of variance (ANOVA) after arcsine square root transformation of percentages. Differences between treatments were determined by Tukey's Honestly Significant Difference (HSD) tests.