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Efficacy of *Spodoptera exigua* multiple nucleopolyhedrovirus as a biological insecticide for beet armyworm control in greenhouses of southern Spain

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Abstract

Chemical control measures targeted at *Spodoptera exigua* in greenhouse sweet pepper crops in Spain have resulted in pest resistance to virtually all commercially available insecticidal products. A multicapsid nucleopolyhedrovirus (SeMNPV), isolated from diseased *S. exigua* in Spain, was produced in laboratory reared larvae, tested for insecticidal activity in a laboratory bioassay, and was then applied in eleven commercial greenhouses planted with sweet pepper. Virus occlusion bodies (OBs) were applied on two occasions, at an interval of $\sim$7 days, at a rate of 5×10^8 OBs/L of spray in a volume of $\sim$600 L/ha, depending on crop phenology and greenhouse area. The percentage of plants showing recent ($<$48 h old) feeding damage fell dramatically in greenhouses with high infestations of *S. exigua*; the same pattern was observed, although less dramatically, in greenhouses with low infestations. Average mortality of larvae collected from treated plants at 4 days after each application, and reared in the laboratory until death, was high (70–89%) and was not significantly affected by the degree of crop infestation. In a separate trial, the rate of acquisition of infection was examined in larvae that fed on plants treated with 1×10^8 or 5×10^8 OBs/L of spray. Of the 27 and 60% of larvae, respectively, that acquired infection in the 48 h period after spraying, about half became infected in the first 6 h post-application, irrespective of application rate. Acquisition of infection proceeded more slowly during the night-time compared to the daytime period, underlining the advantages of early morning applications of the virus. We conclude that the Spanish SeMNPV isolate merits registration as a biological insecticide for use in greenhouse crops in this region.

Keywords: *Acquisition of infection, baculovirus, biological insecticide, greenhouse crops, Spodoptera exigua, sweet pepper*

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Introduction

The beet armyworm, *Spodoptera exigua* (Hübner) is a major pest of sweet pepper, tomato, aubergine, courgette, melon and watermelon crops in greenhouses in Almeria, southern Spain (Moreno et al. 1992). The greenhouses in this region cover an area of over 28 000 ha, making this the most important region for the production of covered crops in Europe.

Almerian growers attempt to control *S. exigua* infestations by applying broad spectrum or new generation biorational insecticides singly, or in cocktails, at weekly intervals. Frequent use of synthetic insecticides has resulted in widespread resistance in many *S. exigua* populations, including those of Almeria (Smagghe et al. 1997, 2003; Mascarenhas et al. 1998; Torres Vila et al. 1998; Moulton et al. 1999; Moulton et al. 2002). The presence of pesticide residues in greenhouse produce from this region also requires continual monitoring (Garrido et al. 2004). At present, there are no effective biological agents commercially available for control of *S. exigua*. This situation leads to repeated use of chemical insecticides and precludes the implementation of biological control programmes targeted at other major greenhouse pests (e.g. thrips, whiteflies, aphids, etc.), for which effective biocontrol agents are readily available (Lara & Urbaneja 2002; Jacas et al. 2006). There is an urgent need to identify safe and effective insecticides for beet armyworm control in greenhouse crops in southern Spain.

A commercial preparation of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) was first registered in United States (Spod-X®), and thereafter in other countries of northern Europe (Smits et al. 1987a; Bianchi et al. 2001) and Thailand (Kolodny-Hirsch et al. 1997). The Floridian strain of SeMNPV that forms the active ingredient of Spod-X contains parasitic genotypes that reduce the insecticidal activity of the product (Muñoz et al. 1998). Populations of *S. exigua* from Almeria are significantly more susceptible to a native Almerian strain of SeMNPV collected during an epizootic of infection in 1992, compared to Spod-X (Belda et al. 2000).

The efficacy of baculovirus applications depends on multiple factors, principally the dose applied, the susceptibility to infection of the pest at each instar, the stage structure of the pest population and rate of inactivation of viral occlusion bodies (OBs) on foliage surfaces (Pinnock & Brand 1981). The proportion of the pest population that acquires a lethal infection will also be influenced by the spatial distribution of OB deposits, which in turn depends on physical factors including application volume, formulation, spray equipment, nozzle characteristics, plant coverage and canopy structure (Smits et al. 1988; Jones & Burges 1998). Larval feeding behaviour will be particularly important to the likelihood of infection in greenhouse crops which are usually subjected to high volume spray applications in an attempt to ensure maximum coverage by spray deposits (Smits et al. 1987b; Bianchi et al. 2000).

Here, we report the results of trials of an Almerian isolate of SeMNPV for control of *S. exigua* on sweet pepper crops under commercial greenhouse conditions. Batches of SeMNPV produced in the laboratory were subjected to quality control procedures to ensure high insecticidal capacity. The prevalence of *S. exigua* feeding damage and insect mortality was evaluated following each of two applications of SeMNPV in eleven commercial greenhouses planted with sweet pepper. In a separate trial, we examined the process of acquisition of infection in *S. exigua* larvae that fed on sweet pepper plants previously treated with two different rates of OBs. The results of these

experiments should prove valuable in the process of registration of this isolate of SeMNPV as a biological insecticide for use in greenhouse crops in southern Europe.

Materials and methods

Laboratory production of SeMNPV

The strain of SeMNPV used in the formulation was originally isolated from a group of larvae collected during a natural epizootic in Almeria, southern Spain (Caballero et al. 1992). SeMNPV was propagated in fifth instar *S. exigua* obtained from a colony maintained at the Universidad Pública de Navarra, Pamplona, Spain. Groups of 100 larvae, of 20–35 mg live weight, were placed in ventilated rectangular ($200 \times 300 \times 60$ mm) plastic boxes. Larvae were infected by feeding on a thin layer of semisynthetic diet ($100 \times 50 \times 2$ mm) that had been previously surface contaminated by spraying upper and lower surfaces with 1.5×10^8 OBs in a volume of 1.5 mL of sterile water using an artist's air-brush (Paasche VL, Paasche Airbrush Co., Chicago, IL). The contaminated diet was placed on a plastic mesh platform with 10-mm rectangular holes to allow feeding from beneath.

Larvae were held in a dark room at $25 \pm 1^\circ$C and $60 \pm 10\%$ RH. Two days after inoculation, a new piece of untreated diet was supplied to larvae. Each box was checked daily for virus mortality from 5 to 8 days post-inoculation to avoid losses due to cannibalism of dead or moribund larvae. Dead and dying larvae were carefully collected daily and stored at -20°C. On the 8th day, when virtually all larvae had died, each box was placed in a -20°C freezer and corpses were harvested in their frozen form to avoid loss of OBs from lysis of the insect tegument.

Virus killed larvae were pooled, triturated and homogenised in phosphate-buffered saline (PBS) containing 0.15% sorbic acid and 5% glycerol, and adjusted to pH 6.5 using dilute hydrochloric acid. The resulting slurry was filtered through a fine steel gauze to remove debris and was suspended in sterile PBS solution to a final concentration of 5×10^{11} OBs/L and stored at 4°C until use. Five samples of OB suspension were each counted twice using a Neubauer Improved chamber (Hawksley, Lancing, UK) under phase contrast microscopy at $\times 400$. A total of 30 L of OB suspension was prepared for greenhouse trials.

Verification of SeMNPV activity

The biological activity of the formulated product was evaluated by bioassay in second instar *Spodoptera exigua* using a modified droplet feeding bioassay technique (Hughes et al. 1986). Thirty newly moulted larvae were starved for 16 h and then allowed to drink from an aqueous suspension containing OBs, 10% (w/v) sucrose and 0.001% (v/v) Fluorella Blue food colouring. Each group of larvae was supplied with an aqueous suspension containing one of the following concentrations: 2.3×10^5, 7.6×10^4, 2.5×10^4, 8.5×10^3, 2.8×10^3 OBs/mL, calculated to result in 10–90% mortality. Previous calibration studies indicated that second instar *S. exigua* consume 0.33 μL of aqueous suspension (Hughes & Wood 1981; Muñoz et al. 1997) so that mean ingested doses of OBs were 75.2, 25.1, 8.4, 2.8 and 0.9 for each concentration of virus supplied, respectively. A virus free solution was also fed to a group of insects as a control. Groups of 25 larvae that ingested the suspension in a period of 15 min were transferred individually to a plastic box divided in 25 square compartments, provided

fresh diet and held at $25 \pm 2^{\circ}$C. Larvae that failed to consume the droplet were discarded. Three repetitions were performed simultaneously. Virus induced mortality was assessed at 7 days post-inoculation, after which time all infected larvae had died (Muñoz et al. 1997). The 50% lethal dose was estimated by logit regression using the Generalized Linear Interactive Modelling (GLIM) program with a binomial error distribution specified (Numerical Algorithms Group 1993).

Greenhouse trial 1: Efficacy of SeMNPV

Tests were performed in eleven commercial greenhouses located in a 25-km radius of the town of El Ejido, Almeria, Spain. The owners of these greenhouses had volunteered to participate in a programme to evaluate greenhouse integrated pest management practices in the region. SeMNPV was supplied to the growers in 1-L polythene bottles containing 5×10^{11} OBs/L. Following usual greenhouse practices, growers were instructed to apply the product in a volume of water sufficient to wet the crop entirely and we recommended the use of a commercial wetter-sticker (Agral®, Syngenta Agro S.A., Madrid, Spain) at 0.05% (v/v) and an acidifier (polycarboxylic acid, Químicas Meristem S.L., Valencia, Spain), to reduce the final pH to ~ 6.5. After shaking the virus suspension well, OBs were added to the spray tank at a rate of ~ 1 mL of SeMNPV suspension per L of spray volume, equivalent to 5×10^{8} OBs/L of spray. Two applications of OBs were made in each greenhouse at an interval of 7–9 days apart, depending on the growers other commitments.

Sweet pepper crops differed in height (0.50–1.40 m) between greenhouses but were always planted at 1-m intervals with a 1-m space between rows. As planted area and crop phenology differed in each greenhouse, the volume required for each spray application also differed in each greenhouse. The planted area varied from 2000 to 10 000 m^2 and the spray volume varied from 300 to 1000 L, equivalent to a spray rate of 63–225 mL/m^2, in the eleven greenhouses used in trials (Table I). As a result, the dose of OBs varied from 2.9×10^{11} to 1.1×10^{12} OBs/ha at each application. In all cases, applications were made using air-assisted hydraulic sprayers with a cone nozzle at a pressure of 2–4 kg/cm^2.

Plant damage. The number of plants with feeding damage was estimated in each greenhouse just before the first application (0 days) and at 7–9 days after each spray application. Systematic sampling for plant damage was performed as follows. All greenhouses were rectangular in shape and were divided into sectors by walkways. Every fourth row of every sector was selected and every plant in that row was examined for signs of defoliation characteristic of feeding by *S. exigua* larvae. The procedure was repeated until the experimenter reached the end of each sector. An average ($\pm$S.D.) of 2470 ± 1458 plants were examined on each occasion (range 680–4500 plants in each greenhouse) giving a total sample size of $N = 81\,532$, considering the three observations in each greenhouse. Feeding by *S. exigua* was classified as recent ($<$48 h old) or old ($>$48 h old) based on the presence of a yellow necrotic zone surrounding the edge of the damaged part of the leaf. As we were mainly concerned with the prevalence of new defoliation, plants that presented a mixture of new and old damage were classified only as newly damaged, until they passed into the old damage category by aging (indicated by the appearance of the necrotic zone). Plants with old feeding damage remained classified in that category for the duration of the study

Table I. Infestation by *Spodoptera exigua*, crop area, spray volume and dose of SeMNPV occlusion bodies applied on two occasions in eleven commercial greenhouses planted with sweet pepper in Almeria, Spain

Infestation level[a] (Greenhouse code)[b]	Treated area (m^2)	Spray volume (L/ha)		Dose of each application[c] (OBs $\times 10^{11}$/ha)
		First application	Second application	
High				
(a)	8000	630	630	3.1
(b)	9000	1100	1100	4.4
(c)	2000	2250	2250	11.2
(d)	4000	750	750	5.0
(e)	9800	1020	1020	5.1
Low				
(f)	10 000	1000	1000	5.0
(g)	6500	620	690	3.1
(h)	8500	590	590	2.9
(i)	6000	750	830	3.8–4.2
(j)	9000	1100	1100	4.4
(k)	4000	750	1000	3.8–5.0

[a] Greenhouses were classified as having a high (>45% plants damaged) or low infestation (<16% plants damaged) of *Spodoptera exigua* larvae based on the prevalence of recent crop damage. [b] Each greenhouse was assigned a letter code that corresponds to the results given in Figure 1 a–k. [c] Single value indicates that OB dose did not change between first and second treatments, whereas two values indicate the dose applied in first and second treatments, respectively.

unless new defoliation occurred. Plant damage estimates were not subjected to statistical analysis due to variation in the scale of the infestations and differences in crop phenology between greenhouses. No other greenhouse pests were observed causing the feeding damage characteristic of *S. exigua* larvae.

SeMNPV mortality. Four days after each application, plants in every greenhouse were checked for *S. exigua* infestation. Groups of 27–118 (mean $\pm$S.D. 52.2 $\pm$ 22.6) larvae were collected from sprayed plants by arbitrarily selecting plants at arbitrary points within the greenhouse. To ensure a minimum sample size of $\sim$30 larvae in each collection, greenhouses with high infestations were searched by three persons for 30 min (1.5 person-hours), whereas greenhouses with low infestations were searched for 4.5 person-hours. Larvae collected from plants were placed individually in plastic pots, taken to the laboratory and reared individually until death or pupation on artificial diet to determine virus mortality (total sample size $N = 1149$ insects). Percentages of mortality were normalised by arcsine transformation and subjected to analysis of covariance with the number of collected larvae (sample size) as a covariable in SPSS ver.12.0 (SPSS Inc., Chicago, IL)

Greenhouse trial 2: Rate of acquisition of infection

In a different trial, greenhouses planted with sweet pepper crops of 1.1–1.5 m height were selected in which virus had not been applied previously. The rate of acquisition of a lethal infection was evaluated after application of 5×10^8 OBs/L of spray in four greenhouses and 1×10^8 OBs/L of spray in three other greenhouses. The planted area of greenhouses ranged from 3500 to 17 000 m^2 in greenhouses treated with 5×10^8

OBs/L applications and 6500–10 000 m^2 in greenhouses treated with 1×10^8 OBs/L applications. Accordingly, spray volumes ranged from 350 to 1000 L and 250 to 400 L in greenhouses treated with the high or low doses of OBs, respectively. Applications were made in the morning (07:00–10:00 h) following the same procedures described above. Groups of 30–74 larvae between third and fifth instars were randomly collected in each greenhouse immediately prior to the application (time point 0) and from treated plants at 6, 24 and 48 h after application. Larvae were individually reared in the laboratory on artificial diet and checked for virus mortality as described above. Percentage of mortality data were normalised by arcsine transformation and subjected to repeat measures analysis of variance in SPSS.

Results

Laboratory virus production

Infected larvae weighed 140–210 mg upon death and produced from 1.2×10^9 to 2.3×10^9 OBs/larva. The corpses of about 80% of the infected larvae were recovered and $\sim$20% were lost to cannibalism resulting in the production of between 1.1×10^{11} and 1.4×10^{11} OBs/box. The application rates used in greenhouse efficacy trials therefore represented the virus production from approximately four boxes of larvae for each hectare treated.

Verification of SeMNPV activity

The LD_{50} value of the experimental batch of SeMNPV in second instars was estimated at 7.8 OBs/larva (95% C.I. 6.1–10.0; log-odds regression slope $= 1.059 \pm 0.105$; intercept $= -2.178 \pm 0.255$; Pearson's $\chi^2 = 0.94$). This value compares favourably with previous estimates of the activity of SeMNPV-SP2 in second instars, reported in two studies as 3.5 and 9.2 OBs/larva (Caballero et al. 1992; Muñoz et al. 1997). The virus batch was therefore approved for use in greenhouse experiments.

Greenhouse trial 1: Efficacy of SeMNPV

Plant damage. Following the first evaluation of plant damage, greenhouses were classified into two groups: high and low larval infestations based on the prevalence of *S. exigua* feeding damage. Thus, five greenhouses had infestations that initially resulted in 45–94% of plants exhibiting signs of recent feeding by *S. exigua* ($<$48 h old) (Figure 1a–e), whereas the remaining six greenhouses had light infestations that initially resulted in $<$16% of plants with signs of recent feeding (Figure 1f–k). The prevalence of recent feeding damage in all the greenhouses with high infestations of *S. exigua* fell dramatically following application of SeMNPV such that the percentage of plants showing recent damage following two applications of SeMNPV ranged from 0.1–9.9%. A concurrent increase was observed in the percentage of plants showing old feeding damage (Figure 1a–e). A decrease in the prevalence of recent feeding damage and an increase in old damage were also observed following SeMNPV applications in greenhouses with low infestations of *S. exigua*, although the effect was not as dramatic as seen in the high infestations (Figure 1f–k).

SeMNPV mortality. A similar number of larvae were collected from sprayed plants in greenhouses with high ($N = 533$) and low infestations ($N = 616$). Virtually all the

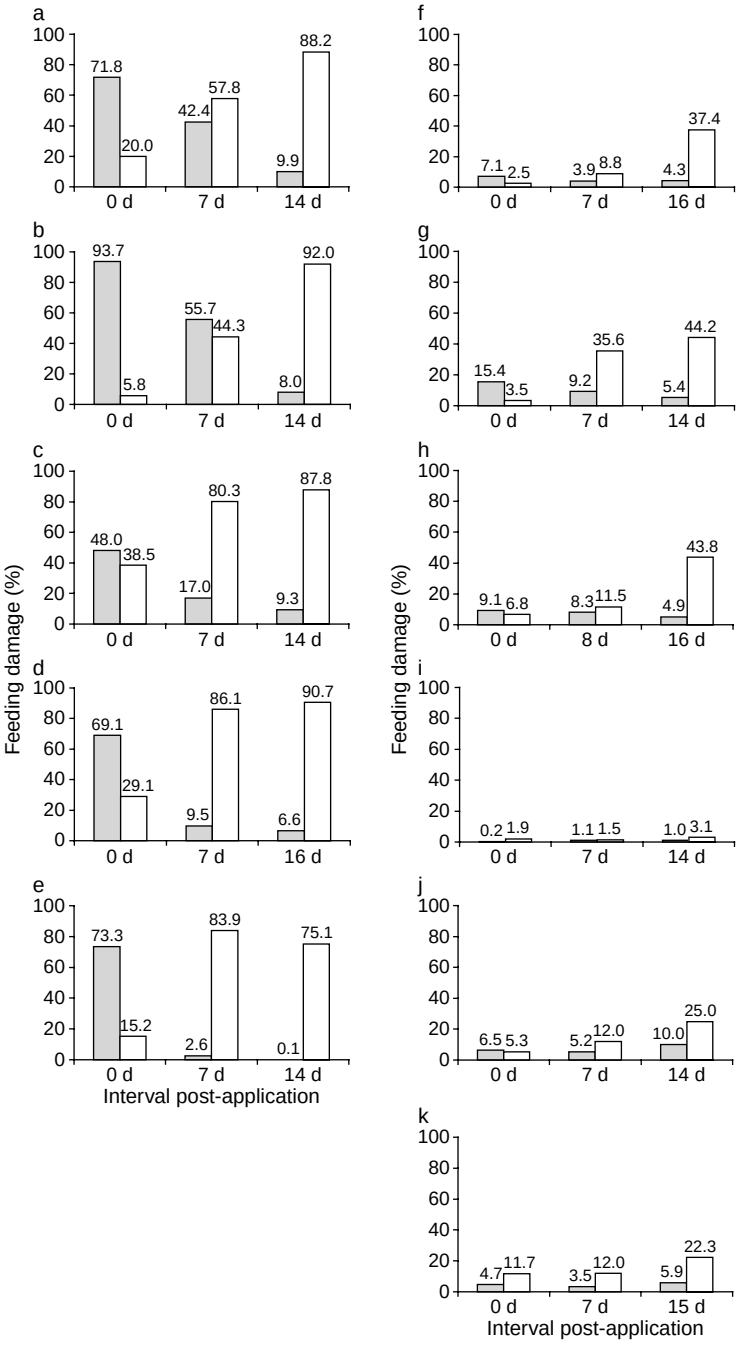

Figure 1. Percentage of sweet-pepper plants showing recent (grey columns) or old (white columns) feeding damage by *Spodoptera exigua* after each of two SeMNPV spray applications of SeMNPV (5×10^8 OBs/L) in eleven commercial greenhouses with high (a–e) and low (f–k) larval infestation conditions in Almeria, Spain. Plant damage was evaluated immediately prior to the first application and at intervals of 7–9 days thereafter. Each graph indicates the results from a different greenhouse.

larvae collected were in the fourth or fifth instar; third instar larvae represented only ~5% of the collected larvae and no earlier instars were collected. Virus induced mortality was high (70–89%) and did not differ between each spray application ($F = 3.01$; df = 1,20; $P = 0.101$) or between greenhouses with high and low infestations of *S. exigua* ($F = 0.431$; df = 1,20; $P = 0.520$) (Figure 2). The sample size of each larval collection from each greenhouse was not a significant covariate ($F = 0.248$; df = 1,20; $P = 0.625$).

Greenhouse trial 2: Rate of acquisition of infection

A low prevalence of virus mortality (mean <1%) was observed in larvae collected from greenhouses prior to virus application, indicating the natural presence of SeMNPV in the *S. exigua* population (Figure 3). The percentage of *S. exigua* larvae that acquired a lethal infection increased significantly over time ($F = 135.81$; df = 3,15; $P < 0.001$). The probability of infection was significantly influenced by application rate ($F = 11.47$; df = 1,5; $P = 0.020$); percentage of infection was 60% in larvae collected at 48 h post-application and reared in the laboratory until death in greenhouses treated with 5×10^8 OBs/L compared to 27% in larvae collected at the same moment in greenhouses treated with 1×10^8 OBs/L. Consequently, the rate at which larvae acquired infection was significantly greater in the high application rate treatment (treatment*time interaction: $F = 10.78$; df = 3,15; $P < 0.001$).

Discussion

Laboratory production of SeMPNV was based on a system in which approximately 320 larvae (four boxes, 80 corpses harvested/box) were required for the production of 5×10^{11} OBs, the rate used in greenhouse trials. The plastic box system could doubtless be improved upon in the future, perhaps by adopting high density rearing techniques, such as those developed and patented by Hughes (1994). The insecticidal activity of laboratory-produced SeMNPV was verified by droplet bioassay in second

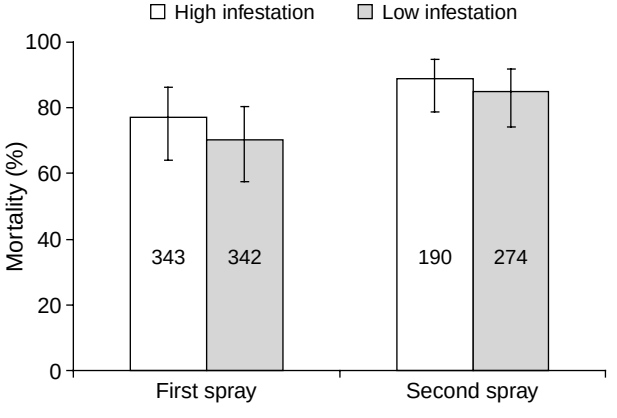

Figure 2. Mean (±S.E.) virus mortality observed in *Spodoptera exigua* larvae collected at 4 days post-application and reared in the laboratory following each of two spray applications of SeMNPV in commercial greenhouses with high and low *S. exigua* infestations. Figures within each bar indicate the total number of larvae collected.

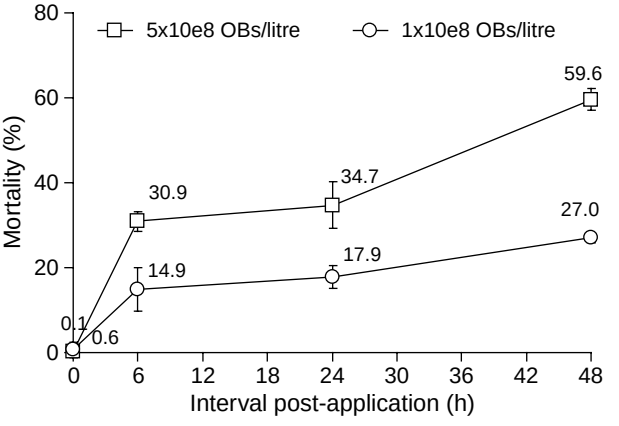

Figure 3. Mean (±S.E.) virus mortality of *Spodoptera exigua* larvae collected at different time points and reared in the laboratory after application of two different concentrations of SeMNPV to sweet pepper plants.

instar *S. exigua* and found to conform to previous reports on the pathogenicity of this Spanish isolate. In contrast, the LD_{50} of a US isolate of SeMNPV, the active ingredient of Spod-X, was calculated to be approximately 50% greater than that of the Spanish isolate (Muñoz et al. 1997).

The prevalence of plants with recent feeding damage fell markedly following application of SeMNPV in greenhouses, despite the slow speed of kill of nucleopolyhedroviruses compared to rapid-acting synthetic insecticides. During the time between consuming a lethal dose of OBs and death, larvae continue to feed and move around in search of suitable foliage. Previous studies have indicated that 95% of the total defoliation of chrysanthemum crops was made by fourth and fifth instar *S. exigua* that consumed between 720 and 1400 mm^2 of leaf area/day, respectively (Smits et al. 1987b). Clearly, it is advantageous to target applications at early instars, before they can cause substantial defoliation. However, detection of infestations of early instars is difficult due to the minor damage to foliar surfaces that they inflict; infestations are not usually detected by the growers until larvae are in the third instar (Smits et al. 1987b). Monitoring systems based on light trap and pheromone trap catches could help in the early detection of significant levels of infestation in each greenhouse.

Insect feeding behaviour is highly influential on the probability of infection of pathogens that are transmitted by ingestion (Hunter & Schultz 1993; Goulson et al. 1995). In pepper crops, *S. exigua* third to fifth instars preferentially feed on young leaves at the growing tips of the plant, to which spray applications of OBs can be easily targeted (Belda et al. 2000). Feeding behaviour differs in other crops, such as Chinese kale or shallots, where *S. exigua* larvae feed in concealed parts plants that provide a refuge against spray deposits, resulting in poor levels of pest control (Smits et al. 1987b; Kolodny-Hirsch et al. 1997). Foliar feeding on sweet pepper represents indirect damage and does not seriously affect the value of sweet pepper harvest. However, when infestation levels are high, *S. exigua* larvae can act as a direct pest by feeding on the pepper fruits causing scaring, rotting, and a significant reduction in the value of the harvest (Moreno et al. 1992; Belda et al. 2000). In this respect, a change in the behaviour of virus infected late instar *S. exigua* was noted as they moved away from pepper fruits and climbed to the upper parts of the plant where they died. Such

behaviour is characteristic of baculovirus infection and increases the likelihood of transmission of these pathogens (Vasconcelos et al. 1996; Goulson 1997).

Approximately 77% of larvae collected following the first application died in the laboratory of virus infection and this rose to around 89% following the second application. The air-assisted spraying system that was used in all cases evidently provided good coverage of spray deposits on crops. The sweet pepper plants have a dense foliage that facilitates the interception of spray droplets and improves the probability that larvae acquire a lethal dose of OBs shortly after each application. The susceptibility of larvae to infection decreases with age but is compensated for by a higher consumption of foliage, and therefore OB deposits, in older instars (Smits 1986). As the prevalence of virus-induced mortality did not increase dramatically in insects collected after the first and second applications, we assume that secondary cycling of virus, which results in a large but spatially heterogeneous increase in inoculum, contributed little to overall insect mortality.

The SeMNPV insecticide provided excellent crop protection, especially in high density infestations seen in five of the eleven treated greenhouses where chemical control measures based had failed, despite the use of modern active materials such as spinosad, indoxacarb, flufenoxuron, lufenuron, tebufenozide and *Bacillus thuringiensis* either singly, or in cocktails containing various products (R. Lasa, unpublished data).

The short life-span of new insecticides in Almerian greenhouses underscores the importance of proactively implementing resistance management strategies to preserve the usefulness of viral insecticides such as SeMNPV. Similar concerns have been noted for control of *S. exigua* populations in horticultural production in S.E. Asia (Kolodny-Hirsch et al. 1997). Alternation of SeMNPV use with novel biorational insecticides, particularly during the mid to late season period when pest pressure is especially high, should provide effective crop protection and reduce the risk of development of resistance. The adoption of the SeMNPV insecticide would also open the way to the use of other biocontrol agents against greenhouse pests.

Previous studies had indicated that the first 48 h of feeding by *S. exigua* larvae was key to the acquisition of infection (Lasa et al. 2007). We therefore examined the rate at which insects became infected at two different application rates. It was clear that many insects acquire infection during the first 6 h following application, whereas in the following 18-h period the proportion of infected insects does not increase substantially irrespective of application rate. This is probably due to low feeding rates during the period of darkness and cool night-time temperatures that coincided with the 6–18-h interval. The probability of infection rose again in the following 24-h period (24–48 h post-application) that included another period of daytime feeding. In such a situation, it would be clearly advantageous to ensure that all virus applications are made early in the morning to achieve maximum pest control. The proportion of the pest population that acquired infection was clearly dose-dependent with the low application rate (1×10^8 OBs/L) resulting in approximately half the number of infected insects compared to the high application rate (5×10^8 OBs/L). Applications of SeMNPV at a rate intermediate between those that we used (3×10^{11} OBs/ha) resulted in $\sim$90% mortality in first to fourth instar *S. exigua* on chrysanthemum crops (Smits et al. 1987a; Bianchi et al. 2000) and provided good control of this pest in Thailand on garden peas, grapes, tomato, pepper and chickpea, but not on Chinese kale or shallot (Kolodny-Hirsch et al. 1997).

In conclusion, greenhouse trials of a SeMNPV insecticide based on a native Spanish isolate resulted in excellent control of *S. exigua* populations on sweet pepper crops in Almeria, Spain. The viral insecticide effectively controlled infestations that could not be controlled by any other commercial insecticide. The number of insects that acquired infection increased rapidly in the 6-h period immediately following application of OBs and subsequently increased at a reduced rate until ~80% of the population were infected at 4 days post-application. The vast majority of infected insects died at 5–9 days post-application resulting in excellent levels of crop protection, especially when a second application was made some 7 days after the first. We recommend the registration of this product as a biological insecticide for *S. exigua* control in the greenhouses of this region.

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References

Belda JE, Mirasol E, Escribano A, Rapallo S, Caballero P. 2000. Eficacia de un nucleopoliedrovirus (SeNPV) en el control de *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae) en pimiento de invernadero. Boletín de Sanidad Vegetal de Plagas 26:619–628.

Bianchi FJJA, Joosten NN, Vlak JM, Van Der Werf W. 2000. Greenhouse evaluation of dose and time mortality relationships of two nucleopolyhedroviruses for the control of beet armyworm, *Spodoptera exigua*, on chrysanthemum. Biological Control 19:252–258.

Bianchi FJJA, Joosten NN, Vlak JM, Van Der Werf W. 2001. The influence of greenhouse chrysanthemum on the interaction between the beet armyworm, *Spodoptera exigua*, and the baculovirus SeMNPV: parameter quantification for a process based simulation model. Journal of Applied Entomology 125:557–562.

Caballero P, Zuidema D, Santiago-Alvarez C, Vlak JM. 1992. Biochemical and biological characterization of four isolates of *Spodoptera exigua* nuclear polyhedrosis virus. Biocontrol Science and Technology 2:145–157.

Garrido A, Martínez JL, Lopéz T, Cotés S, Martinez I. 2004. Monitoring multi-class pesticide residues in fresh fruits and vegetables by liquid chromatography with tandem mass spectrometry. Journal of Chromatography A 1048:199–206.

Goulson D. 1997. Wipfelkrankheit: Modification of host behaviour during baculoviral infection. Oecologia 109:219–228.

Goulson D, Hails RS, Williams T, Hirst ML, Vasconcelos SD, Green BM, Carty TH, Cory JS. 1995. Transmission dynamics of a virus in a stage-structured insect population. Ecology 76:392–401.

Hughes PR. 1994. High density rearing system for larvae. US Patent no. ,351 5:643.

Hughes PR, Wood HA. 1981. A synchronous peroral technique for the bioassay of insect viruses. Journal of Invertebrate Pathology 37:154–159.

Hughes PR, Vanbeek NAM, Wood HA. 1986. A modified droplet feeding method for rapid assay of *Bacillus thuringiensis* and baculoviruses in noctuid larvae. Journal of Invertebrate Pathology 48:187–192.

Hunter MD, Schultz JC. 1993. Induced plant defenses breached – phytochemical induction protects an herbivore from disease. Oecologia 94:195–203.

Jacas JA, Urbaneja A, Viñuela E. 2006. History and future of introduction of exotic arthropod biological control agents in Spain: A dilemma? BioControl 51:1–30.

Jones KA, Burges HD. 1998. Principles of formulation and application. In: Burges HD, editor. Formulation of microbial biopesticides: beneficial micro-organisms, nematodes and seed treatments. Dordrecht: Kluwer.

Kolodny-Hirsch DM, Sitchawat T, Jansiri T, Chenrchaivachirakul A, Ketunuti U. 1997. Field evaluation of a commercial formulation of the *Spodoptera exigua* (Lepidoptera: Noctuidae) nuclear polyhedrosis virus for control of beet armyworm on vegetable crops in Thailand. Biocontrol Science and Technology 7:475–488.

Lara L, Urbaneja A. 2002. Control biológico de plagas en pimiento en la provincia de Almería. Horticultura 165:86–90.

Lasa R, Ruiz-Portero C, Alcázar MD, Belda JE, Caballero P, Williams T. 2007. Efficacy of optical brightener formulations of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) as a biological insecticide in greenhouses in southern Spain. Biological Control 40:89–96.

Mascarenhas VJ, Graves JB, Leonard BR, Burris E. 1998. Susceptibility of field populations of beet armyworm (Lepidoptera: Noctuidae) to commercial and experimental insecticides. Journal of Economic Entomology 91:827–833.

Moreno R, Téllez MM, Benítez E, Gomez J, Rodriguez MD, Sáez E, Belda JE, Cañero R, Cabello T. 1992. Lucha integrada: aplicación en los cultivos bajo plástico del sur de España. Horticultura 1:41–54.

Moulton JK, Pepper DA, Dennehy J, Dugger P, Richter D. 1999. Studies of resistance of beet armyworm (*Spodoptera exigua*) to spinosad in field populations from the southern USA and southeast Asia. Proceedings of the Beltwide Cotton Conferences. Orlando, FL, USA 2:884–887.

Moulton JK, Pepper DA, Jansson RK, Dennehy TJ. 2002. Pro-active management of beet armyworm (Lepidoptera: Noctuidae) resistance to tebufenozide and methoxyfenozide: Baseline monitoring, risk assessment, and isolation of resistance. Journal of Economic Entomology 95:414–424.

Muñoz D, Vlak JM, Caballero P. 1997. *In vivo* recombination between two strains of the genus Nucleopolyhedrovirus in its natural host, *Spodoptera exigua*. Applied and Environmental Microbiology 63:3025–3031.

Muñoz D, Castillejo JI, Caballero P. 1998. Naturally occurring deletion mutants are parasitic genotypes in a wild-type nucleopolyhedrovirus population of *Spodoptera exigua*. Applied and Environmental Microbiology 64:4372–4377.

Numerical Algorithms Group 1994. In: Francis B, Green M, Payne C, editors. The GLIM system: Release 4 Manual. Oxford, UK: Clarendon Press.

Pinnock DE, Brand JR. 1981. A quantitative approach to the ecology of the use of pathogens for insect control. In: Burges HD, editor. Microbial control of pests and diseases. New York: Academic Press. pp 655–665.

Smagghe G, Auda M, Vanlaecke K, Degheele D. 1997. Significance of penetration and metabolism on topical toxicity of diflubenzuron in *Spodoptera littoralis* and *Spodoptera exigua*. Entomologia Experimentalis et Applicata 82:255–260.

Smagghe G, Pineda S, Carton B, Del Estal P, Budia F, Viñuela E. 2003. Toxicity and kinetics of methoxyfenozide in greenhouse-selected *Spodoptera exigua* (Lepidoptera: Noctuidae). Pest Management Science 59:1203–1209.

Smits PH. 1986. Calculations on the polyhedra intake by beet armyworm larvae feeding in virus sprayed chrysanthemums. In: Samson RA, Peters D, Vlak JM, editors. Fundamental and applied aspects of invertebrate pathology. Wageningen: Veldhoven. pp. 616–619.

Smits PH, Van De Vrie M, Vlak JM. 1987a. Nuclear polyhedrosis virus for control of *Spodoptera exigua* larvae on glasshouse crops. Entomologia Experimentalis et Applicata 43:73–80.

Smits PH, Vanvelden MC, Vandevrie M, Vlak JM. 1987b. Feeding and dispersion of *Spodoptera exigua* larvae and its relevance for control with a nuclear polyhedrosis-virus. Entomologia Experimentalis et Applicata 43:67–72.

Smits PH, Rietstra IP, Vlak JM. 1988. Influence of application techniques on the control of beet armyworm Larvae (Lepidoptera: Noctuidae) with nuclear polyhedrosis-virus. Journal of Economic Entomology 81:470–475.

Torres Vila LM, Rodrigues Molina MC, Lacasa A, Palo EJ, Mejias M, Guerrero M. 1998. Susceptibilidad de 20 insecticidas de *Helicoverpa armigera y Spodoptera exigua* en la Vega del Guadiana (Extremadura). Boletín de Sanidad Vegetal de Plagas 24:353–362.

Vasconcelos SD, Cory JS, Wilson KR, Sait SM, Hails RS. 1996. Modified behavior in baculovirus-infected lepidopteran larvae and its impact on the spatial distribution of inoculum. Biological Control 7:299–306.