

## Application of juvenile hormone analogue and optical brightener technologies to the production of *Spodoptera frugiperda* multiple nucleopolyhedrovirus

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**Abstract:** Final instar larvae of *Spodoptera frugiperda* grew to a maximum weight of  $622 \pm 13$  mg on diet treated with 1% piriproxyfen, a juvenile hormone analogue (JHA), or  $695 \pm 17$  mg on diet treated with 1% fenoxycarb, both of which were significantly greater weights than observed in larvae that developed on untreated diet ( $512 \pm 9$  mg). Virus mortality in insects inoculated with SfMNPV occlusion bodies (OBs) was approximately 50% in all treatments, reflecting the resistance of late instar *S. frugiperda* larvae to infection. However, JHA treatment did not result in a significant increase in the total OB yield, or OBs per mg larval weight, compared to untreated infected insects. We determined whether resistance to infection could be overcome by inoculation with mixtures of OBs and optical brighteners. Potentiation of OB activity was >2500-fold in mixtures with 1% Leukophor AP or Blankophor BA, or >15-fold in mixtures with 0.1% of either brightener, compared to SfMNPV OBs alone. We conclude that SfMNPV production was not increased in JHA treated larvae because the JHA did not result in a supernumerary instar in *S. frugiperda*. However, optical brighteners were highly effective in overcoming developmental resistance to infection in this species.

**Key words:** baculovirus production, piriproxyfen, fenoxycarb, leukophor AP, blankophor BA

### Introduction

Baculovirus-based insecticides have to be produced in living insects. This represents a constraint in the commercial development of baculovirus-based products because mass rearing of insects is costly, and because insect cultures often show changes in vigor that result in temporal variation in the quality and quantity of insects that can be infected during the virus production process. Recently, we showed that treatment of late instar *Spodoptera exigua* larvae with juvenile hormone analogues (JHA), such as fenoxycarb or methoprene resulted in the production of a supernumerary instar that was larger and weighed more than untreated final instar insects (Lasa et al., 2007). Infection of the JHA-treated larvae with high doses of virus occlusion bodies (OBs) resulted in 2.7 to 2.9-fold increases in total OB yields per larva compared to yields from untreated infected insects. In the present study we examined whether JHA technology could be effectively applied to increase the production of a multiple nucleopolyhedrovirus (SfMNPV) of the fall armyworm, *Spodoptera frugiperda*, that is a serious pest of maize and sorghum in the Americas. We also examined the potential of two stilbene optical brighteners to reduce the concentration of OBs required for lethal infection of this pest.

## Material and methods

### *Insects, virus, JHA and optical brighteners*

Larvae of *S. frugiperda* were obtained from a laboratory colony maintained in the insectary of the Universidad Pública de Navarra, Pamplona, Spain. The insects were subjected to the following conditions during rearing and during the experiments described below:  $26 \pm 2^\circ\text{C}$ ,  $60 \pm 5\%$  R.H., 16 h: 8 h L:D photoperiod. Larvae were reared using a wheatgerm-based semisynthetic diet described previously (Muñoz et al., 2001). OBs of a Nicaraguan isolate of SfMNPV were fed to fourth instars that were subsequently reared on uncontaminated semisynthetic diet. Patently diseased larvae were collected, triturated in 0.1% SDS and OBs were collected by filtering through muslin and centrifugation at 90 g for 5 min to eliminate insect debris. Two JHAs were obtained in the form of commercial insecticide products. Fenoxycarb was obtained as a water dispersible granule formulation (Zambu, 25% WG, Agro Artes, Castellón, Spain), whereas pyriproxyfen was obtained as a liquid formulation (Juvinal, 10% EC, Kenogard, Barcelona, Spain). The stilbene optical brighteners used in this study were Blankophor BA (Bayer, Barcelona, Spain) and Leukophor AP (Croma, Gipuzcoa, Spain).

### *Effects of JHAs on insect growth and OB production*

Recently-moulted fifth instars were placed individually in 25 ml plastic pots that had been filled to a depth of 2 mm with semisynthetic diet. The surface area of the exposed diet was  $625 \text{ mm}^2$ . Groups of 30 larvae were randomly assigned to one of the following treatments involving applications to the surface of the diet: (i) water (control) (ii) 1% piriproxyfen, (iii) 1% fenoxycarb, (iv) SfMNPV OBs alone (v) SfMNPV OBs + 1% piriproxyfen, (vi) SfMNPV OBs + 1% fenoxycarb. In all cases virus treatments were based on a concentration of 1000 OBs/ $\text{mm}^2$  diet surface (625,000 OBs/pot). All treatments were applied in a volume of 55  $\mu\text{l}$  of water. Larvae were weighed individually 12 days after starting the experiment. To determine OB production, ten infected larvae from each treatment were randomly selected, individually homogenized in 600  $\mu\text{l}$  sterile distilled water, washed twice, suspended in a total volume of 2 ml and subjected to counting in triplicate in a Neubauer haemocytometer. The experiment was performed three times.

### *Effects of optical brighteners on the lethal concentration of SfMNPV OBs*

To determine the effect of two optical brighteners on 50% lethal concentration values of SfMNPV, trays of semisynthetic diet, 2 mm in depth, were subjected to surface contamination treatments involving water alone (control), one of five concentrations of SfMNPV OBs estimated to result in between 10 and 90% mortality, and mixtures of SfMNPV OBs and either Leukophor AP or Blankophor BA at concentrations of 0.1 or 1.0% (wt./vol.). Each treatment was applied to the surface of the diet, spread gently over the diet surface, and allowed to dry for 45 mins, after which time the diet was cut into  $400 \text{ mm}^2$  squares and placed into 25 compartment plastic dishes. Groups of 25 recently moulted forth instars were individually placed in each of the compartments of each dish and incubated for 7-8 days until pupation or death. The experiment was performed three times.  $\text{LC}_{50}$  values were calculated by logit regression in GLIM.

## Results and discussion

For insects that were not infected by virus, treatment with either JHA resulted in a significant increase in the maximum weight attained by larvae compared to the controls ( $\chi^2 = 83.1$ ; d.f. =

2;  $P < 0.05$ ). The average maximum weight of untreated insects was  $512 \pm 9$  mg at 5 days after the start of the experiment, after which the insects began to pupate. This compares to maximum a weight of  $695 \pm 17$  mg in fenoxycarb-treated insects, that were significantly heavier than piriproxyfen-treated insects ( $622 \pm 13$  mg) ( $Z = 3.70$ ;  $P < 0.05$ ), both at 9 days post-treatment. Unlike the situation in *S. exigua*, JHA treatment did not induce a supernumerary moult, but instead extended the duration of the final instar, which continued to grow until death, an effect that has also been observed in *S. littoralis* (Gelbič & Nemec, 2001) and *Bombyx mori* (Miranda et al., 2002).

Virus mortality in insects inoculated with SfMNPV OBs was approximately 50% in all treatments, reflecting the resistance of late instar *S. frugiperda* larvae to NPV infection (Table 1). Infected larvae that were treated with piriproxyfen were significantly heavier immediately prior to death than untreated or fenoxycarb-treated insects (Kruskal-Wallis  $\chi^2 = 6.45$ ; d.f. = 2;  $P = 0.04$ ). However, treatment with piriproxyfen or fenoxycarb did not result in a significant increase in the total OB yield per insect compared to untreated insects (Kruskal-Wallis  $\chi^2 = 0.97$ ; d.f. = 2;  $P = 0.62$ ), and no differences were observed in the production of OBs per mg larval weight in any of the treatments (Table 1).

Table 1. Effect of JHA treatments on larval weight, total OB yield and OB yield per mg larval weight in *Spodoptera frugiperda* larvae inoculated with SfMNPV.

Treatment	Weight of larvae (mg)	Total OB yield per larva ( $\times 10^9$ )	OBs per mg larval weight ( $\times 10^6$ )
SfMNPV alone	$328.3 \pm 12.9$ a	$2.73 \pm 0.19$ a	$8.30 \pm 0.53$ a
SfMNPV + 1% piriproxyfen	$393.4 \pm 18.7$ b	$2.61 \pm 0.22$ a	$6.94 \pm 0.55$ a
SfMNPV + 1% fenoxycarb	$357.9 \pm 25.4$ a	$2.90 \pm 0.24$ a	$8.47 \pm 0.69$ a

Figures followed by similar letters did not differ significantly for comparisons within columns (Mann-Whitney U-test,  $P > 0.05$ ).

To determine whether resistance to SfMNPV infection could be overcome by inoculating insects with mixtures of OBs and optical brighteners, the concentration-mortality response was determined in fourth instars inoculated with SfMNPV and Leukophor AP or Blankophor BA. The estimated  $LC_{50}$  value of SfMNPV OBs alone was  $384$  OBs/mm<sup>2</sup> of diet surface (Table 2). OBs in mixtures with optical brighteners were significantly more pathogenic than SfMNPV OBs alone ( $\chi^2 = 111.9$ ; d.f. = 4;  $P < 0.05$ ). Potentiation of OB activity was >2500-fold in mixtures with 1% of either brightener, or >15-fold in mixtures with 0.1% of either brightener, compared to SfMNPV OBs alone. The brighteners were equally effective synergists; no significant differences were detected in the potentiation activity of the optical brighteners when applied at a concentration of 1% ( $t = 0.076$ ; d.f. = 1;  $P > 0.05$ ) or 0.1% ( $t = 1.28$ ; d.f. = 1;  $P > 0.05$ ).

These results showed a clear effect of optical brightener concentration on the degree of potentiation in mixtures of optical brighteners and OBs, in line with previous observations on SfMNPV (Martínez et al., 2003). The cost of these products has also been highlighted as an issue of concern (Martínez et al., 2000), but at 3.16 €/kg for Blankophor BA and 3.10 €/kg for Leukophor AP, these compounds can be considered economical. It seems likely that increased pest control and/or reduced quantities of OBs necessary in each application mean that the use of optical brightener formulations of SfMNPV may represent an attractive option for control of this important pest.

Table 2. Logit analysis of the concentration-mortality response of *Spodoptera frugiperda* fourth instars fed SfMNPV OBs alone or in mixtures with one of two optical brighteners.

Treatment	Regression <sup>a</sup>	LC <sub>50</sub> (OBs/mm <sup>2</sup> )	Range of 95% C.I.		Relative potency
			upper	lower	
SfMNPV	$y = 0.7175 x + 8.57$	384.8	1105.2	134.0	1
SfMNPV + 1% Blankophor BA	$y = 0.7175 x + 2.89$	0.14	0.24	0.08	2738
SfMNPV + 0.1% Blankophor BA	$y = 0.7175 x + 6.30$	16.2	37.1	7.1	23.8
SfMNPV + 1% Leukophor AP	$y = 0.7175 x + 2.92$	0.14	0.25	0.08	2640
SfMNPV + 0.1% Leukophor AP	$y = 0.7175 x + 6.58$	24.1	59.7	9.7	16.0

<sup>a</sup>A test for non-parallelism was not significant ( $\chi^2 = 9.30$ ; d.f. = 4;  $P > 0.05$ ).

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