

Juvenile hormone analogs greatly increase the production of a nucleopolyhedrovirus

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Abstract

The commercial production of baculovirus insecticides is limited by the need to produce the virus in living insects. The influence of juvenile hormone analogs (JHA) on the growth and survival of *Spodoptera exigua* larvae placed on treated diet in the fifth instar was examined. Weight increases observed in methoprene- and fenoxycarb-treated larvae were over three-fold greater than that of control insects, whereas other compounds resulted in lower weight gains (pyriproxyfen) or highly variable responses (hydroprene). Approximately 90% and 70% of fenoxycarb and methoprene-treated larvae, respectively, molted to a supernumerary sixth instar and attained a final weight at 8–10 days post-treatment that was approximately double the maximum weight observed in control larvae. Inoculation of fenoxycarb and methoprene-treated sixth instars with a nucleopolyhedrovirus (SeMNPV) resulted in 2.4- or 2.9-fold increases in final weights, compared to control larvae inoculated in the fifth instar. The total yield of SeMNPV occlusion bodies (OBs) per larva was 2.7- and 2.9-fold greater in fenoxycarb- and methoprene-treated larvae, respectively, compared to fifth instar controls. A significant but small increase in the yield of OBs/mg larval weight was observed in fenoxycarb-treated insects but not in the methoprene treatment. The LC_{50} value of OBs harvested from fenoxycarb-treated insects was slightly higher than that of OBs from control insects, whereas no such difference was observed in OBs from methoprene-treated insects. We conclude that appropriate use of JHA technology is likely to provide considerable benefits for the mass production of baculoviruses.

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1. Introduction

The commercial production of baculovirus insecticides is limited by the need to produce the virus in living insects. This necessitates a large and productive insect colony as a source of healthy larvae that can be inoculated with lethal amounts of virus occlusion bodies (OBs) and later harvested as moribund or dead infected insects. Previous attempts to improve the efficiency of virus production processes have focused on the development of suitable insect rearing containers (Hughes, 1994; Ignoffo and Boening, 1970; Vail et al., 1973), reducing the handling times required for certain aspects of the process (Bell et al., 1981),

optimization of the quantity of inoculum (Cherry et al., 1997), the rearing temperature (Ignoffo, 1966; Shapiro et al., 1981c), and the timing of harvesting of infected insects (Cherry et al., 1997; Shapiro, 1986).

Similar efforts to reduce the direct costs of virus production have examined the benefits of modifying the ingredients and their relative abundance in artificial diets for rearing infected larvae (Bell et al., 1981; Hunter-Fujita et al., 1998; Shapiro et al., 1981a), and the use of alternative host species that are easy to rear or that produce large larvae (Kelly and Entwistle, 1988; Tompkins et al., 1981).

The key parameters of OB production per larva and OB production per milligram of host weight are sensitive to OB dose, duration of infection, growth rate, total weight gain during the infection period, and final weight at death (Orlovskaya, 1980; Shapiro, 1986; Sherman, 1985). There

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is also clear evidence that baculoviruses can manipulate the host endocrine system to block molting via the expression of an ecdysone glycosyltransferase (*egt*) gene, which extends the duration of the infection, resulting in increases the final yield of viral OBs from each host (O'Reilly, 1997). The penultimate instar is often selected for inoculation as the final instar tends to be refractive to infection and may pupate rather than die from virus (Grzywacz et al., 1998).

Here, we demonstrate that the *per capita* production of a nucleopolyhedrovirus can be greatly increased by producing the virus in a supernumerary host instar resulting from the application of juvenile hormone analogs. This technology is the subject of a patent application for the production of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV), that is currently being tested as a biological insecticide for use in greenhouse crops in southern Spain (Lasa et al., 2007a,b).

2. Materials and methods

2.1. Insect colony, virus strain and juvenile hormone analogs (JHAs)

Larvae of *S. exigua* were obtained from a laboratory colony maintained at 25 ± 2 °C temperature, 70 ± 5 % humidity and 16:8 light/dark photoperiod in the Universidad Pública de Navarra, Spain. A Spanish nucleopolyhedrovirus isolate named SeMNPV-SP2 (Caballero et al., 1992) was grown in fifth instar *S. exigua* larvae, purified by filtration and centrifugation as described previously (Muñoz et al., 1997), quantified by counting in a Neubauer chamber (Hawksley, Lancing, United Kingdom), and stored in aliquots of 100 µl at -20 °C until required. Four commercial insecticides based on JHAs were tested, two terpenoidal compounds: methoprene and hydroprene (both supplied as technical grade material [95% purity] by Dr. Xavier Belles, Instituto de Biología Molecular, Barcelona, Spain), and two aromatic non-terpenoidal compounds: fenoxycarb (Zambu[®] 25 WG, Agro Artés, Castellón, Spain), and pyriproxyfen (Juvinal[®] 10% EC, Kenogard, Barcelona, Spain). Detailed studies on the relationship between JHA treatment and virus production were performed using only fenoxycarb and methoprene, in 2005 and 2006, respectively. Although quantitative data are not available, larvae from the laboratory colony took approximately one day longer to complete their development in 2006 compared to 2005 and it was apparent that the colony was less productive and less vigorous in 2006 than in the previous year.

2.2. Preliminary study for selection of JHAs

Preliminary studies were performed using four different JHA compounds and a range of concentrations. Plastic cups of 25 ml capacity were filled to a depth of 3 mm with artificial diet (625 mm² surface area). The diet surface of

each cup was treated with a volume of 55 µl of JHA solution using a micropipette. The solution was distributed uniformly over the surface of the diet by gently moving the cup from side to side. The following compounds were applied to diet cups (i) pyriproxyfen, (ii) fenoxycarb, (iii) methoprene, (iv) hydroprene, each at a concentration of 2.5 mg a.i./l. Fenoxycarb and pyriproxyfen were dissolved in distilled water whereas methoprene and hydroprene were dissolved in analytical grade acetone. An identical number of control cups were treated with water or acetone alone. After application, diet cups were allowed to dry for 40 min before introducing insects. Groups of 10 *S. exigua* fifth instars that had molted in the previous 10 h were individually weighed (35–50 mg live weight) and a single larva placed in individual treatment or control cups. Larvae were incubated at 25 ± 2 °C for 48 h, the JHA treated diet was replaced with untreated diet and larvae were incubated for a further 8 days. JHA treatment and control larvae were then weighed individually each day for 10 days after the start of the experiment. The experiment was terminated after 10 days due to a high mortality in JHA treated larvae at >10 days post-treatment observed in previous trials (data not shown). The experiment was performed twice on separate days in 2005. The increase in weight that occurred between the day of treatment and the day on which maximum weight was observed (8–10 days after the start of the experiment) was calculated for each insect and subjected to analysis of variance (ANOVA), using the package SPSS v.12.0 (SPSS Inc., Chicago, IL), with day of experiment specified as a block. Multiple comparisons were performed by the Games-Howell post hoc test which is appropriate for handling unequal variances (Sokal and Rohlf, 1995).

2.3. Larval growth response to JHA treatments

Two compounds, fenoxycarb and methoprene, were selected for larval growth studies based on the results of preliminary tests. Plastic cups containing a layer of insect diet were treated with an aqueous solution containing 2.5 mg/l fenoxycarb as described in the section on preliminary studies. An identical number of control cups were treated with distilled water alone. Groups of 10 *S. exigua* fifth instars that had molted in the previous 10 h were weighed individually and placed singly in individual treatment or control cups. Larvae were reared at 25 ± 2 °C and weighed every 24 h during a 10-day period. Fenoxycarb-treated diet was replaced with untreated diet after 48 h of exposure to the JHA. The experiment was performed four times using different batches of insects. The maximum weight attained by larvae was normalized by log_e transformation and subjected to analysis of variance (ANOVA), with insect batch considered as a block, in SPSS v. 12.0.

Larval growth studies with methoprene were nearly identical to those involving fenoxycarb, but with the following differences. Diet cups were treated with an acetone solution containing 2.5 mg/l of methoprene. Identical

numbers of cups were treated with acetone alone or water alone as controls. Larvae were transferred to untreated diet 24 h after placing larvae on methoprene-treated diet. Maximum weights attained could not be normalized by transformation and were subjected to a non-parametric Kruskal–Wallis test in SPSS v.12.0.

2.4. Influence of JHA treatments on OB production

Untreated larvae were inoculated with OBs as fifth instars that had molted in the previous 10 h (35–50 mg live weight), whereas JHA-treated larvae were inoculated as recently molted supernumerary sixth instars (210–270 mg live weight), at 4 or 5 days after being placed on fenoxycarb- or methoprene-treated diet, respectively. In each case, groups of 30 larvae were placed individually in 25 ml cups containing a small cube of diet ($4 \times 4 \times 3$ mm) contaminated with 1×10^6 OBs. Larvae that consumed the diet cube in a 24-h period were individually reared on untreated diet. Larvae were weighed and examined daily for signs of polyhedrosis disease each day until death. Between 10–15 larvae per repetition were considered as uninoculated controls to evaluate control mortality. To facilitate collection of OBs, cups containing dead infected larvae were placed in a -20°C freezer until completely frozen. The frozen cadavers were placed individually in 2 ml plastic Eppendorf tubes containing 1.2 ml sterile distilled water, allowed to thaw and then filtered through fine steel gauze to remove insect debris. The resulting OB suspension was adjusted to a final volume of 1.5 or 2.0 ml using sterile water for control and JHA-treated larvae, respectively. The experiment was performed three times using different batches of insects on different days.

The number of occlusion bodies recovered from each larva was determined using a Neubauer Improved counting chamber under phase contrast microscopy at $400\times$. Two samples were taken for each larva, counted in triplicate, and used to calculate a mean value. The relationship between OB production and host weight (OBs/mg) was estimated based on larval weight recorded on the day prior to death. OB production and larval weight data were normalized by \log_e transformation and subjected to analysis of variance with starting day of experiment considered as a block (ANOVA) in the study involving fenoxycarb, whereas these variables were subjected to Mann–Whitney test for the experiment involving methoprene. Values of OBs per mg of larval weight were normally distributed and were subjected to ANOVA in SPSS, v.12.0.

2.5. Pathogenicity of OBs produced in JHA-treated insects

Virus occlusion bodies (OBs) were collected from 20 randomly selected larvae from each experiment. OB pathogenicity (*sensu* Thomas and Elkinton, 2004) was evaluated in second instar *S. exigua* using a modified-droplet bioassay technique (Hughes et al., 1986). Late first instars were starved for 16 h at $25 \pm 2^\circ\text{C}$ and

allowed to molt to the next instar over a period of 10 h. Groups of 30 recently molted larvae were allowed to feed on droplets of 10% (wt/v) sucrose, 0.001 (v/v) fluorella blue food dye and one of a range of five concentrations of OBs, in the range of 3.8×10^3 – 3.1×10^5 OBs/ml, previously calculated to result in mortalities between 10% and 90%. Control larvae were treated identically but fed on a solution of sucrose and food dye alone. Each of 25 larvae that had ingested the OB suspension within 10 min were placed individually in the cells of a 25 cell tissue culture plate containing diet and incubated at $25 \pm 2^\circ\text{C}$. Mortality was noted at 5 days post-inoculation. The bioassay was performed three times. Results were subjected to logit regression with the Generalized Linear Interactive Modeling (GLIM) program with a binomial error distribution specified (Numerical Algorithms Group, 1993). Minor overdispersion in the mortality results was taken into account by scaling the error distribution where necessary. Pathogenicity was expressed as the 50% lethal dose based on an average ingested volume of $0.33 \mu\text{l}$ per larva in this instar (Chaufaux and Ferron, 1986; Muñoz et al., 1997).

3. Results

3.1. Preliminary study for selection of JHAs

A fraction of the experimental larvae died within 48 h of treatment; this was lowest in the water control (1/20 larvae) and highest in the pyriproxyfen treatment (7/20 larvae). Maximum weights were observed at 2 and 8 days after the start of the experiment for the acetone and water control groups and the fenoxycarb-treated group, respectively, whereas maximum weight was attained at 10 days for all remaining JHA treatments. Acetone treatment resulted in a small but significant reduction in weight gain compared to the water control (Fig. 1). The greatest weight gains were observed in larvae treated with fenoxycarb or methoprene and these two compounds were selected for further study. Pyriproxyfen treatment resulted in the lowest weight gain among the JHA treatments. Hydroprene resulted in an intermediate weight gain, but a high degree of variation was evident in the larval growth response to this compound (Fig. 1).

3.2. Larval growth response to JHA treatments

Larval mortality in the control and fenoxycarb treatment did not exceed 5% over the 10 days of the experiment. Approximately 90% of the fenoxycarb-treated larvae molted into a supernumerary sixth instar at 3–4 days post-treatment. The maximum mean (\pm SD) larval weight observed in fenoxycarb-treated larvae (Fig. 2a) was 599 ± 106 mg on day 8 of the experiment, which was significantly higher than the maximum mean weight observed in control larvae (232 ± 40 mg) on day 2 of the experiment ($F_{1,72} = 480$, $P < 0.001$).

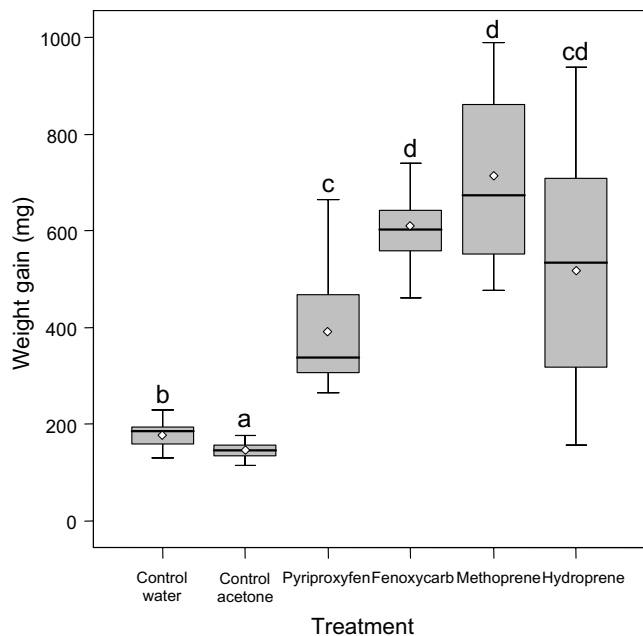


Fig. 1. Box plot indicating results of preliminary study on weight gain response of *Spodoptera exigua* larvae placed on diet treated with four juvenile hormone analogs or acetone or water controls. Maximum weights were observed at 2 days post-treatment for water and acetone controls, 8 days post-treatment for fenoxycarb-treated group and 10 days post-treatment for all remaining treatments. Vertical bars indicate maximum and minimum weight gain, shaded boxes indicate interquartile range, horizontal line indicates the median and white diamond indicates the mean of each treatment. Bars headed by identical letters were not significantly different (ANOVA, Games-Howell test $P > 0.05$).

The prevalence of mortality in larvae treated with acetone or water did not exceed 3% whereas 15% of the larvae treated with methoprene were dead at 48 h post-treatment. Approximately 70% of the surviving methoprene-treated larvae molted into a sixth instar at 4–5 days post-treatment. The maximum mean (\pm SD) larval weight observed in methoprene-treated larvae (Fig. 2b) was 456 ± 202 mg on day 10 of the experiment, which was significantly higher than the maximum mean weight observed in water control larvae (256 ± 34 mg) on day 3 of the experiment or acetone control larvae (199 ± 36 mg) on day 2 of the experiment (Kruskal–Wallis, $\chi^2 = 59.4$, $df = 2$, $P < 0.001$). In both experiments water control larvae lost weight prior to pupation on day 5 or 6 of the experiment. Acetone treatment resulted in lower maximum weight and pupation one day earlier than observed in control larvae that had been treated with water alone.

3.3. Influence of JHA treatments on OB production

Ingestion of 1×10^6 OBs resulted in >90% mortality of fifth and sixth instars in the experiment involving fenoxycarb and 74% mortality in sixth instars that had been subjected to methoprene treatment (Table 1). Less than 6% mortality was observed in uninoculated controls. The final weight of SeMNPV-infected sixth instar larvae was

2.4- and 2.9-fold greater than the corresponding fifth instar infected larvae for the experiments involving fenoxycarb and methoprene, respectively (Fig. 2c and d). Similarly, the total yield of OBs from sixth instars was 2.7- and 2.9-fold greater than the yields from insects inoculated as fifth instars for the experiments involving fenoxycarb and methoprene, respectively. A significant but small increase in OB production per mg larval weight was observed in fenoxycarb-treated larvae compared to the fifth instar controls ($F_{1,171} = 4.15$, $P = 0.043$), whereas OB production/mg larvae weight did not differ significantly between fifth instar control and sixth instar treated larvae in the experiment involving methoprene ($F_{1,150} = 0.595$, $P = 0.442$). Although larvae used in experiments had a similar size and weight at inoculation in both experiments, the final weight at death, *per capita* OB yield and OB yield/mg weight were all higher in the experiment involving fenoxycarb performed in 2005, compared to the experiment involving methoprene performed in 2006, that is likely due to a reduction in the vigor of the laboratory insect colony in 2006.

3.4. Pathogenicity of OBs produced in JHA-treated insects

The slopes of individual dose–mortality logit regression lines (Table 2) did not differ significantly between treatment and controls and could be fitted in parallel with a common slope of 1.15 ± 0.06 in the experiment involving fenoxycarb ($\chi^2 = 0.350$, $df = 1$, $P = 0.554$) and a common slope of 0.84 ± 0.05 in the experiment involving methoprene ($\chi^2 = 0.924$, $df = 1$, $P = 0.336$). The LD_{50} value of OBs produced in sixth instar fenoxycarb-treated larvae was significantly higher than that of OBs produced in fifth instar control insects ($\chi^2 = 6.77$, $df = 1$, $P = 0.009$); the relative potency of OBs from the fenoxycarb treatment was 0.66 in second instar *S. exigua*. In contrast, the LD_{50} value of OBs produced in methoprene-treated insects was significantly lower than that of OBs produced in control insects ($\chi^2 = 5.89$, $df = 1$, $P = 0.018$); the relative potency of OBs produced in methoprene-treated insects was 1.49.

4. Discussion

All the JHAs tested resulted in extended larval development times, prevented pupation and increased larval weights between 2.3- and 4-fold over that of control insects. Considerable variation was observed in larval supernumerary molting and growth responses. This was particularly evident in preliminary studies involving hydroprene, but was also observed in the subsequent detailed growth studies in which the molting and growth responses of larvae treated with methoprene were more variable than those treated with fenoxycarb. These results may have been affected by the duration of exposure and the dose of JHA acquired by insects that were treated with fenoxycarb or methoprene over periods of 24 and 48 h, respectively. The timing of JHA treatment in relation to the previous

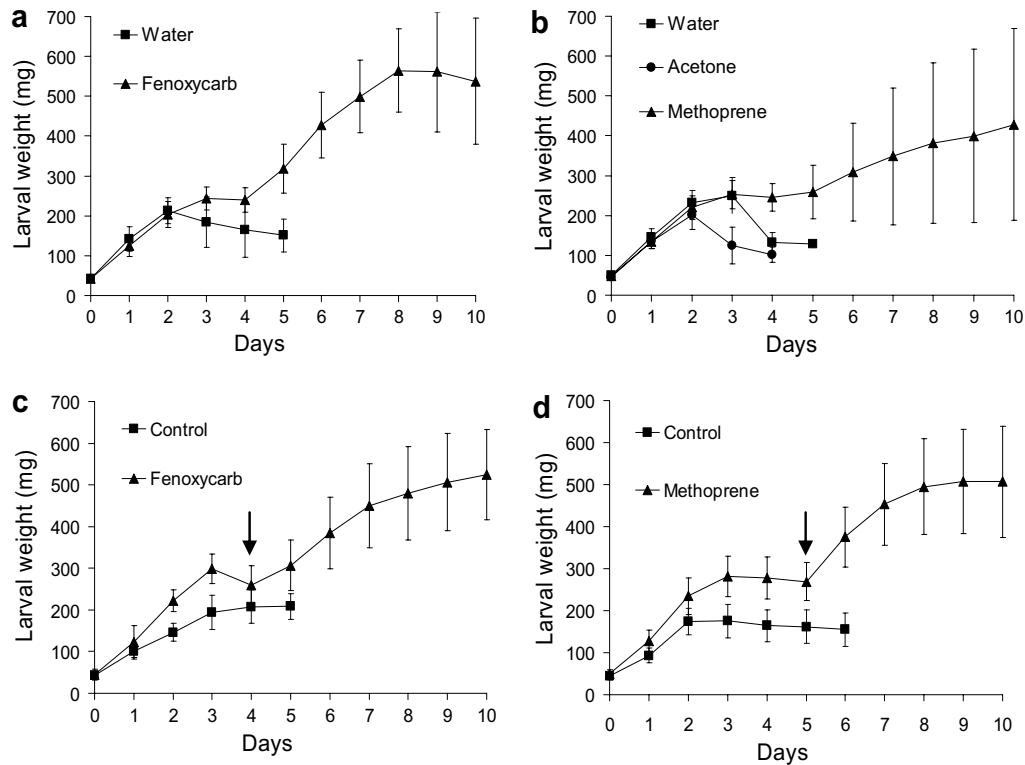


Fig. 2. Weight of healthy *Spodoptera exigua* larvae placed on diet treated with (a) water (control) or an aqueous solution of fenoxycarb (48 h exposure), (b) water, acetone (controls) or methoprene dissolved in acetone (24 h exposure). The influence of SeMNPV infection on mean weight of larvae inoculated with 1×10^6 OBs in the fifth instar (controls, inoculated at time zero) or as mostly sixth instars after being placed on diet treated with (c) fenoxycarb (48 h exposure) or (d) methoprene (24 h exposure). Vertical arrow indicates the moment of inoculation of fenoxycarb- and methoprene-treated larvae. The absence of control data points after (c) day 5 or (d) day 6 was due to virus mortality. Vertical bars indicate SD.

molt, type and concentration of JHA and the target species of insect all strongly influence the likelihood of molting to a supernumerary instar (Hatakoshi et al., 1986, 1988; Rountree and Bollenbacher, 1986).

Larvae inoculated with OBs in the very late fifth and early sixth instars, at 4–5 days following treatment with

JHA developed to a greater weight and produced a significantly greater quantity of OBs compared to control larvae inoculated in the early fifth instar. Total OB yields from control larvae were between 1.6×10^9 and 2.3×10^9 OBs/larva which were similar to yields reported previously for this species (Cherry et al., 1997; Huang and Kao, 1994),

Table 1

Occlusion body production in *Spodoptera exigua* larvae inoculated in the fifth instar (controls) or in a supernumerary sixth instar following treatment with fenoxycarb or methoprene

Instar (treatment) ^a	Number of insects inoculated	Number of insects that died from virus infection	Weight (\pm SD) when OBs collected (mg) ^b	Means (\pm SD) OBs per larva ($\times 10^9$) ^b	Means (\pm SD) OBs per mg larval weight ($\times 10^7$) ^c
Fifth instar (control)	90	85	197.6 \pm 42.5a	2.29 \pm 0.77a	1.20 \pm 0.40a
Sixth instar (fenoxycarb)	90	88	476.8 \pm 116b	6.20 \pm 2.13b	1.31 \pm 0.36b
Fifth instar (control)	90	87	156.3 \pm 37.9a	1.60 \pm 0.45a	1.04 \pm 0.23a
Sixth instar (methoprene)	90	67	453.5 \pm 105b	4.65 \pm 2.18b	1.00 \pm 0.35a

Values followed by identical letters did not differ significantly from that of their respective controls ($P > 0.05$).

^a Statistical comparisons were performed separately for experiments involving fenoxycarb and methoprene.

^b Results of fenoxycarb treatment were \log_e transformed and subjected to ANOVA whereas results of methoprene treatment were subjected to Mann–Whitney U test. In both cases, values followed by identical letters did not differ significantly from that of their respective controls ($P > 0.05$).

^c Results were subjected to ANOVA without prior transformation.

Table 2

Logit regression of dose-mortality response of second instar *Spodoptera exigua* following consumption of SeMNPV OBs produced in larvae inoculated in the fifth instar (controls) or in the sixth instar following treatment with fenoxycarb or methoprene

Source of OBs	Slope \pm SE	Intercept \pm SE	LD ₅₀ (OBs)	Range of 95% CI of LC ₅₀		Scale parameter
				Upper	Lower	
Control	1.19 \pm 0.09	-12.08 \pm 0.96	8.2a	9.8	6.8	1.0
Fenoxycarb	1.12 \pm 0.08	-11.79 \pm 0.94	12.5b	15.2	10.3	1.0
Control	0.77 \pm 0.09	-7.98 \pm 0.98	10.1a	14.4	7.2	1.8 ^a
Methoprene	0.87 \pm 0.07	-8.64 \pm 0.73	6.8a	8.5	5.4	1.0

LD₅₀ values followed by identical letters did not differ significantly for comparisons between treatments and their respective controls ($P > 0.05$).

^a Error distribution was scaled to account for overdispersion in the mortality results.

whereas 2.7- to 2.9-fold greater quantities of OBs were recovered from JHA-treated larvae.

Previous attempts to increase OB yields from individual insects have focused on sexual dimorphism in the number of instars and preferential inoculation of *Lymantria dispar* female larvae that grow larger, and produce more OBs, than males (Shapiro, 1986), and the use of vitamin and protein-rich diets (Shapiro et al., 1981a,b). Studies aimed at increasing baculovirus production using JHAs have focused exclusively on the use of methoprene and have resulted in relatively modest increases (1.2- to 1.5-fold) in larval body weight and OB production compared with untreated infected larvae (Table 3). In contrast, the key aspect to achieving the greatly increased production in the present study was the inoculation of the host insect when many had molted to a supernumerary instar. Infection of a large proportion of late instar larvae can be difficult as late stages tend to be refractive to baculovirus infection. This resistance to infection may be overcome by administering OBs in mixtures with stilbene derived optical brighteners that greatly reduce the dose of OBs required to initiate a lethal infection (Hamm and Chandler, 1996; Shapiro and Argauer, 2001). However, the use of optical brightener technology in combination with JHA

treatment should be carefully evaluated as inocula containing optical brighteners can result in decreased OB yields (Murillo et al., 2003; Shapiro and Robertson, 1992). Moreover, JHA treatment can itself alter insect susceptibility to infection (Longworth and Singh, 1980).

OB yield per mg larval weight was increased slightly in fenoxycarb-treated larvae compared to control insects, but not in methoprene-treated larvae. Modest increases in OB yields/mg tissue have been observed following methoprene treatment in 15- and 17-day-old *L. dispar* + LdMNPV (Kolodny-Hirsch et al., 1995) and *Cydia pomonella* infected with its granulovirus (deduced from graphical data given in Glen and Payne, 1984), whereas OB yields/mg were significantly decreased, compared to those of control insects, following treatment of 25-day-old *L. dispar* larvae or *Pseudoplusia includens* + PiSNPV (Kolodny-Hirsch et al., 1995; Mohamed et al., 1984).

It appears that under certain circumstances, JHA treatments may alter the quantity or availability of host resources for allocation to OB production, although the mechanism by which this occurs is unclear. The mode of action of JHAs has been reviewed in detail (Gilbert et al., 2000; Wilson, 2004). Studies with *Drosophila* and *Manduca sexta* indicate that JHA treatment disrupts the

Table 3

Summary of studies on baculovirus production in insects treated with juvenile hormone analogs

Host species, virus	Compound	Instar treated	Increase in larval weight compared to controls	Increase in yield OBs/larva	Reference
<i>Cydia pomonella</i> , CpGV	Methoprene	First	1.2–1.3	1.8	Glen and Payne (1984)
<i>Epiphyas postvittana</i> , EppoMNPV	Methoprene	First ^a	1.0–1.3	1.8	Longworth and Singh (1980)
<i>Helicoverpa zea</i> , HzSNPV	Methoprene	Second/third ^b	1.28	1.16	Shieh (1989)
<i>Heliothis virescens</i> , AcMNPV	Methoprene	Fifth ^c	1.4	1.4–1.6	Nordin (1981)
<i>Lymantria dispar</i> , LdMNPV	Methoprene	Various ^d	1.5	1.2	Kolodny-Hirsch et al. (1995)
<i>Pseudoplusia includens</i> , PiSNPV	Methoprene	Fifth	1.4–1.5	1.2–1.4	Mohamed et al. (1984)
<i>Spodoptera exigua</i> , SeMNPV	Fenoxycarb	Fifth	2.4	2.7	This study
<i>S. exigua</i> , SeMNPV	Methoprene	Fifth	2.9	2.9	This study

^a Inoculated with OBs at 10 days old.

^b 5-day-old larvae.

^c Inoculum was injected because fifth instars are refractive to AcMNPV peroral infection.

^d 15- to 25-day-old larvae.

metamorphic reorganization of the central nervous system, salivary glands, and musculature in a dose-dependent manner (Restifo and Wilson, 1998). Although severely hampered by the lack of information on the identity of the JH receptor (Wilson, 2004), it is clear that JHAs modulate the expression of the 20-hydroxyecdysone-inducible ecdysone receptor (Hiruma et al., 1999), and influence ecdysone-mediated patterns of gene expression (Restifo and Wilson, 1998). The complexity of the interaction between JH and ecdysone means that the physiological and developmental consequences of endocrinological changes will be particularly difficult to predict in insects infected by baculovirus pathogens. This is because many baculoviruses interfere with the insect endocrine system by modulating ecdysone titers to increase larval biomass and improve the total yield of OBs per host, and thereby enhance the likelihood of transmission (Cory et al., 2004).

The pathogenicity of OBs from methoprene-treated larvae did not differ from that of the control whereas the pathogenicity of OBs from fenoxycarb-treated insects was lower than that of the control, as indicated by the non-overlap of 95% confidence intervals of the estimated LC₅₀ values (Table 2). Changes in the size, number of occluded virions or maturation of OBs can result in increased pathogenicity of OBs harvested *post mortem*; (Bell, 1991; Grzywacz et al., 1998; Ignoffo and Shapiro, 1978; Shapiro and Bell, 1981), whereas these variables may have been altered in JHA-treated insects. No changes in OB pathogenicity were observed in methoprene-treated *P. includens* (Mohamed et al., 1984) or *L. dispar* (Kolodny-Hirsch et al., 1995), compared to OBs produced in control insects.

The use of JHAs in mass production of baculoviruses requires additional study, particularly with regard to reducing the magnitude of variation in larval growth and molting responses, probably by perfecting the concentration, timing and application method required to achieve a uniform insect response. Treated larvae fed for longer and the duration of the rearing-infection-harvesting process was extended by 8–10 days that would likely lead to increased costs of production in JHA-treated insects compared to standard OB production procedures in fifth instars. However, our study indicates that appropriate use of JHA technology is likely to provide considerable benefits for the mass production of baculoviruses.

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