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High level production of polyhedra in a scorpion toxin-containing recombinant baculovirus for better control of insect pests

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ABSTRACT. A sufficient occlusion body yield by a scorpion toxin-containing baculovirus is important to the success of a pest management program. In this study, recombinant baculoviruses, AcMNPV, vAPcmIT$_2$, by which the scorpion toxin (LqhIT$_2$) is driven by an early phase promoter (*p-PCm*); and another recombinant baculovirus vAP10IT$_2$, by which LqhIT$_2$ is driven by a very late *p10* promoter, were tested for the efficiency of their polyhedral production. In Sf21 cells, the yield of polyhedra by vAPcmIT$_2$ is significantly better than that of vAP10IT$_2$. Although in *Trichoplusia ni* (Hübner) and *Spodoptera exigua* (Fabricius) the polyhedra yields by vAPcmIT$_2$-infected larvae were not as good as those of the wild type virus, they were about tenfold higher than those produced by vAP10IT$_2$-infected larvae. To test the insecticidal activity of these recombinant baculoviruses, vAPcmIT$_2$ and vAP10IT$_2$ were applied against two major pesticide-resistant vegetable pests, *Plutella xylostella* (Linnaeus) and *S. exigua*. Our results demonstrated a significant shortening of the lethal time (LT$_{10}$ and LT$_{50}$) compared to those larvae infected with wild type AcMNPV. In field trials, larvae of *S. exigua* infected with the toxin-recombinant viruses provided more than 90% control efficacy and resulted in a 58.7~67.4% reduction in leaf area consumed compared to wild type AcMNPV. Based on the efficacy of polyhedral production and crop protection, the superiority of vAPcmIT$_2$ over both vAP10IT$_2$ and wild type AcMNPV renders it a better candidate to serve as a useful biopesticide.

Keywords: *Autographa californica* nucleopolyhedrovirus; AcMNPV; *Leiurus quinquestriatus hebraeus*; Insecticidal efficacy; Neurotoxin; *Plutella xylostella*; Polyhedral production; Recombinant virus; *Spodoptera exigua*; *Trichoplusia ni*.

INTRODUCTION

The baculovirus has long been used as a useful microbial agent for the control of insect pests. However, infected insects can still feed on crops during the incubation period, resulting in significant economic loss. Therefore, if a toxin gene sequence can be inserted into the genome of baculoviruses and then expressed by promoters which can drive the insect-specific toxin earlier and/or stronger, the insecticidal efficacy can be well accelerated. Recombinant viruses containing scorpion toxin or mite toxin can paralyze infected larvae and prevent them from further damaging crops. These toxin genes can not only make infected larvae stop eating and prevent the loss of crops, they also cause the infected larvae to die earlier, hence strengthening the effect of pest management (Stewart et al., 1991; Tomalski and Miller 1991; Hoover et al., 1995; Hughes et al., 1997; Hernandez-Crespo et al., 1999).

An insect specific neurotoxin from *Leiurus quinquestriatus hebraeus*, LqhIT$_2$, has effective toxicity and shortened time to paralysis (Zlotkin et al., 1993; Benkhalifa et al., 1997; Prikhod'ko et al., 1998; Gershburg et al., 1998; Regev et al., 2003; van Beek et al., 2003). However, the key factors that determine the expression of foreign genes and lethality of neurotoxins are the timing and intensity of promoter expression (Lu et al., 1996; Jarvis et al., 1996; Gershburg et al., 1998; van Beek et al., 2003; Tuan et al., 2005). Previously, the toxin LqhIT$_2$ was expressed by early promoters, including *CMV* minimal (Tuan et al., 2005), *pag90* (Jinn et al., 2006) and *p6.9*

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(Fujita et al., 2006) promoters. In these experiments, the early promoters were shown to be more efficient insecticides than very late promoters (*p10*, vAP10IT$_2$).

Mass production of baculovirus is a bottleneck in the application of microbial insecticides. The yields can vary with pathogenicity of the virus, inoculum concentration, larval stage, body weight, neurotoxin expressed, and the timing and intensity by which the promoter is expressed (Shapiro, 1986; Bonning et al., 1995; Tuan et al., 1995; Ignoffo and Garcia, 1996; 1997; Burden et al., 2000; Harrison and Bonning, 2000). Thus, if an engineered baculovirus is to become an effective biological control agent, its proper production is necessary.

In previously studies, *p10,* the very late promoter, was used most often to drive heterologous proteins in recombinant baculoviruses because of its powerful expression capability (Stewart et al., 1991; Hughes et al., 1997; Burden et al., 2000; Regev et al., 2003). However, the use of Ro10AaIT and Ro10LqhIT$_2$, AcMNPV *p10* promoter resulted in a reduction of polyhedral production *in vitro* (Harrison and Bonning, 2000). The reduced yield of polyhedra can be due to competition among *p10* and *polh* promoters, both strong very late promoters, for transcriptional and/or translation factors (Chaabihi et al., 1993; Volkman et al., 1996). Presumably such competition would be resolved if the expression time of the toxin and polyhedra genes could be separated.

In this report, we compared the effect of the early *p-PCm* and very late p10 promoters on their production of polyhedra. The *p-PCm* promoter contains the human cytomegalovirus minimal (*CMVm*) promoter ligated *in cis* with the polyhedrin upstream (*pu*) sequence. This results in a high-level of expression for the foreign genes at the early infection stage of the baculovirus (Wu et al., 2000; Lo et al., 2002). Different insect species and cell lines were used to produce polyhedra of recombinant viruses that express the toxin protein LqhIT$_2$ using *p10* and *p-PCm* promoters. The insecticidal efficacy and the management potential of the recombinant viruses for different insects using *p10* and *p-PCm* promoter were also evaluated. Importantly, in the expression of toxin gene using *CMVm* and *p10* promoters in different recombinant viruses, the former was found to produce more polyhedra than the latter. These results will be useful for polyhedral production in the practical application of toxin gene-containing recombinant baculoviruses for pest control in the fields.

MATERIALS AND METHODS

Cell lines and viruses

Spodoptera frugiperda IPLB-Sf21AE (Sf21, Vaughn et al., 1977) cells were maintained and propagated in a modified TNM-FH medium containing 8% heat-inactivated fetal bovine serum at 26°C (Lee et al., 1998; Lin et al., 1999). The C6 strain of wild-type AcMNPV (C6-AcMNPV), and two toxin-gene-containing viruses, vAP10IT$_2$, and vAPcmIT$_2$ (Tuan et al., 2005) were propagated in the Sf21 cell line under the conditions mentioned above. On day 7 post infection (p.i.), the polyhedra were collected and quantified (O'Reilly et al., 1992; Tuan et al., 2005). vAP10IT$_2$ and vAPcmIT$_2$ were recombinant AcMNPVs which contained LqhIT$_2$ expressed by two temporal promoters, the very late *p10* and the early *p-PCm*, respectively (Tuan et al., 2005).

Insects

Larvae of the diamondback moth *P. xylostella* and the cabbage looper *T. ni* were collected from Shi-hu County, Taiwan and reared on an artificial diet (Tuan et al., 1997). Colonies were maintained at 22±1°C with 70±5% relative humidity (RH) and a 12L:12D photoperiod. Larvae of the beet armyworm *S. exigua* (Hübner) were collected from Shi-hu County, Taiwan and reared on an artificial diet (Tuan et al., 1997). Colonies were maintained at 25±1°C under the above-mentioned conditions. All insects tested were 2nd, 3rd, or 4th instar larvae after rearing for three generations. Sanitation procedures for maintaining colonies were applied as a precaution against contamination with microorganisms (Tuan et al., 2005). The insects in all experiments were observed daily by smears under a phase-contrast microscope and checked for evidence of baculovirus infection. For bioassays, all molting larvae of the same age were selected and enclosed individually in a 30-well plate overnight. Next morning, the newly molted larvae were starved for 8 h to synchronize larval growth.

Quantification of polyhedra

Sf21 cells were seeded at 10^6 cells/well into 6-well plates (Falcon®) in TNM-FH medium and allowed to attach for 2 h at 26°C. Following removal of the spent medium, 0.5 ml C6-AcMNPV, vAP10IT$_2$, and vAPcmIT$_2$ were added to each well at m.o.i.=1 in triplicate. The inoculated cells were re-incubated at 26°C for 8 days. Visual examination of the infection processes was performed daily until all samples were harvested. Collection and quantification of polyhedra was carried out on day 5, 6, 7, and 8 p.i. (O'Reilly et al., 1992; Tuan et al., 2005). Polyhedra were isolated from Sf21 cells infected with C6-AcMNPV, vAP10IT$_2$, and vAPcmIT$_2$, and diluted to 10^5, 10^6, 10^7, and 10^8 PIBs/ml. Third-instar *S. exigua* and 4th-instar *T. ni* were fed on an artificial diet contaminated with polyhedral suspensions at the doses of 7.8, 78, 780, and 7800 PIBs/mm^3 diet, and reared individually in 30-well plastic plates (Tuan et al., 1997). Inoculated larvae were collected on day 4, 5, and 6 p.i. The polyhedra were quantified according to the method mentioned above. Thirty larvae in three replicates were inoculated in each treatment. All treated larvae were weighed on day 5 p.i.

Measurement of lethal times

Third-instar larvae of *P. xylostella* and *S. exigua* were fed on a diet contaminated with C6-AcMNPV, vAP10IT$_2$,

and vAPcmIT$_2$ at a dose of 780 PIBs/mm^3. Three days post-inoculation, all assayed larvae were fed a virus-free diet. The initial lethal time (10% lethal time, LT$_{10}$) and median lethal time (LT$_{50}$) were determined. Ninety larvae in three replicates were inoculated in each treatment. The larvae were stimulated with a hairbrush and scored as responders if they were paralyzed or dead (Tuan et al., 2005).

Field trial

Forty newly molted 2nd-instar larvae of *S. exigua* were transferred to potted cabbage ca. 60 cm^2 in size with 12~14 leaves. Each pot was confined in a fine-mesh nylon cage on the trial farm of Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Wufeng, Taichung, Taiwan. The cabbages were treated individually with C6-AcMNPV, vAP10IT$_2$, vAPcmIT$_2$ at 10^7 PIBs/ml, and sterile water (as a control) using a handheld sprayer. The spraying volume was 1 ml per leaf. Tween-20 (0.05%) was added to all viral suspensions and controls. Three potted cabbages were tested for each treatment. The insects were checked twice daily to record signs and symptoms of disease and death using the protocol referred to by Tuan et al. (2005). Two applications were conducted at an interval of 7 days. Survival rate, average body weight, and leaf area eaten were calculated on day 7 after the second application of viral suspensions as they were after the first application. All leaves were xeroxed and scanned with a leaf area meter (Model Li-3100 Area Meter, Li-Cor, USA), the area eaten by larvae was calculated as referred to by Tuan et al. (2005). The survival rate was equal to the number of survival larvae of treated group divide by the number of tested larvae, and the value was then multiplied by 100%. The control efficacy was equal to the leaf area eaten by the larvae of the virus-treated set divided by the leaf area eaten by larvae of control, and the value was then multiplied by 100%.

Statistical analysis

All of the bioassays, polyhedral production assays, and field trials were performed at least thrice. Mortalities were corrected with Abbott's formula, and LTs values were calculated by the probit analysis (Finney, 1971). The polyhedral production and field trial values for each virus were averaged and analyzed by one-way ANOVA followed by the Least Significant Difference Test (Steel and Torrie, 1980).

RESULTS

Comparison of polyhedral production *in vitro*

On day 7 p.i., all the cells infected with C6-AcMNPV (Figure 1A) became extremely swollen and filled with dozens of polyhedra of regular size. Moreover, some cells were lyzed to release polyhedra. However, significantly fewer polyhedra were formed in the vAP10IT$_2$-infected cells (Figure 1B) than those produced by the vAPcmIT$_2$-infected cells (Figure 1C). The production curves of all infected cells continued to rise until day 7 p.i., then plateaued on day 8 p.i. (Figure 2). Sf21 cells infected with C6-AcMNPV, vAP10IT$_2$, and vAPcmIT$_2$ viruses had polyhedral production of 3.48×10^7, 1.33×10^7, and 3.93×10^7 PIBs/10^6 cells on day 7 p.i., respectively. Among these viruses, vAP10IT$_2$, the recombinant virus expressing LqhIT$_2$ under the very late *p10* promoter produced the

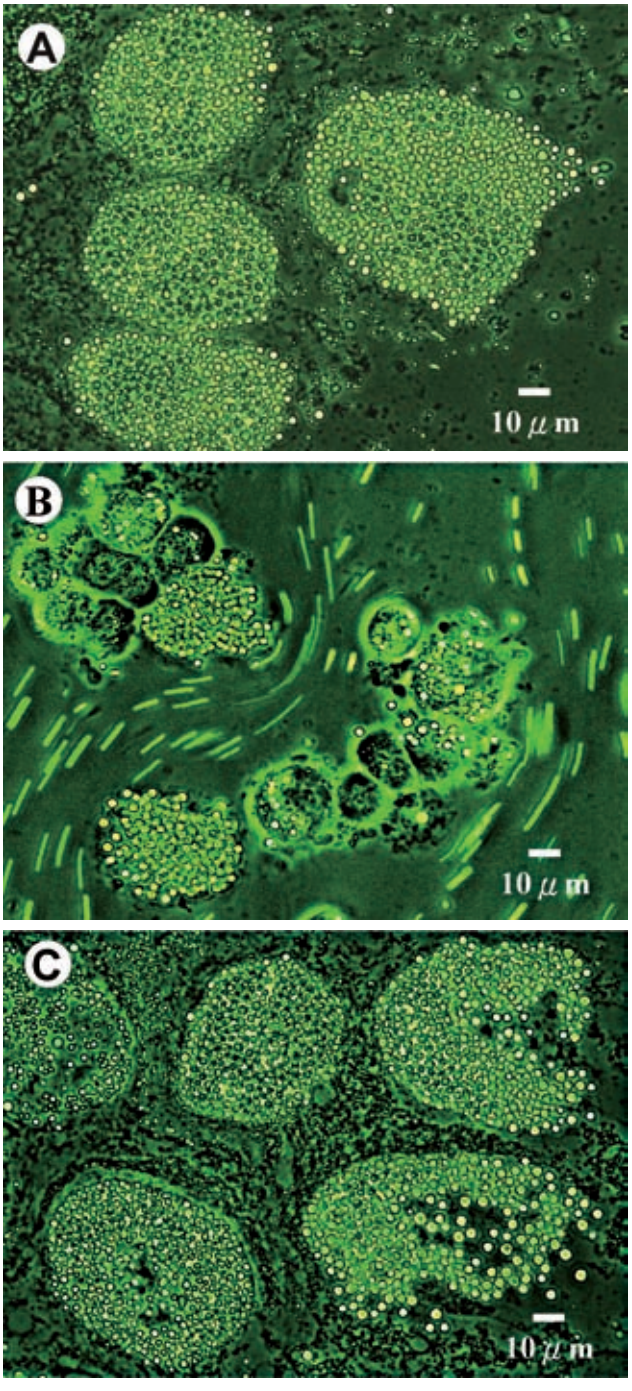

Figure 1. The micrographs of the occlusion bodies produced in the Sf21 cells infected with wild type AcMNPV (Panel A), and toxin-expressing recombinant viruses, vAP10IT$_2$ (Panel B) and vAPcmIT$_2$ (Panel C), on day 7 p.i.

least polyhedra in all the infection stages. On day 8 p.i., compared with wild type C6-AcMNPV, the polyhedra produced by vAP10IT$_2$ was reduced by 57% in Sf21 cells. vAPcmIT$_2$-infected Sf21 cells produced 12.7% more polyhedra than C6-AcMNPV-infected cells although this difference was not significant based on results of the Least Significant Difference Test with a 95% confident limit. The vAPcmIT$_2$-infected Sf21 cells produced polyhedra amounts as high as 2.82-fold that of vAP10IT$_2$-infected Sf21 cells (Figure 2).

Comparison of polyhedral production *in vivo*

On day 5 p.i., the average weight of *T. ni* larvae infected with C6-AcMNPV, vAP10IT$_2$, and vAPcmIT$_2$ was 54.0, 38.8, and 36.4 mg/larva, respectively. All weighed less than the control group larvae, averaging 108.9 mg/larva. The average weights of larvae infected with the scorpion toxin-containing viruses were 30~50% less than those infected with wild type viruses. Among the larvae infected with toxin gene-containing viruses, no significant difference emerged between the use of early and late promoters (Figure 3). On day 6 p.i., the polyhedral production in vAP10IT$_2$ and vAPcmIT$_2$-infected larvae was far less than that in the wild type viruses-infected larvae. The yields of polyhedra in insects with a low inoculated concentration of C6-AcMNPV, vAP10IT$_2$, and vAPcmIT$_2$ viruses were 3.79×10^8, 3.50×10^7 and 1.33×10^8 PIBs/larva, respectively. These data showed that the polyhedral productions in the larvae infected with vAP10IT$_2$ and vAPcmIT$_2$ were only 9.2% and 35.1% of those in larvae infected with the C6-AcMNPV.

The larvae treated with higher concentrations of these viruses had higher overall polyhedral production than the larvae treated with lower viral concentrations. After the inoculation of the C6-AcMNPV, vAP10IT$_2$, and vAPcmIT$_2$ viruses, the average polyhedral production of each infected larva was 7.91×10^8, 3.86×10^7 and 3.58×10^8 PIBs/larva, respectively. These data showed that the polyhedral productions by vAP10IT$_2$ and vAPcmIT$_2$ were 4.1% and 40.3% of that produced by wild type virus (Figure 4). Even after raising the inoculum concentration 100 fold, the yield of polyhedra in *S. exigua* larvae is still around one-fourth that of *T. ni* (Figure 5). Therefore, *T. ni* is better than *S. exigua* for polyhedral production. The average yield of polyhedra per mg body weight of *T. ni* larvae infected with vAPcmIT$_2$ was around 10 fold that with vAP10IT$_2$, and about 64% of that with wild type virus C6-AcMNPV (Figure 6).

Determination of the lethal activity of recombinant viruses

The lethal activity of recombinant viruses was determined and compared with wild-type AcMNPV (Table 1). The infection of *P. xylostella* 3rd-instar larvae by vAPcmIT$_2$ showed that the LT$_{10}$ was 11% and LT$_{50}$ was 22% earlier than that of C6-AcMNPV-infected larvae. However, the acceleration of LTs in *S. exigua* larvae was

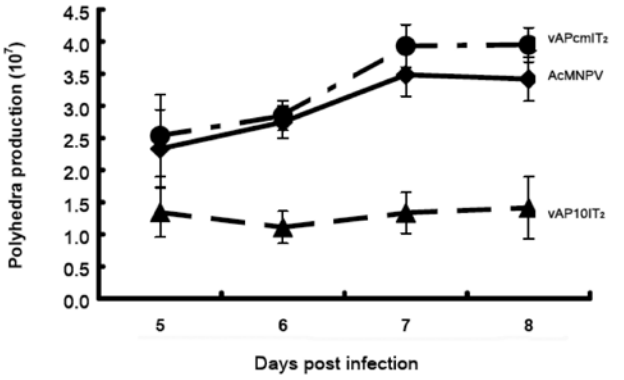

Figure 2. Comparisons of polyhedral production in Sf21 (10^6) cells infected with recombinant and wild-type AcMNPVs. The polyhedra were harvested from 5 to 8 days post inoculation.

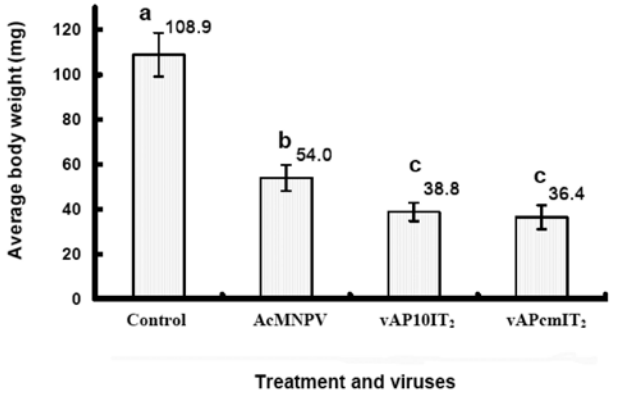

Figure 3. Average body weight of *Trichoplusia ni* larvae after infection with different viruses. Average body weights (means ± SD) of *T. ni* larvae on day 5 post inoculation with recombinant virus or wild type AcMNPV at a concentration of 78 PIBs/mm^3. Each bar labeled with different letters arise significantly different at $P<0.05$, Least Significant Difference.

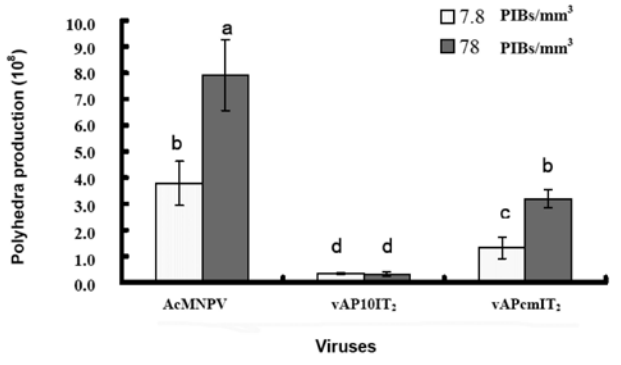

Figure 4. The polyhedral production in *Trichoplusia ni* larvae infected with wild-type AcMNPV or recombinant viruses at a concentration of 7.8 and 78 PIBs/mm^3, and then harvested on day 6 post inoculation. Each bar in the same color labeled with different letters is significantly different at $P<0.05$, Least Significant Difference.

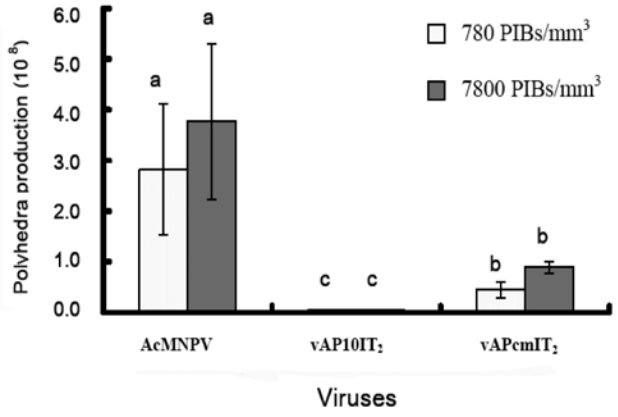

Figure 5. The polyhedral production in *Spodoptera exigua* larvae infected with wild-type AcMNPV or recombinant viruses at a concentration of 780 and 7,800 PIBs/mm^3 and then harvested on day 6 post inoculation. Each bar in the same color labeled with different letters is significantly different at P<0.05, Least Significant Difference.

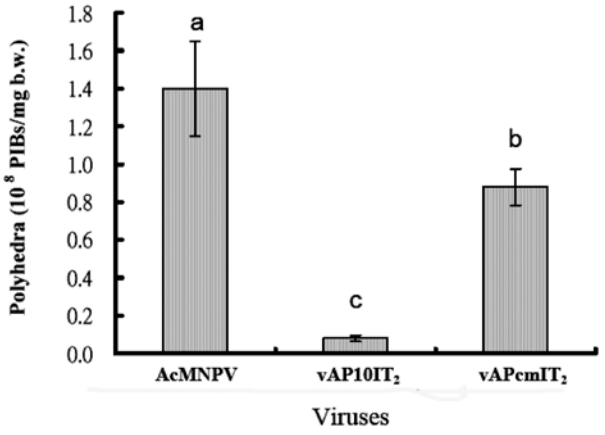

Figure 6. The average yield of polyhedra per mg body weight of *Trichoplusia ni* larvae infected with wild-type AcMNPV or recombinant viruses at a concentration of 78 PIBs/mm^3, and then harvested on day 6 post inoculation. Each bar labeled with different letters are significantly different at *P*<0.05, Least Significant Difference.

much less significant than in *P. xylostella*. The LT50 of toxin recombinant virus-infected *S. exigua* larvae was reduced by 8% to 13% compared to C6-AcMNPV-infected larvae. Tests on the larvae of these two lepidopteran species, which are susceptible to AcMNPV, demonstrated that expression of scorpion toxin could accelerate the lethal effect (Table 1).

Field efficacy of recombinant viruses

Beginning from day 3 post application (p.a.), the 2nd or 3rd instar larvae of *S. exigua* were found to fall from the potted cabbages sprayed with vAP10IT$_2$ and vAPcmIT$_2$. These larvae were paralyzed, but did not show the symptoms of swelling or liquefying typical of wild-type AcMNPV-infected larvae. The infected larvae even remained on cabbage leaves and stopped eating while the larvae treated with C6-AcMNPV or Tween-20 (0.05% in sterile water) continuously ingested leaves and gained weight. Seven days after the 2nd application with viruses, the vAPcmIT$_2$ group had the lowest larval survival rate at 20.3%, followed by the vAP10IT$_2$ group at 27.3%. The survival rates of the C6-AcMNPV and control groups were 35.4% and 100%, respectively. There were significant differences in survival rates among all groups (Table 2). However, there was no significant difference between vAP10IT$_2$ and vAPcmIT$_2$ treated groups with regard to the average weight of surviving larvae or the leaf area eaten. Both groups had a much lower average weight than those groups treated with wild type viruses. In addition, the leaf areas eaten by the larvae sprayed with vAP10IT$_2$ and vAPcmIT$_2$ were 58.7% and 67.1% smaller than those sprayed with wild type virus. All virus-treated groups had less feeding activity than the non-virus treated control (Table 2). These results indicated that the insecticidal efficacies of the toxin-recombinant viruses are considerably better than those of the wild type virus based on the larval survival rate in the field trial, the average weight of survived larvae, and the leaf area eaten. vAPcmIT$_2$ showed significantly better insecticidal efficacy than vAP10IT$_2$ with respect to larvae survival rate, but these two viruses showed no significant differences in average body weight or leaf area eaten.

Table 1. Comparison of lethal time of *Spodoptera exigua* and *Plutella xylostella* 3rd instar larvae inoculated with recombinant or wild type baculoviruses[1, 2, 3]

Virus (10^7 PIBs/ml)	Spodoptera exigua		*Plutella xylostella*	
	LT_{10} (h)	LT_{50} (h)	LT_{10} (h)	LT_{50} (h)
AcMNPV	137.1^b	182.3^b	89.0^b	138.2^b
vAP10IT$_2$	127.5^a	167.9^a	79.5^a	116.4^a
vAPcmIT$_2$	123.4^a	168.4^a	78.9^a	107.6^a

[1] Time in hours when larvae were placed on the diet contaminated with 780 PIBs/mm^3.
[2] LT_{10} and LT_{50} values were calculated using probit analysis with 95% confident limits.
[3] Means within a column labeled with different letters were significantly different at *P*<0.05, Least Significant Difference.

DISCUSSION

AcMNPV, a pathogen of more than 30 species of lepidopteran pests, has developed into a useful microbial insecticide over the past few decades (Bonning and Hammock, 1996). However, an infected larvae can continue to cause economic damage during the incubation period before its death (Ignoffo, 1973; Granados and Williams, 1986; Groner, 1986; Smits and Vlak, 1988; Granados and Williams, 1994; Tuan et al., 1998). Improvement to the efficiency of AcMNPV as an

Table 2. Comparison of survival and body weight of *Spodoptera exigua* 2nd instar larvae inoculated with recombinant viruses or wild type AcMNPV and leaf area eaten [1, 2].

Virus (10^7 PIBs/ml)	Survival rate (%)		Average body weight (mg)		Leaf area eaten (cm^2)	
	Mean	SD	Mean	SD	Mean	SD
AcMNPV	35.4[c]	4.1	30.4[b]	5.3	70.0[b]	13.6
$vAP10IT_2$	27.3[b]	3.2	20.1[a]	3.2	28.9[a]	6.7
$vAPcmIT_2$	20.3[a]	2.4	22.9[a]	4.4	22.8[a]	3.5
Control	100.0[d]	0.0	118.2[c]	11.8	337.4[c]	33.8

[1] All viral suspensions were 10^7 PIBs/ml, adjuvant with Triton® at 2,000-fold dilution, and data were calculated on day 7 after the 2nd application at the interval of 7 days from the 1st application on day 0.

[2] Means within a column labeled with different letters are significantly different at $P<0.05$, Least Significant Difference. SD, standard deviation.

insecticide is dependent upon reduction of the incubation period. Scorpion and spider neurotoxins have been the most widely used foreign toxin proteins and have transformed baculoviruses into potent bio-insecticides (Maeda et al., 1991; McCutchen et al., 1991; Cory et al., 1994; Hoover et al., 1995; Hughes et al., 1997; Gershburg et al., 1998; Harrison and Bonning, 2000; Burden et al., 2000). *Leiurus quinquestriatus hebraeus* excitatory and inhibitory neurotoxins ($LqhIT_1$ and $LqhIT_2$) are both considered promising toxins able to accelerate the speed of kill of baculoviruses, and they can even work synergistically to reach a higher effect when coexpressed (Regev et al., 2003).

In order to successfully engineer these toxin genes into insect viruses for better insecticidal efficacy, strong early expression of the toxin gene is critical (Difalco et al., 1997; Gershburg et al., 1998; Harrison and Bonning, 2000; van Beek et al., 2003). It has been demonstrated that high level expression of $LqhIT_2$ and $LqhIT_1$ in recombinant viruses by the strong very late promoters *polh* and *p10* can improve the effective paralysis time 50% (ET_{50}) significantly, and their efficacy is better than that of the early *p35* promoter (Gershburg et al., 1998). In the expression system of insect cells using recombinant baculovirus, the *p-PCm* promoter showed a high level of foreign protein expression at the early stage (Lo et al., 2002) and expressed scorpion toxin around 12 h post infection, approximately 12~18 h earlier than that observed under the *p10* promoter (Tuan et al., 2005). This is also supported by luciferase expression of the *p-PCm* promoter, which resulted in higher luciferase activity than the *p10* promoter at early stages of infection, although the latter expressed more proteins than the former at the very late stage (Wu et al., 2000; Lo et al., 2002). It has been previously reported that only a trace amount of scorpion toxin is required to cause paralysis and death in larvae (McCutchen et al., 1991; Gershburg et al., 1998). Thus, the early expression of toxin by the *p-PCm* promoter is still sufficient to lead to early paralysis and larval death.

Our experiments showed that the expression of the $LqhIT_2$ by $vAP10IT_2$ and $vAPcmIT_2$ accelerated the insecticidal effect on the larvae of *T. ni*, *P. xylostella*, and *S. exigua*. The LT_{50} of *T. ni* infected with $vAPcmIT_2$ was significantly shorter than that of $vAP10IT_2$-infected larvae (Tuan et al., 2005), but in the case of *P. xylostella*, and *S. exigua* larvae, there were no significant differences between larvae infected with $vAPcmIT_2$ and $vAP10IT_2$. Previously, both RoMNPV recombinant viruses expressing AaIT and $LqhIT_2$ from the *p10* promoter produced a lower quantity of viral occlusions than other recombinant viruses from the *p6.9* promoter. Presumably, the relative position of the strong *p10* promoter in those viruses relative to the *polh* promoter may be accountable for the decreased polyhedrin protein accumulation and occlusion assembly (Harrison and Bonning, 2000). Studies have shown that expression from the *p10* promoter can have a negative effect on the expression from the polyhedrin promoter *polh* due to a resource competition for protein synthesis (Roelvink et al., 1992; Chaabihi et al., 1993; Bonning et al., 1994; Volkman et al., 1996).

Economically sound production of polyhedra is important for the practical application of toxin-gene engineered baculoviruses. The scorpion toxin causes the infected larvae to cease eating in a shorter time relative to wild type, resulting in a significant reduction in polyhedral production (Cory et al., 1994; Ignoffo and Garcia, 1996; Fuxa et al., 1998; Burden et al., 2000). Similarly, when propagating polyhedra of AcAaIT toxin-recombinant viruses in the larvae, the yield of polyhedra obtained was only 20% of that for C6-AcMNPV (Kunimi et al., 1996). In our study, the toxin-containing recombinant viruses caused most infected larvae of *S. exigua* or *T. ni* to stop eating, become paralyzed, or even die early before swelling. At 5 days p.i., the average weight of *T. ni* larvae infected with recombinant viruses was 30% less than that of the larvae infected with wild type virus. Polyhedral production in $vAP10IT_2$ and $vAPcmIT_2$ infected larvae were about 96% and 60% less than that observed for wild type AcMNPV. Bonning et al. (1995) pointed out that the polyhedral production in cells *in vitro* is 1.5 times greater than that in larvae, but neither sets of polyhedra showed any significant difference in the lethal time to *T.*

ni and *Heliothis virescens*. In our study, the production of $vAPcmIT_2$ in Sf21 cells was similar as that of wild type virus AcMNPV.

Cory et al. (1994) and Hoover et al. (1995) indicated that recombinant virus with AaIT toxin paralyzed larvae, stopped their eating, and even caused them to fall from the plants, reducing the leaf areas consumed on host plants. In this study, we found that batches of *S. exigua* larvae fall to ground 2~3 days p.a., and $vAP10IT_2$ could further reduce the leaf area consumed to 67.4% of that consumed using the wild type virus. Based on the damage to area of the leaves, the control efficacy performed by $vAPcmIT_2$ reached 93.3% as compared with the control group. In conclusion, our studies showed that scorpion toxin driven by the early phase promoter is superior to that driven by the very late promoter in killing lepidopteran larvae. More importantly, expression of the toxin gene by the early phase promoter gives rise to better polyhedral yields in insect cells than that by the very late promoter. A higher polyhedral production will be useful for the future application of this virus in the fields for effective control of insect pests.

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提高多角體產量以增加具蠍神經毒表現能力之桿狀病毒的殺蟲效果

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含蠍毒桿狀病毒之多角體量產的成功與否，是成為害蟲防治利器的關鍵因素。本研究中兩種由加州苜蓿夜蛾核多角體病毒（*Autographa californica* (Speyer) nucleopolyhedroviruses, AcMNPVs）改造而成含以色列黃蠍之鎮定神經毒（*Leiurus quinquestriatus hebraeus* (Ehrenberg), $LqhIT_2$）之重組病毒 $vAPcmIT_2$ 及 $vAP10IT_2$ （Tuan et al., 2005），分別利用早期起動子（*p-PCm*）及非常晚期起動子（*p10*）來表現蠍毒基因。試驗目在於探討兩種重組病毒多角體的量產效率，發現無論係在體內或體外系統，早期表現蠍毒之重組病毒均顯著優於晚期表現蠍毒之重組病毒。以 Sf21 細胞株生產病毒，$vAPcmIT_2$ 的量產效果較 $vAP10IT_2$ 顯著良好，以擬尺蠖（*Trichoplusia ni* (Hübner)）及甜菜夜蛾（*Spodoptera exigua* (Fabricius)）兩種幼蟲繁殖病毒時，發現雖然 $vAP10IT_2$ 的產量尚不如野生型 AcMNPV，但卻是 $vAP10IT_2$ 多角體產量的十倍。含蠍毒重組病毒對兩種具抗藥性的蔬菜害蟲-甜菜夜蛾及小菜蛾（*Plutella xylostella* (Linnaeus)）幼蟲之起始致死時間（LT_{10}）及半致死時間（LT_{50}）均較野生型病毒有顯著提早之效果。在甘藍盆栽試驗，含蠍毒重組病毒導致甜菜夜蛾幼蟲提早死亡，表現 90% 以上的防治效果，且較野生型病毒處理組顯著減少 58.7~67.4% 之葉片受害率。以病毒多角體量產的效率及防治效果而言，早期表現蠍毒之重組病毒 $vAPcmIT_2$ 較晚期表現蠍毒之重組病毒 $vAP10IT_2$ 及野生型病毒更具開發成生物農藥的優勢。

關鍵詞：加州苜蓿夜蛾核多角體病毒；重組病毒；以色列黃蠍；鎮定神經毒；甜菜夜蛾；擬尺蠖；小菜蛾；多角體量產；防治效果。