

## A Genetically Modified Nucleopolyhedrovirus: Effectiveness and *in vivo* Expression in the Black Cutworm, *Agrotis ipsilon* (Huf.) (Lepidoptera: Noctuidae)

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### ABSTRACT

Baculoviruses are orally infectious to insects and are considered as viral insecticides and safe for the environment. To enhance their speed-of-kill, these viruses were genetically engineered to express insect enzymes. Efficiency of a recombinant virus, *Autographa californica* multiple nucleopolyhedrovirus expressing *Manduca sexta* juvenile hormone esterase (*AcMsJHE*), and its wild type (*AcMNPV*) for killing 1<sup>st</sup> instar larvae of *Agrotis ipsilon* (Huf.) was analyzed. Mortality rate of *A. ipsilon* larvae was higher with recombinant virus than wild type by 1.4 times on the level of LC<sub>50</sub>; at the same time it was 16.7% faster in killing than its wild type. Effect of virus infection on larval weight indicated that 3<sup>rd</sup> instar larvae infected with 1000 PIBs/larva were dramatically reduced in weight with either recombinant or wild type viruses, although the percentage of reduction was higher with recombinant than wild type virus. Isoelectric focusing analysis showed that there were expression of juvenile hormone esterase *in vivo*, and it is believed that it is related to gene expression. These results indicated that the genetically engineered baculovirus, *AcMsJHE*, could provide effective control compared with its wild type for *A. ipsilon* and further support the potential use of genetically engineered baculoviruses in insect pest control.

**Key words:** *Agrotis ipsilon*, Recombinant Nucleopolyhedroviruses, bioassay, JHE Expression.

### INTRODUCTION

Baculoviruses are a group of arthropod-specific viruses that have been isolated mostly from larvae of Lepidoptera (Bonning, 2005). All baculoviruses belong to a single family, Baculoviridae, which is currently composed of the genera *Nucleopolyhedrovirus* and *Granulovirus* (Theilmann *et al.*, 2005). Baculoviruses have a narrow host range, are highly pathogenic and have occlusion bodies which make them more environmentally stable than some other families of entomopathogenic viruses (Entwistle and Evans, 1985). These characteristics enable baculoviruses to be used as microbial control agents against insect pests. There are many examples of successful use of baculoviruses in this role (Entwistle, 1998 and Moscardi, 1999).

The black cutworm, *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae) is a worldwide pest. It lives on the ground, where it feeds on seedlings of nearly all vegetable and field crops, most important crops include tobacco, cotton, tomato, potato, cabbage, barley and oats (Rings *et al.*, 1975). All instars of *A. ipsilon* feed on the leaves of corn seedlings but the most serious damage results from leaf and stem cutting by late instars (Clement and McCartney, 1982). They are often detected only when the plants are already severely damaged. In the past, several species of entomopathogenic bacteria, protozoa, fungi and viruses were isolated and evaluated as possible biological control agents of cutworm pests (Lipa, 1971; Ignoffo and Garcia,

1979 and Cossentine and Lewis, 1986). Cutworms could be controlled with different baculoviruses isolated from diseased insects (Zethner *et al.*, 1987 and Caballero *et al.*, 1991a, b).

Baculoviruses have been considered as viral insecticides and are safe for the environment (Cheng and Lynn, 2009). Although several viral insecticides with advantageous characteristics have been registered, their use is still limited. A major disadvantage of viral insecticides is that the speed of insect killing is relatively slow, allowing infected pest larva to continue to cause economical damage to crops. Recombinant DNA technology used to eliminate the disadvantages of viral insecticides by contributing to the acute insecticidal effects induced by inserted or deletion genes in baculoviruses, several innovative and successful approaches have been taken to improve the speed of kill of baculoviruses through genetic modification (Kamita *et al.*, 2005). Most examples of GM viruses utilize viruses of the Baculoviridae family and include exogenous gene insertions such as those encoding insect specific toxins (Merryweather *et al.*, 1990; Maeda *et al.*, 1991; Gershburg *et al.*, 1998; Harrison and Bonning, 2000 and Regev *et al.*, 2003), hormones (Eldridge *et al.*, 1991, 1992a and b), neuropeptides (Ma *et al.*, 1998) and enzymes (Bonning *et al.*, 1992 & 2002; Gopalakrishnan *et al.*, 1993 and Harrison and Bonning, 2001).

In this study, the susceptibility of *A. ipsilon* to a recombinant nucleopolyhedrovirus (*Autographa*