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Review

Baculoviruses and insect pests control in China

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China is rich in insect viruses. *Helicoverpa armigera* nucleopolyhedrovirus (HaSNPV), was the first commercial insecticide registered in 1993. Subsequently, 13 viral insecticides were registered and released into the market as pesticides, including nine nucleopolyhedrovirus (NPV), three granulovirus (GV), one densonucleosis virus (DNV), and one cytoplasmic polyhedrosis virus (CPV). Industrial exploitation of viruses in China started more than three decades ago. To date, at least 24 baculovirus insecticides have been or are currently used in field trials in China, 12 of which are unregistered. Examples of successful applications and benefits of entomopathogenic viruses as microbial insecticides are presented in the current paper. However, chemical prevention is still the main measure for the management of insect pests in China. Effective public extension services, government policies, and farmer education are important to expand the use of viral insecticides as well as for the further development in the production and use of these insecticides.

Key words: Microbial control, baculoviruses, viral insecticide, sustainable agriculture.

INTRODUCTION

The use of pathogenic microorganisms for pest control is not a new concept. A considerable number of economic insect pests were controlled with fungal diseases since the late 19th century (Charnley, 1991). However, all forms of biological control of insect pests declined because of the dramatic impact of synthetic chemical insecticides in the 1940s and 1950s (Charnley, 1991). The use of synthetic chemical insecticides, which can affect the non-target organisms (e.g., humans, natural enemy, and livestock), pollute the environment, and induce insecticide-resistant pests, has serious social implications (Yasuhisa, 2007). Insect pest control is critical to human health. Hence, an ecological pest management that is environmentally friendly and sustainable must be established.

Baculoviruses belong to a virus family, that is pathogenic to arthropods with large circular, covalently closed, and double-stranded DNA genomes that are packaged into nucleocapsids. More than 700 baculoviruses have been identified from insects of the orders Lepidoptera, Hymenoptera, and Diptera (Moscardi, 1999; Herniou and Jehle, 2007). baculoviruses as microbial insecticides are ideal tools in integrated pest management (IPM) programs as they are usually highly specific to their host insects, thus, they safe to the environment, humans, other plants, and natural enemies (Yasuhisa, 2007; Ahmad et al., 2011).

Over 50 baculovirus products have been used to control different insect pests worldwide (Ahmad et al., 2011). The use of *Anticarsia gemmatalis* NPV (AgMNPV) to control *A. gemmatalis* in soybean in Brazil was a very successful program and was considered as the most important one in the world (Moscardi, 1999). This virus was used in about 2 million ha during the 2003/2004 growing season in Brazil, approximately 10% of the

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Table 1. Commercially available viral insecticides in China

Host insect	Baculovirus	Target crop(s)
Pieris rapae	GV	Vegetable
Gynaephora spp.	NPV	Pasture
ddf	NPV	Tea
Euproctis pseudoconspersa	NPV	Tea
Helicoverpa armigera	NPV	Cotton, pepper, soybean, tobacco, tomato, pea
Autographa californica	NPV	Vegetable
Spodoptera exigua	NPV	Vegetable
Plutella xylostella	GV	Vegetable, wheat, Corn
Spodoptera litura	NPV	Vegetable
Buzura suppressaria	NPV	Tea, tung oil tree
Leucania separata	NPV	Wheat, Corn
Pseudaletia separata	GV	Wheat, corn
Periplaneta fuliginosa	DNV	Cockroach
Dendrolimus punctatus	CPV	Pine moth

This list is not comprehensive. Data are based on electronic Pesticide Manual information of the Ministry of Agriculture the People's Republic of China in July, 2011.

soybean cultivation area in the country (Moscardi, 2007). In China, *Helicoverpa armigera* SNPV (HaSNPV), which has been produced and applied to cotton, soybean, pigeon pea, maize, and tomato crops since, was first authorized as a commercial microbial insecticides in 1993 (Zhang et al., 1995; Sun and Peng, 2007).

USE OF BACULOVIRUSES AS MICROBIAL INSECTICIDE CONTROL IN CHINA

Registration of baculoviruses developed as microbial insecticides

Since the comprehensive review by Moscardi (1999) on the use of baculoviruses for control of insect pests, other studies have been published on the state of virus application against insect pests in agricultural, forest, and vegetable production systems (Copping and Menn, 2000; Szewczyk et al., 2006, 2009; Souza et al., 2007; Zhang et al.,2007; Erlandson, 2008). The registration of HaSNPV in 1993 was the first real commercialization of viral insecticide application in China. To date, authorized commercial insecticides involve 14 species, including nine nucleopolyhedroviruses (NPV), three granuloviruses (GV), one densonucleosis virus (DNV), and one cytoplasmic polyhedrosis virus (CPV) (Table 1). The commercial viral insecticides in China are much developed when compared to other countries.

Main examples of baculoviruses developed as microbial insecticides applied in field trials

One of the most important successes in the commercial production and use of a baculovirus in China is the *H. armigera* NPV (HaSNPV) for use in several crops and vegetables. In China, NPVs of *H. armigera* are used on over 100,000 ha annually involving at least 12 HaSNPV producers (Sun and Peng, 2007).

Other important viruses that are currently employed, to control insect pests in larger areas, including *Euproctis pseudoconspersa*, *Buzura suppressaria*, *Spodoptera litura* NPV in China. Aside the 12 species that have been registered as baculovirus insecticides, more than 12 species have not been registered. At least 24 baculovirus have been utilized in field trials in China (Table 2) since the beginning of insect pest biological control by insect virus in 1973.

USES OF BACULOVIRUSES FOR IMPORTANT FOREST INSECT PESTS

Hyphantria cunea NPV (HcNPV), *Apocheima cinerarius* NPV (AciNPV), and *Lymantria dispar* NPV (LdMNPV) are presently used in forest systems in China. These viral microbial insecticides have achieved a remarkable success in the biological control of forest insect pests. These viruses have been produced in insects reared on artificial diets. Biological missile, which indicates that the virus was transmitted by egg parasitic wasps to control forest pest, was first created by the Wuhan Institute of Virology, Chinese Academy of Sciences in 1997.

Since the introduction of the *H. cunea* into China in 1979, it has spread Liaoning, Shandong, Shanxi, Hebei and Tianjin and continues to spread (Zhang et al., 2007). Larvae of the *H. cunea* with high reproductive ability, mixed feeding habits and strong capacity to migrate, and during cyclic outbreaks have been responsible for

Table 2. Main examples of baculoviruses applied in field trials in China.

Host insect	Baculovirus	Target crop(s)	Key References
Helicoverpa armigera	NPV	Cotton, pepper, soybean, pigeon, tomato, pea	Yang et al. (2001)
Buzura suppressaria	NPV	Tea, tung oil tree	Qi et al.(1993) and Gan (1981)
Prodenia litura	NPV	Vegetable	Yang et al. (2001)
Spodoptera exigua	NPV	Vegetable	Jiang et al. (2004)
Autographa californica	NPV	Vegetable	Yang et al. (2001)
Mamestra brassicae	NPV	Vegetable	Ni and Zhang (1991)
Euproctis pseudoconspersa	NPV	Tea	Hong and Yin (1991) Qi et al. (1986)
Ectropis obliqua	NPV	Tea	Hong and Yin (1991)
Ectropis grisescens	NPV	Tea	Chen et al. (2000)
Eranhis ankeraria	NPV	Forest	Sun et al. (1990)
Sucra jujube	NPV	Jujube	Ji et al. (1999)
Apochemia cinerarius	NPV	Forest	Wang et al. (2003)
Lymantria dispar	NPV	Forest	Yue et al. (1984)
Lymantria xylina	NPV	Forest	Chen et al. (1987)
Leucoma salicis	NPV	Forest	Zhang et al. (2000)
Hyphantria cunea	NPV	Forest	Qiao (2007);Yang and Zhang (2007)
Clania Variegata	NPV	Forest	Zhao (1993)
Eriogyna Pyretotum	NPV	Forest	Wu et al. (1987)
Plutella xylostella	GV	Vegetable	Lv (1998)
Pieris Rapae	GV	Vegetable	Wen (2007)
Agrotis segetum	GV	Vegetable	Chen (1994)
Andraca bipuncte	GV	Tea	Ding et al. (1999)
Clostera anachoreta	GV	Poplar, willow	Zhang et al. (1992)
Ostrinia furnacalis	GV	Corn	Yin and Chang (1993)

This list is not comprehensive. The year indicated in the key references is not necessarily the first time that the baculovirus insecticides were applied in field trials.

defoliation of trees, crops and vegetables (Zhang et al., 2007).

HcNPV has been vastly produced on insects reared on an artificial diet. This virus was used in approximately 3,000 ha during 1983 and 1985 in China (Zhang et al., 2007; Ye et al., 1985). Vertical trans-mission, or parent-to-offspring passage of a pathogenic microorganism, is a phenomenon commonly observed with insect viruses, and vertical transmission may be potentially useful in microbial control (Fuxa et al., 2002). The HcNPV has been detected subsequently in the population of *H. cunea* in several areas (Liaoning, Shandong, and Hebei provinces), where HcNPV has been used to control *H. cunea* 6 years ago (unpublished).

Lymantria dispar is an important pest that is widely distributed worldwide and attacked more than 500 species of plants in China (Schaefer et al., 1984). LdMNPV has been developed as a viral insecticide under the trade names Gypcheck and Disparvirus since the 1980s (Reardon et al., 1999). In China, this insecticide has been utilized in field trials in 1980s. This insecticide was used to control *L. dispar* in the Jilin Province and applied to 1700 ha during 1994 and 1995 (Yue et al., 1984; Zhao, 1996). LdMNPV has emerged as an important factor affecting the *L. dispar* population in various regions, and generally existed in the Chinese forest ecosystem. However, *L. dispar* outbreak recurred in recent years. One reason for the decline in the biological control by LdMNPV is the other improper implementation of measures to control *L. dispar* (Zhang et al., 2007).

The *A. cinerarius* represent a key pests of several trees in China, especially responsible for the three north shelter-forest trees during cyclic outbreaks, an NPV of *A. cinerarius* was found in 1979 and developed as a viral insecticide in the 1980s (Wang et al., 1983a, b and 1988). Vertical transmission is common in cypoviruses (CPV) (Belloncik and Mori, 1998) and especially in nucleopolyhedroviruses (NPVs) (Kukan, 1999). AciNPV can sustainable control *A. cinerarius* in up to 3 to 5 years, which can even be extended up to 20 years after AciNPV was applied on forest (Wang et al., 1983a, b and 1988). Forest ecosystems tend to be more stable than agricultural systems, allowing for natural or applied baculoviruses to remain in the environment for long periods of time. (Ahmad et al., 2011).

FURTHER PROSPECTS ON THE USE OF BACULOVIRUSES AS MICROBIAL INSECTICIDE

Use of baculoviruses in China is the greatest worldwire, regarding the number of viruses being registered for insect control (Ahmad et al., 2011), and its industrialized exploitation started more than 30 years ago. The technologies in the production and application of this pest control have undergone considerable development. At the same time, scientists have been conducting research on genetically modified baculoviruses for a number of years, and remarkable progress has been made in China. For example, a chimeric promoter p6.9 was constructed by insertion of a p6.9 promoter downstream of the polyhedrin promoter, and this dual promoter was used for the expression of AaIT scorpion toxin gene in the *egt* locus of HaSNPV (Sun et al., 2004). Recombinant HaSNPV-expressing AaIT scorpion toxin gene is safe to non-target organisms and the environment (Sun et al., 2002).

However, the use of baculovirus insecticides has not been optimized in insect pest control, and chemical prevention is still the main measure used for the management of insect pests in China. For example in China, the amount of chemical insecticides produced in 2003 was 47.8,000 t, whereas that of for viral insecticides was 2,000 t. Only 4 t of raw viral insecticides were produced by converting 500 billion PIB, which was 1/100,000 of viral insecticides, resulting in a great disparity in the proportion of pesticides (http://www.pingyin.gov.cn/contents/304/6425.htm).

Various factors may limit the use of baculoviruses. Most baculoviruses generally have a special or more restricted host range. For example, *Lymantria xylina* multiple nucleopolyhedrovirus (LyxyMNPV) replicates only in *L. xylina* and *L. xylina* cell (Wu and Wang, 2005), and HcNPV replicates strongly only in their own larvae. Solar radiation is the major factor that affects the field persistence of baculoviruses, ultraviolet radiation in region B (UV-B) (280 to 310 nm) inactivates baculoviruses. However, UV-A (320 to 400 nm) may also be critical in baculovirus deactivation (Ahmad et al., 2011). Viral activity can be completely lost in less than 24 h, but the mean half-life has varied generally from 2 to 5 d (Ahmad et al., 2011). In addition, the temperature, chemicals, microorganism, and a small amount of active viruses of the insect population are also prerequisites to the epidemic of viruses (Lv, 1982). Determination of the time to kill the insect host is crucial in the case of bacluvirus application (Moscari, 1999; Szewczyk et al., 2006, 2009; Souza et al., 2007; Erlandson, 2008). Larvae, which were infected by viruses, may last for at least 1 wk before they die. Also, an IPM program is not adopted by farmers (Ahmad et al., 2011). Thus, farmers prefer to use chemical insecticides in pest prevention. The lack of related educations for farmers and the unreasonable pesticide market management system in China set barriers to baculovirus use. For example, many supposed viral insecticides have no viral component, and only pesticide manufactures speculate the effective use of viral insecticides. As a consequence, farmers lose their confidence in the use of these insecticides.

Despite these barriers and the low use of viral insecticides, the role of microbial insecticides in the integrated management of insect pests has been reviewed for agriculture (Lacey and Goettel, 1995; Dent, 1997; Georgis, 1997; Tatchell, 1997; Yasuhisa, 2007), forestry (Evans, 1997; van Frankenhuyzen et al., 2000; Zhang et al., 2007) and public health (Skovmand et al., 2000), and the market for microbial insecticides is growing worldwide. In the future, we anticipate a bright prospect for the attributes of entomopathogens. We also expect to see synergistic combinations of microbial control agents with semiochemicals, soft chemical pesticides, other natural enemies, resistant plants, chemigation, and remote sensing. Hopefully, effective and sustainable integrated control strategies will be achieved (Lacey et al., 2001). Microbial insecticides will face an even stiffer competition with new pesticide chemistries and transgenic plants in the future (Gaugler, 1997). Therefore, improved microbial products, farmer education, and government policy on the application of bio-pesticides are important for the use of viral insecticides in controlling pests in China.

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