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Biology of Baculovirus and Use of Baculovirus in Pest Control

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by

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Abstract

Members of the baculovirus family *Baculoviridae* have circular double-stranded DNA and can be used as biopesticide agents. This virus causes cell death inside the midgut of insects, specifically the insects that belong to the order *Lepidoptera*. Here we will be reviewing recent discoveries related to the baculovirus, the biology of these viruses, current research on baculovirus, and future directions in the field of baculovirus research, and practical applications baculovirus. Specifically, we will focus on the ways the baculovirus can be genetically modified to serve as a biopesticide agent in agricultural and forest management practices against invasive species. The baculovirus can cause changes in behavioral patterns in insect hosts; however, our main focus will be on understanding the baculovirus as an efficient way to control pests in agricultural and forest areas.

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Introduction

Viral particles are widespread parasites that infect many eukaryotic and prokaryotic organisms. Humans have known about diseases caused by the parasitic baculovirus for over 2000 years (Federici, 1997). Baculovirus was first discovered in the silkworm *Bombyx mori* (Yue *et.al,* 2008). The first description of the effect the virus has on the silkworms was written in Latin by Marco Vida of Cremona, an Italian bishop. He described the disease as "De Bombyce" translating to: "the weak ones, the skin appears yellow. Then insects swell up, and a foul inactivity comes in the bodies of those who have fallen down. Finally insects break open and everything is infected with repulsive putrid gore: diseased blood from all sides flows from the bodies" (Miller, 1997). The baculovirus is specific only to certain groups of insects, and do not cause any diseases in humans (Miller, 1997; Upadhyay, 2003). Around 500 different species of insects that the baculovirus infects live in forests and agricultural land. Prior to 1975, studies were focused on the baculovirus because of the potential the virus could provide for pest control in agriculture and forest management (Steinhaus, 1963). The U.S. Forest Service carried out one of the first successful biological control uses of the baculovirus. A population of invasive Douglas fir tussock moths *(Orgyia pseudotsugata)* was controlled, by using the viruses as a pesticide (Martignoni, 1984). This review will focus on current research and future directions the field of baculovirus research may take.

Classification and Biology of Baculovirus

Carstens (1980) described all baculovirus particles as rod-shaped, and Upadhyay (2003) later showed them to contain double-stranded DNA (dsDNA). The family *Baculoviridae* includes two main groups of occluded viruses that differ in their structural characters: *Nuclearpolyhedrosisviruses* (NPVs), and the *Granuloviruses* (GVs) (Federici 1997; Upadhyay, 2003). The NPVs consist of multiple enveloped virus units that form a crystal-like protein matrix, or a large polyhedral (Carstens, 1980; Upadhyay, 2003). During

the 19th century, these polyhedral crystals were first observed under the microscope (Miller, 1997). Federici (1997) explained that this specific group of baculoviruses infects over 400 insect species, most commonly the species from genus *Lepidoptera*, which includes moths and butterflies. NPVs are further divided into two subgroups: AcMNPV, consisting of several genera including *Autographa californica* , and OpMNPV, that includes *Orgyia pseudotsugate*.. AcMNPV is the most studied, standard virus. This virus was originally isolated from the alfalfa looper (*Autographa californica*). This particular virus was initially used as an infectious agent in biopesticide control efforts (Dr. Slavicek, personal communication). The OpMNPV virus is isolated from the Douglas fir tussock moth (*Orgyia pseudotsugata*) (Chen *et al.*, 2001; D'Amico & Slavicek, 2011).

Viruses in GV group contain only one or two small occlusion bodies called granules and lack multiple enveloped particles (Federici, 1997; Upadhyay, 2003). GVs are less common among insect species, infecting only about one hundred different species (Federici, 1997). Both NPVs and GVs follow the same path of entry when invading an insect host.

During infection, two forms are present: the oculsion-dervied virus (ODV) and the budded virus (BV). ODVs are encapsulated in the protein matrix called polyhedrin. ODV is accountable for the primary infection within the host. As demonstrated in Figure 1, the polyherin-enveloped structure, covering the ODV facilitates the infection within the host and allows the virus to survive and adapt to the environment of the insect midgut. The BV, are not occluded. BV is released during the secondary infection. During the secondary infection, without having a polyherin envelope, the BV is able to move and infect the tissues inside the host (Funk 1993).

Use of the Baculovirus in Invasive Species Control

With an increase of global trade and freight transport of goods, many invasive species were introduced to the U.S. by means of shipping containers from other countries. Invasive species later spread from harbors to the rest of the country (Dix et. al, 2009).

In 1869, *Lymantria dispar* also known as gypsy moths and originally from Europe, spread to the North and South Central American states (Metcalf & Metcalf, 1993). Gypsy moths attack deciduous and evergreen trees and shrubs by feeding on foliage. Barron (2008) refers to gypsy moths causing the destruction of the pine oak woodlands of North America, one of important wildlife areas. Another, agricultural moth problem began around the same time in the 19th century when the brown tail moth (*Nygmia phaerrhoea*) spread from Europe to eastern Massachusetts. Brown tail moths attack various plants such as apple (*Malus domestica*), cherry (*Prunus avium*), oak (*Quercus sp.*), willow (*Salix sp.*) and other trees and shrubs (Metcalf & Metcalf, 1993). These moths feed on the leaves, and when humans come in direct contact larval bristles, dermatitis and respiratory problems occur (Dix et. al, 2009).

These insects also disrupt other vegetation. Velvet caterpillars (*Anticarsia gemmatalis*) are defoliators of legumes. This moth migrated to Alabama, Georgia, and South Carolina. The main agriculture products this caterpillar attacks are soybean (*Glycine max*), velvet bean (*Mucuna pruriens*), cowpea (*Vigna unguiculata*), peanut (*Arachis hypogaea*), alfalfa (*Medicago sativa*), and other legume plants (*Fabaceae*). Another species that attacks a different plant type is the cabbage looper (*Trichoplusia ni*), these insects have spread throughout the United States, Canada and Mexico. These moths affect production of cabbage (*Brassica oleracea var. capitata)*, lettuce (*Lactuca sativa*), spinach (*Spinacia oleracea*), beet (*Beta vulgaris)*, pea (*Pisum sativum*), celery (*Apirum graveolens* var. dulce), parsley (*Petroselinum crispum*), potatoes (*Solanum tuberosum*), and tomatoes (*Solanum lycopersicum*). Another invasive species that impacts agriculture is the moth *Helicoverpa zea*. *Helicoverpa zea* is known as tomato fruitworm. It infests about

five to 25% of tomatoes in commercial growing areas. The larvae of the tomato fruitworm are known to be aggressive, and they also attack and consume other larvae of the same species (Metcalf & Metcalf, 1993).

Many invasive insect species negatively affect forest areas. In 2006 the United States Department of Agriculture Forest Services (USDA) came up with a plan for Forest Services Research and Development (R&D) to control invasive species. This area of research focused on about 36% of invasive species, developing tools for restoring forest land, and preventing reinvasion. The Strategic Program Area (SPA) plan of action was developed for invasive species. With the invasive species destroying crops and foliage, the public pays about $138 billion for damages and control against invasive species. With an increasing number of nonnative invasive species in forest and agriculture in the United States, USDA and Forest Services Research and Development have to be strategic in controlling such species. Figure 2 shows the estimates of world and U.S. population growth from 1950 to 2050. With populations and the economy increasing, demands on natural resources increase. To control invasive species both pesticides and biocontrol are used. Biocontrol is a feasible option for managing invasive species in forests and inagricultural areas (Dix *et al.,* 2009).

Digestive System of Insects and its Susceptibility to Virus

Digestive systems of insects may be adapted for breaking down cellulose, wood, fur, or other substances depending on their diets (Capinera, 2011). In general, digestive systems function as a storage, mechanical breakdown, biochemical break down, absorption and excretion (Gullan and Cranston, 2010). An insect's digestive tract (Figure 3), also known as the alimentary system consists of three main parts: foregut, midgut, and hindgut. The main purpose of the foregut is to transport, store, and filter food that has been ingested. The foregut includes a buccal cavity, pharynx, esophagus, crop, proventriculus, and esophageal valve. The midgut contains a stomach area where digestion and absorption

occurs. Aside from the stomach, the midgut also has gastric caeca and bladder pouches. Many insects have a peritrophic membrane composed of mucin lining the midgut (Upadhyay, 2003). This pertrophic membrane protects the midgut from harmful chemicals in the ingested food; however, pertrophic membrane also allows enzymes to be secreted to digest the food. Further absorption occurs in the hindgut that contains the intestine, the colon, and the ends at the rectum (Capinera, 2011). The insect body cavity, housing the digestive organs, is called the hemocoel. The cavity is filled with hemolymph, the fluid insects have in their circulatory system. The hemolymph is an aqueous phase that immerses all internal organs, delivers nutrients, removes metabolites, and performs immune functions protecting the insect body from foreign particles (Gullan and Cranston, 2010; Capinera, 2011).

Several of these digestive structures inside the insect body are affected by and affect the baculovirus during infection. Both types of baculovirus, NPVs and GVs, infect insect species. However, individual baculoviruses are very specific to an insect host species. After the consumption of either NPV or GV occlusion bodies, a special environment is needed within the midgut of a host. Activation of the baculovirus in a host requires high pH levels and the presence of alkaline proteinase (Upadhyay, 2003). With these two factors, the occlusion bodies dissolve, release, and bind to the midgut epithelial tissue.

The Process of Infection

The baculovirus has a very specific way of entering an invertebrate host. First, the insect larvae ingest viral particles that lie on the leaves or soil surface: once particles are consumed, the host is infected (Upadhyay, 2003). After consumption of the baculovirus, the viral envelope is dissolved, and the virus takes action spreading via endocytosis into insect tissue. After crossing the peritrophic membrane lining the insect's midgut, the

baculovirus arrives at the microvilli in the midgut where it fuses with epithelial cells (Federici 1997; Upadhyay, 2003). The viruses inside the cell travel with the help of microtubules, and replication of the baculovirus then occurs inside the cells. Microtubules are cytoskeletal structures inside the midgut epithelium. Federici (1997) describes another pathway for the spread of virus particles in the host. After consumption of the baculovirus, viral particles can enter into the host's midgut through hemolymph. Circulating hemolymph carries the baculovirus throughout the infected host causing a rapid spread among tissues and cells (Federici, 1997). Transport via hemolymph and replication of infected cells within the midgut allow viral particles to invade the host's body rapidly.

Between 12 to 16 hours after infection, replication of the baculovirus peaks in the host (Federici, 1997). Next, the baculovirus increases polyhedrin manufacturing inside the host. After many replications of the virus inside the host, the infected host's internal tissues start swelling and developing discolored patches (Faulkner, 1997). Within five to 11 days following the initial infection, the baculovirus causes apoptosis in the host's tissues, a form of cell degradation (Upadhyay, 2003). After the host dies, the body liquefies (Faulkner 1997), which allows viruses to infect new hosts. The timeline of infection with the baculovirus is shown in Figure 4.

In 1981, Granados and Lawler examined the pathway of infection within the larvae host by studying *Autographa californica* MNPV (AcMNPV) the baculovirus. After the polyhedral dissolves in the midgut, virions enter the host cells by virion envelops fusing with microvilli. Nucleocapsids are transported into nucleus where the virus replicates during the early phase. At this stage, enlarged nucleus is observed, and after 12 hours, nucleocapsids begin to bud out from the nuclear membrane. The budded virus proceeds to infect other tissues and cells, such as fat body, muscle, tracheal matrix, hemocytes, and the epithelial cells. This infection produces a second replication phase. Around the 24-hour period, the first occlusion bodies are observed (Blissard & Rohrmann, 1990).

Approximately on the fifth day of infection, larva stops feeding from infected cells within the host occlusion bodies lyse. Lysis of polyhedral accumulates in the basement membrane. Before death, the infected host climbs on the top of leaf, and the insect liquefies (Blissard & Rohrmann, 1990; Bonning & Hammock, 1996). Liquefaction of the dead host in infected areas increases the transmission of the virus around multiple hosts (Bonning & Hammock, 1996).

Host-Parasite Interactions

Different organisms have different defense mechanisms against viral particles. When the host is infected with the baculovirus, it defends itself from infection by adapting and creating barriers against viral infection. The first lines of defense are the physical barriers. The cuticle protects the host cells and tissues from a damaging external environment. The best way for the virus to pass this barrier is to enter the insect through the mouth, the anus or the spiracles. After viral particles are ingested, the conditions of the midgut of the insect host can inhibit growth or kill ingested baculovirus. The baculovirus particles have to survive the pH, presence of different digestive enzymes, redox potential and ionic strength of the midgut (Upadhyay, 2003).

Therefore, having a specific pH level is an important criterion for the viral particle to infect certain hosts. After the virus is ingested by specific host, the polyhedral dissolves inside the midgut when the environment there is at a high alkalinity pH 9.5-11.5. Insect species with low midgut pH are less susceptible to the baculovirus. Organisms that are not susceptible to the infection simply pass the virus through the alimentary canal. For example, birds, earthworms, mammals, and wasps are some of the organisms that are not susceptible to the baculovirus this way (Upadhyay, 2003; Inceoglu *et al.*, 2006).

The peritrophic membrane surrounds and protects the midgut creating a barrier between the epithelial layer and the midgut lumen the baculovirus infection. The host has this barrier to help protect against any chemical damages or infections. To penetrate this

membrane, NPV and GV occlusion bodies use a metalloprotease called enhancin (Figure 4). Enhancin enzymes are incorporated into the baculovirus polyhedrin matrix. This enzyme degrades the inner mucin layer of the peritrophic membrane exposing the midgut cells, increases the fusion of the virus to host cell, and causes disruption of the lining of the peritrophic membrane allowing the viral particles to be absorbed by the villi in the gut (Upadhyay, 2003; Popham *et al.*, 2001). Popham and his colleagues (2001) bioengineered virus particles that lacked functional enhancin (E1) gene, to investigate enhancin function further. Enhancin proteins were found in GV occlusion bodies, promoting the infection in the host. Popham and his colleagues (2001) suggested that enhancin improves the fusion of virus to the host cell and that enhancin causes disruption of the lining of the peritrophic membrane. The research with the *Trichoplusiani* GV (TnGV) demonstrated that enhancin damages the peritrophic membrane lining of the midgut of the host, resulting in exposure of the gut wall allowing the viral particles to be absorbed by the villi in the gut (Popham *et al.*, 2001).

After the baculovirus infects the host, the virus replicates; and the conditions inside the host cells have to be favorable for assemblage of viral particles. An insect's immune system can inactivate the baculovirus (Upadhyay, 2003; McNeil et al, 2010; D'Amico & Slavicek, 2011). However, hosts that are infected with the baculovirus have been shown to have reduced hemolymph activity, meaning that the viruses weaken the host immune responce. The innate immunity in insects is the first line of defense involving specific responses against pathogens (Abe et al, 2003; McNeil et al, 2010). The virus inhibits both humoral and cellular responses in the host. When pathogens pass through the tracheal system and into the hemolymph, the humoral defenses come into play (Figure 5). The humoral response is a defense mechanism that causes induction of bactericidal substance that kills bacteria, and agglutions (coagulation of foreign particles). This response involves recognition of foreign particles such as bacteria and the destruction of these invading

particles by means of toxins (such as melanin and cytokines). Three types of cellular responses are recognized: phagocytosis, encapsulation, and nodulation. Phagocytosis is a major mechanism by which foreign particles are engulfed and destroyed by hemocytes of the hemolymph. Encapsulation is initiated when the foreign particles are too large to be phagocytized by hemocytes. Hemocytes adhere and flatten against the target, enclosing the foreign particles from the hemocoel (Lavine & Beckage, 1995). Nodulation is an important defense mechanism against viral particles. A microaggregation takes place within hemocytes. Virus particles and bacteria are encased with hemocytes, melanized and removed by circulation (Lavine & Beckage, 1995; Gandhe et al, 2007).

As mentioned, the baculovirus causes apoptosis in infected host cells. Apoptosis is the programmed cell death (Hughes, 2002; Hay et al, 1995). When the process of apoptosis occurs, the cells exhibit the following morphological changes: surface blebbing (irregular bulging), cell shrinkage, chromatin condensation and nuclear disassembly (Hay et al, 1995). Generally, apoptosis plays an important role in immune response and development (Wyllie, 1987). Apoptosis occurs in larval cells as a defense mechanism against a viral infection. Baculovirus can prevent apoptosis in the host cells. The p35 gene and inhibitor of apoptosis (*iap*) was observed AcMNPV, showing the p35 preventing some, but not all, insect cell lines from apoptosis during infection. Mechanism of p35 was observed by detecting p35-homologous genes in other baculoviruses. *Spodoptera frugiperda (*fall armyworm) cells infected with Ac*M*NPV mutant lacking p35 exhibited apoptosis and reduction of viral particles. Therefore, insect larvae that are infected with Ac*M*NPV expressing the p35 gene do not undergo apoptosis, helping the virus to spread successfully inside the host (Means, et al. 2003; Crook, et al. 1993; Clem & Miller, 1994).

Trudeau and his colleagues (2001) initially argued that hemocytes are important for the baculovirus particles to be amplified and to disseminate infections. However, studies performed by these authors on the two hosts *Manduca sexta* and *Helicoverpa ze*a disproved

their hypothesis. Hemocytes found in the hemolymph play an important role in the immune systems of invertebrates, by phagocyting foreign particles. Trudeau and his colleagues (2001) demonstrated three events associated with hemocytes and melanization: the infection of midgut cells, the infection of tracheal cells, and the presence of the baculovirus in the circulatory system. After six to ten hours after infection, tracheal cells of the hosts were infected and baculovirus appeared in the hemolymph. Trudeau and his colleagues (2001) observed that melanization and encapsulation responses were involved in resisting the infection from AcMNPV. This suggested that insect immune system has the ability to recognize and respond to viral infections, but that hemocytes did not promote viral replication.

The history of host and parasite interactions involves a coevolutionary relationship. Coevolution is the coadaptation of a parasite and its host. Research has been performed on a baculovirus strain to determine the phylogeny of nine genome sequences among the genes that are important for this coadaptive process. Herniou and his colleagues (2004) stated two hypotheses of coadaptation. The first hypothesis is that the baculovirus originated and evolved within one group of arthropods, such as the *Lepidoptera* and later switched to additional insect hosts. The second hypothesis states that the association between baculovirus and the host is much older, and dates back to ancestral groups from which arthropods originated. To test these hypotheses, phylogenetic analysis was performed. Phylogenetic trees included the baculovirus isolated from *Lepidoptera, Hymenoptera*, and *Diptera* hosts. Following this analysis, the first hypothesis stated by Herniou and his colleagues (2004) was supported. This suggested that NPVs or GVs arose from a *Lepidoptera* host. The second hypothesis was tested by examining lineages of viruses associated with different ancestral lineages of hosts. Viruses affiliated with the orders *Diptera* and *Lepidoptera* were closely related to viruses affiliated with *Hymenoptera*. Herniou and his colleagues (2004) suggested that the ancestral baculovirus

infected different hosts through horizontal transmission. Figure 6 shows the separation of the viruses into groups according to host the virus had infected. Adaptations that are taking place between the host and the virus constrains the range of hosts available to the virus. The coevolution has limited the range of host availability to the baculovirus. For example, only specific baculoviruses infect the gypsy moth. As the gypsy moth evolved over time, the virus evolved with the insect.

Use of Biotechnology to develop Baculoviruses as Pest-control Agents

Genetic engineering can dampen negative effects of the host on the baculovirus (Upadhyay, 2003). During the first trials of the baculovirus biopesticides, there were problems with cost and variable chemical quality. Over time and with new research techniques, genetic engineering has helped to make the application of the baculovirus more effective (Miller, 1997). The first improvement was speeding up the process of the baculovirus infection by removing unnecessary genes that might benefit the host. Another important improvement was gene addition: adding a gene suitable for improving the insecticidal properties of the baculovirus (Black *et al.,* 1997). Black et al. (1997) describe the improvement in the distribution system of a gene in a host that creates a difference in functional characteristics of that gene (Carstens, 1980). Since the baculovirus can infect a large number of insects, there is a third improvement researchers developed: the addition of insect-specific toxins that disturb specific insect functions during the infection process (Tomalski and Miller, 1992; Black *et al.,* 1997). For example, an NPV *Autographa californica* contains an insect- specific toxin that acts to alter the sodium conductance of the neurons (Lorna & Stewart, 1991)**.**

The baculovirus use for insect pest control was first recognized and introduced around the 1970. Since then, the baculoviruses have been used as biocontrol agents against different insects such as Lepidopteran and Coleopteran pests of crops such as cotton and cabbage (Bonning & Hammock, 1996; Black *et al.,* 1997). When the host is infected with

the virus, it takes anywhere from days to weeks from the time of infection to kill the host. The speed of infection depends on the temperature, dosage, age of the insect, host and virus species. Genetic engineering can be applied to the baculovirus to reduce the time that infection takes to kill the insect.

Infecting invasive species with genetically engineered baculovirus kills the species that harm forest and agricultural crops. The baculovirus genome can accommodate up to 50 kbp of foreign DNA, and foreign genes can be easily expressed by the baculovirus. Recombinant baculovirus with genetic material from multiple sources can be constructed. The baculovirus improvement is done through the use of heterologous genes coding for toxins or hormones introduced into a baculovirus transfer vector (Tomalski and Miller, 1992).

Before using recombinant baculovirus as a biopesticide agent, it is important to understand risk-assessment studies of the genetically modified organisms. There are advantages and disadvantages of using insecticides recombinant baculovirus insecticides. The advantages of recombination provide important characteristics of baculovirus to be more host-specific. This characteristic allows the virus to be ideal for integrated pest management (IPM). The IPM program targets pests that damage vegetation. Moreover, using the baculovirus in IPM programs along with other biological-control agents or with traditional chemical insecticides helps reduce pesticide resistance in pests. Recombinant viruses are an effective way to enhance the effects of traditional insecticides. One of the disadvantages of using recombinant baculoviruses for what is its reduced recyclability in the environment, as a recombinant virus produces fewer polyhedral units (Bonning & Hammock, 1996).

In order to create the baculovirus as an effective insecticide, genetic engineering combines pathogenicity of the virus with the insecticidal action of a toxin, hormone or enzyme. The recombinant approach improves the production, modifing host range, and

enhances the usefulness of the biopesticide. One way to enhance the baculovirus as a biopesticide is by reducing the time from infection with the recombinant virus to the death of the insect. This reduction is an incentive for farmers and other users to accept the baculovirus as an insecticide (Bonning & Hammock, 1996). For example, toxin gene tox34 produces a neurotoxin venom protein known as TxP-1 causing an immediate muscle paralysis (Tomalski and Miller , 1992). TxP-1 is a toxin derived from the straw itch mite *Pyemotes tritici* (Bonning & Hammock, 1996). To determine the ability of AcMNPV tox34 gene to produce a similar toxin, insect larvae were injected with the same dosage of the toxin as is delivered during infection. After 24 hours, AcMNPV baculovirus recombinants expressing tox34 in insect larvae hosts paralyzed about 10% of insects. The rest of the infected larvae were dead. Larvae hosts that were mock infected died after 96 hours. Larvae that were infected with tox34 quickly melanized and changed coloration after death. With the expression of tox34 gene in the virus, the larval weight gain was dramatically reduced during infection. This reduction in weight gain indicates that less food was consumed by larvae, and thus less damage was done to the crops (Tomalski and Miller, 1992).

Recombinant baculovirus can cause other detrimental symptoms in infected larvae. AaIT is derived from the North African scorpion *Androctonus australis*. Larvae infected with recombinant baculovirus expressing this toxin showed signs of dorsal arching, increased irritability and stopped feeding (Bonning & Hammock, 1996). Another recombinant baculovirus was developed that enhanced the virus to express the diuretic hormone from the tobacco hornworm, *Manduca sexta*. Larvae that were injected with it died 20% more rapidly (Bonning & Hammock, 1996).

Another interesting effect on the host that helps virus propagate among the insect population is associated with the expression of *egt* gene in a modified virus. Eclosion hormone is expressed in bioengineered AcNPV. This hormone is involved in ecdysis,

which is the molting of the cuticle in caterpillars. The baculovirus AcNPV produces ecdysteriod UDP-glucosyltransferase (*egt*). Expressing the *egt* protein inhibits molting and pupation in larvae, allowing the larvae to keep feeding, prolonging the insect feeding time, and allowing the insect to gain weight (Bonning & Hammock, 1996; Burand et al, 1996). However, by producing the *egt* protein, the virus inhibits the normal behavior of the host. When *egt* protein is not expressed, the insect behaves normally. During normal behavior larvae feed at night and migrate down to the trunk of the tree to hide from predators during the day. Normally, infected insects molt within the trunk of the tree, thus reducing the propagation of the virus. A virus enhanced with *egt* gene affects the larva behavior by making the insects stay on the leaves, and continue to stay on the leaves until they die. The expression of the *egt* gene in the virus increases the rate of its propagation and the persistence in the environment (Dr. Slavicek, personal communication). *Egt* conjugation of ecdysteriods with UDP-glucosyltransferase and inactivation of ecdysteroid hormones suppress molting, pupation and alter the behavior of the larvae (Burand et al, 1996; Dr. Slavicek, personal communication).

Invasive species of insects disrupt vegetation and change the balance in ecosystems by outcompeting native species. To reduce the numbers of gypsy moth species, Gypchek, the market name for the biologically engineered baculovirus, is applied to the gypsy moth species in the area. Gypcheck is one of the examples of marketed baculovirus. Two strategies are utilized in this type of pest control. Areas that are infected with invasive species are either sprayed with concentrated baculovirus to decrease the pest numbers, or sprayed with lower concentration of the baculovirus, establishing the virus among the pest species for multiple generations (Moscardi, 1999). Gypchek is also used as a powder produced from killed larvae (LdNPV). Before application, the virus powder is mixed with commercial lignosulfonate-based formulation. This mixture can be applied through aerial methods or through the ground in parks and urban infected areas. Presently, Northern

Research Station (NRS) personnel at Ansonia CT in partnership with Animal and Plant Health Inspection Service (APHIS) personnel are producing Gypchek at Otis, MA. The use of Gypchek on infected trees within areas causes larval numbers to decrease within several years. More than 100,000 acres have been treated with Gypchek under state/federal cooperative suppression programs. The demand for the Gypcheck product has dramatically increased throughout the years, resulting in a decrease of production cost, and improvements in the lethal time of infection (Dr. Slavicek, personal communication).

Forest areas present a more stable environment for the baculovirus for longer periods of time compared to agricultural environments. For example, in agriculture, *Spodoptera* genus is important to control. In Brazil, approximately 20,000 hectares of maize were under control against *Spodopters frugipedera* NPV (Moscardi, 1999). In the same country, over 2,000,000 hectares of soybean plants were subjected to the baculovirus treatment. For this crop the numbers of invasive species have decreased. However, because of technical problems in laboratory s, the use of the baculovirus in these areas was terminated. This treatment was also tested on the cabbage crops in India. Granulovirus GpGV is used for approximately 250,000 hectares against coddling moth *Cydia pomonella* to protect apple and pear orchards in Germany, France, Switzerland, and Russia (Lacey *et al.*, 2008). A new virus currently used is *Homona magnanima* GV in Japan. About 5,850ha tea, field areas were sprayed with GV. China uses the baculovirus to protect cotton, pepper and tobacco. Therefore, using these different species of baculovirus around the world brings up economical, ecological and social benefits (Moscardi, 1999).

The use of baculovirus in different regions has allowed for the stability of the virus. A stable virus has high effectiveness. As previously mentioned, baculovirus can generally be affected by temperature, pH, humidity, additives and ultraviolet light. Ultraviolet light can be detrimental to baculovirus. Within the different environment fields, the maximum activity of the virus is under the shaded regions of plant canopy. Recently

UV protectants have been added to genetically engineered viruses, and stilbene fluorescent brighteners were marketed under Gypcheck (Zou and Young, 1994).

Future use of the baculovirus pesticides depends onbiotechnology. In agriculture however, only naturally occurring baculovirus is used to treat crops. Improvements can be made to the virus by developing *in vitro* cultures and constantly changing the formulae of the biopesticide (Bonning & Hammock, 1996). Another important development is formulating brighteners that reduce the cost of baculovirus. Baculovirus may become inactive when it gets affected by plant metabolites like peroxidase generating free radicals, however these free radicals do not cause much of an effect on the virus (Hoover *et al.*, 1998; Dr. Slavicek, personal communication).

Technology used to produce the baculovirus is carried out *in vivo* by applying the virus in the insect host, collecting diseased or dead larvae, and giving an artificial environment and diet to the target insects in the labs. Advantages of using *in vivo* technology are that the process is more controlled, sterilizing techniques are used, and the resulting product is highly pure. *In vitro* process (using cell cultures instead of live hosts) of baculovirus production is used for agricultural invasive species and is efficient in affecting the insect hosts. The product of the *in vitro* process is highly pathogenic to the target host. Both *in vivo* and *in vitro* methods have been successfully used for production of the baculovirus (Moscardi, 1999).

Other current research is focused on increasing the effectiveness of *in vitro* technique by developing formulations suitable for *in vitro* product and developing strong viral strains agreeable to *in vitro* production. Using an *in vitro* system decreases the cost of the product. Using the Wave cell culture bioreactor produces Gypcheck on a larger scale, enough to treat 20,000 acres per year. Compared to current ways of production in a stirred tank bioreactors, the wave bioreactor has a greater potential to produce polyhedral. The wave bioreactor also lowers the cost of production and eliminates downtime and

autoclaving. The system of the wave bioreactor is created by using sterile disposable plastic bags instead of stainless steel piping/fittings. Another important criterion that the wave bioreactor presents is the movement of the liquid, which allows greater oxygen transfer. When the stirr tank is used, bioreactors created shear stress from agitation and sparger. Shear stress is when the cell membrane is destroyed causing the cells to die. Wave bioreactor eliminates shear stress and sparger caused by agitator allowing healthier cells and increased amounts of polyhedral production. The wave bioreactor yields twice as much polyhedral. During the recent study of the wave bioreactor, a decrease in polyhedra production was initially observed, but it stabilized around tenfold. The cell densities in the wave bioreactors declined from 8×10^6 cells/ml to 2×10^6 cells/ml. This decline was the reason for the decreased amounts of polyhedra production (Dr. Slavicek, personal communication).

Conclusion

For over 2000 years, humans have known about diseases caused by the family *Baculoviridae*, parasitic baculovirus**.** Baculovirus particles are rod-shaped and contain double-stranded DNA (dsDNA). Baculovirus includes two groups of occluded viruses that differ in their structural characters, *Nuclearpolyhedrosisviruses* (NPVs), and the *Granuloviruses* (GVs)**.** These two groups of the baculovirus are used as a biopesticidie agent in areas infested by invasive species harming agricultural and forest areas. There are many different groups of the baculovirus created to kill a specific host in the area. The most studied are *Autographa californica* AcMNPV and *Orgyia pseudotsugate* OpMNPV. Many insects that are attacked by the baculovirus belong to the order *Lepidoptera.* The moths feed on foliage or cadavers on the leaves and get infected with the baculovirus. After the feeding the budded viruses infect other cells in the host body, such as epithelia, and muscle cells. Around the fifth day larva stops feeding and dies becoming a liquefied cadaver. The host is capable of protecting itself against the virus by having first line of

defense, immune system, or by lowering their pH. To increase the viability of the virus in the host, genetic engineering is used. Genetically engineered baculovirus kills the species that harm forest and agricultural crops. Biotechnological improvement of the baculovirus is done through inserting heterologous genes coding for toxins or hormones into the naturally occurring baculovirus. Going in the route of recombinant baculovirus as biopesticide agent is a great way to manipulate the virus to attack invasive insect species. To increase the likelihood of the virus successfully spreading among the host populaiton, *egt* protein expressed in the virus inhibits molting and pupation in larvae, and the p35 gene prevents insect cell lines from apoptosis during infection. Expressed *egt* allows the virus to be propagated across the infected environment, while p35 inhibits apoptosis allowing the virus to infect the host cells and to replicate successfully without interference. Formulated baculovirus is called Gypchek. This is the market name for the baculovirus, which is commercially used. Gypchek has been applied to forest and agriculture fields in Brazil, Germany, France, Switzerland, Russia, China, and Japan. Efforts of enhancing the baculovirus are made by producing polyhedra in the wave bioreactor. Such production lowers the cost of the product, and is much more efficient compared to the stirred tank bioreactor previously utilized. However, initially there have been some problems, as the cell densities declined from 8×10^6 cells/ml to 2×10^6 cells/ml. Using bioreactors decreases the amount of polyhedra production. Bioreactors can produce the polyhedra virus but cannot produce the virus economically. For example, in order to generate enough of the virus to treat an acre of forest by producing polyhedra within the invasive species and extracting the virus costs about 80 dollars. The same amount of the virus produced in bioreactors costs around 500 dollars. Dr. Salvicek and many other researchers in other countries have tried to reduce the cost of bioreactor production, but have not been successful. Therefore, Dr. Salvicek and his team are working on finding new ways of making this process more efficient and inexpensive.

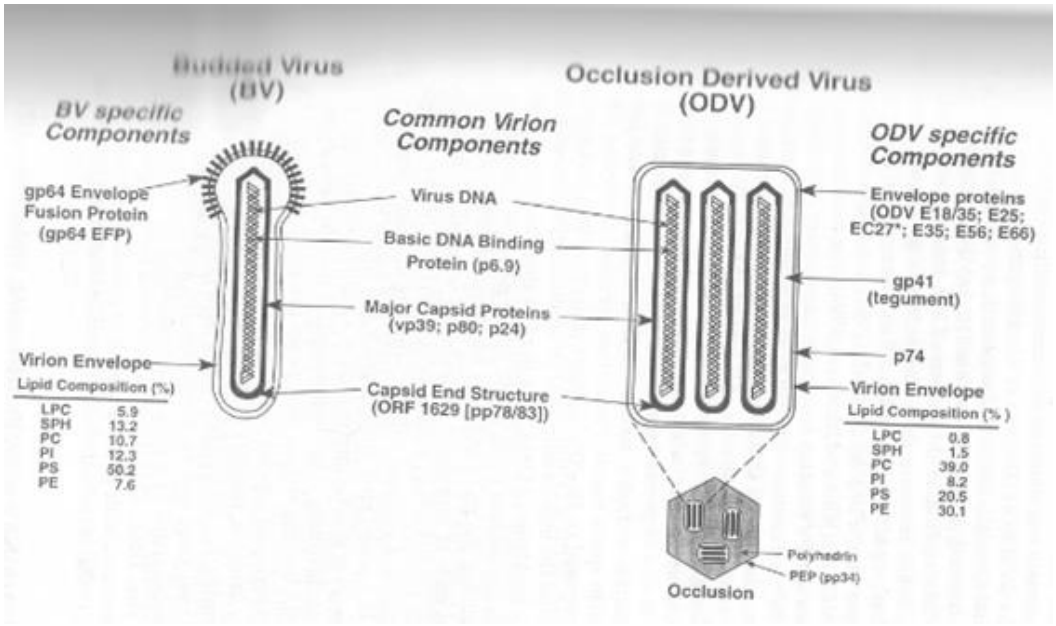

Figure 1. Image of the Budded Virus and the Occlusion Derived Virus. Occlusion derived virus (ODV) present in a protein matrix polyhedrin. ODV is responsible for the primary infection of the host while the budded virus (BV) that is not enclosed in a protein matrix is released from the infected host cells later during the secondary infection (Funk 1993).

Figure 1.—*World population and estimates, 1950–2050 (United Nations 2007).*

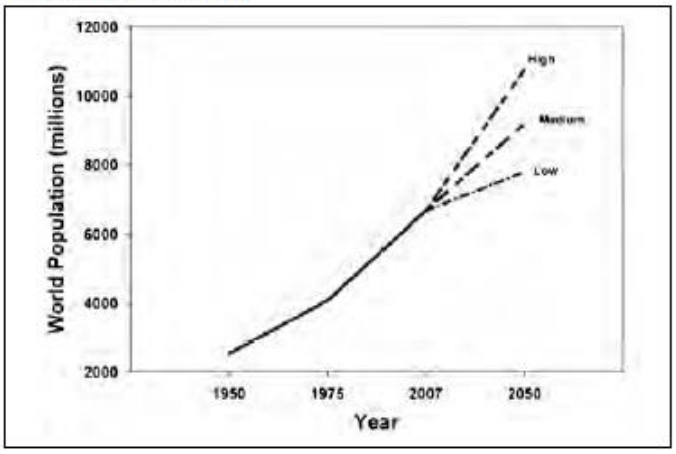

Figure 2.—*U.S. population and estimates, 1950–2050 (U.S. Census Bureau 2004).*

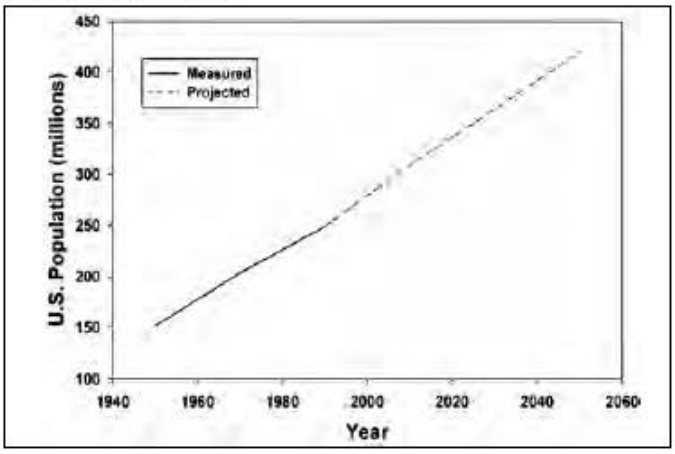

Figure 2. Estimates of world and U.S. populations from 1950 through 2050. As populations and economy increases, demands on natural resources also increase. This increase shows the demand to control the invasive species (Dix *et. al*, 2009).

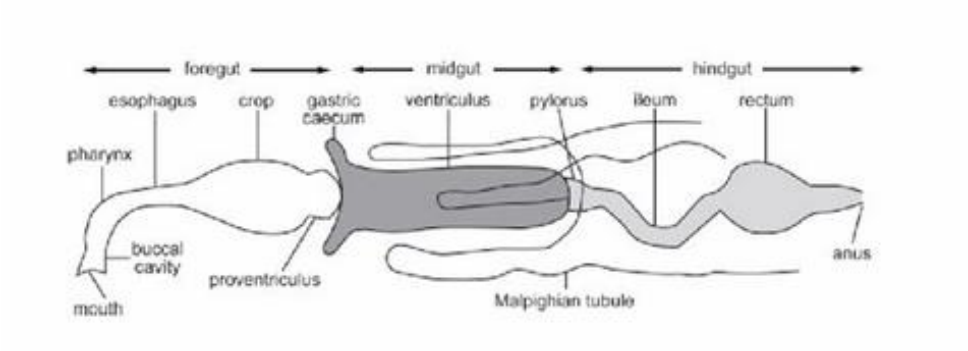

Figure 3. Diagram of the insect digestive system also known as the alimentary system. (Capinera, 2011).

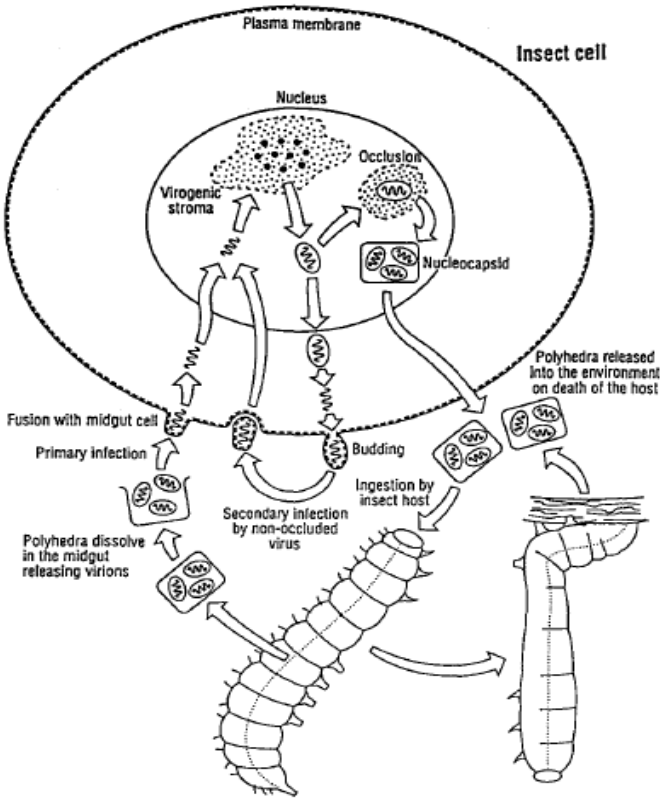

Figure 4. The life cycle of nucleopolyhedrovirus (NPV). After ingestion of the virus, polyhedral dissolve in the midgut. Primary infection fuse with the midgut cells. The viral particles replicates inside the nucleus and buds out through the cytoplasm membrane to spread the infection throughout the insect. When the insect dies, the polyhedra are released into the environment (Bonning & Hammock, 1996).

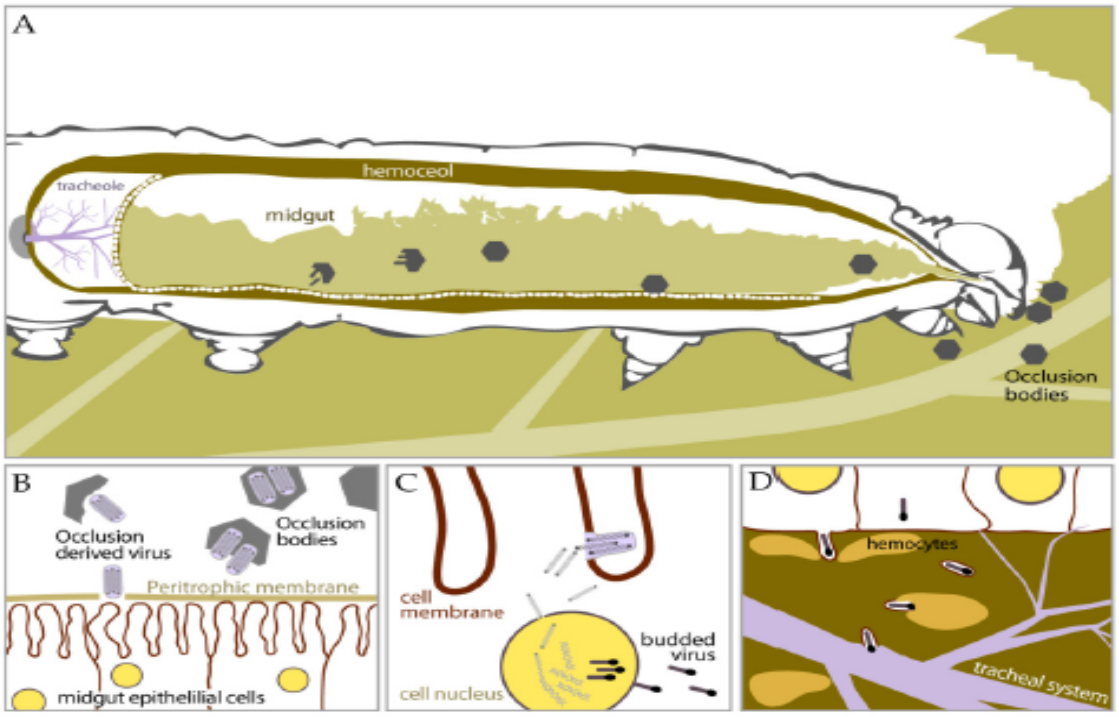

Fig. 1. Nucleopolyhedrovirus transmission. (A) NPV infections in larval Lepidoptera are initiated when caterpillars ingest viral occlusion bodies on foliage. (B) These dissolve in the alkaline conditions of the midgut and release occlusion derived nucleocapsids, which penetrate the peritrophic membrane. (C) Nucleocapsids fuse with the membrane of epithelial cells and pass through nuclear pores to begin production of the budded form of NPV. (D) Budded virus leave the midgud cells and infects hemocytes and other cell types.

Figure 5. A detailed process of Nucleopolyhedrovirus transmission. (A) After ingesting viral occlusion bodies on foliage. (B) Occlusion bodies dissolved in alkaline environment within the insect. The virus particles penetrate the peritrophic membrane to get inside the midgut epithelial cells. (C) Nucleocapsids fused with the membrane of epithelial cells and passed through nuclear pores to begin production of the budded form of NPV. (D) Budded virus leaves the midgut cells and infected hemocytes, tracheal system and other cells (D'Amico and Slavicek, 2011).

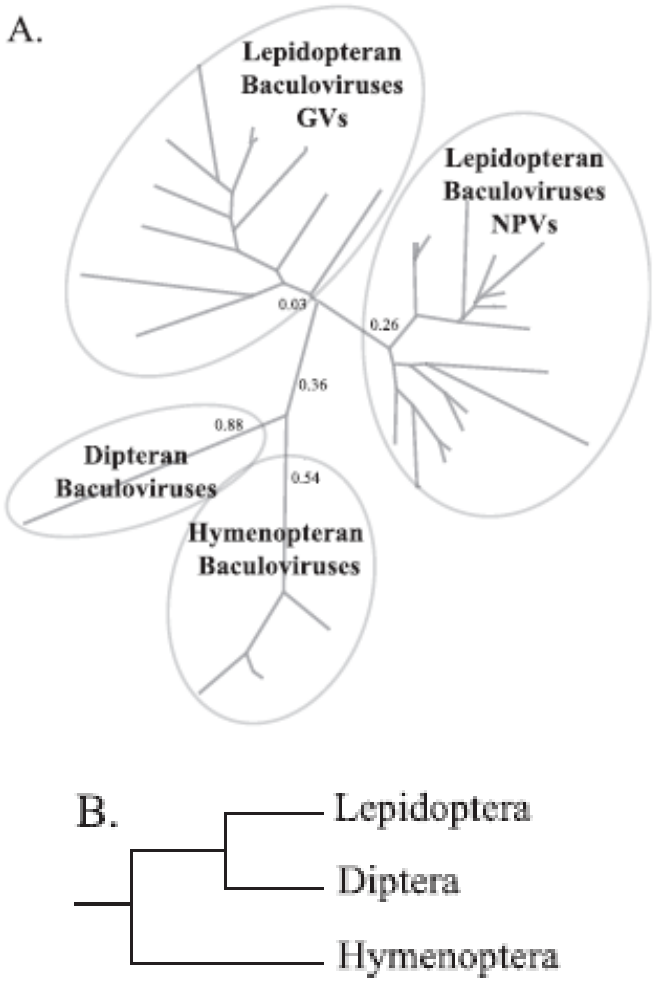

Figure 6. The evolution relationship of the family *Baculoviridae*. (A)Phylogeny of the baculoviruses highlights four main groups of the unrooted tree. (B) Shows the relationships of the three arthropod orders infected by the baculoviruses (Herniou, 2004)

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