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9. Baculoviruses as insecticides: Four examples

One of the earliest references for using natural pathogens for insect control was reported by V. Audouin in 1839, who tells of a sericulturist who emptied fungus-contaminated silkworm rearing trays out a window onto trees infested with defoliating insect larvae. Within a few days all of the defoliating insects had died of the fungus. More explicit suggestions for the microbial control of insects were made by J.L. LeConti in 1874 in an address to the AAAS, in which he recommended the study of epidemic diseases of insects and advocated their use to control insects. At the same time, Louis Pasteur, who had spent considerable effort investigating a microsporidian parasite of silkworms, recommended the use of this pathogen against an insect pest of grapes, described in (1).

In ecosystems, baculoviruses often play a major role in the suppression of a variety of different types of insects. For example, the virus of the gypsy moth, *Lymantria dispar,* is considered to be the major natural regulator of dense populations of this moth (2). Likewise, baculoviruses of the Douglas fir tussock moth, *Orgyia pseudotsugata*, are also major factors in the control of this insect (3). They also are found to naturally control pest insects of cultivated crops. For example, they were found to be major contaminants of cabbage purchased from supermarkets in the Washington, D.C. area, and it was estimated that an average serving of cole slaw would contain over 100 million occlusion bodies (4). Once the role these viruses played in controlling natural insect populations was understood, they were considered for a variety of insect control programs, particularly of forest pests (5).

There have been numerous reviews on the development of baculoviruses to control insects, e.g., (6-10), and despite this widespread interest and intrinsic attractiveness of their application, the acceptance and use of viruses for insect control has been limited. This can be attributed to their slow speed of kill, their limited host range (such that one preparation can only be used on a few insects), and to a certain degree, the complexity of producing standardized viral preparations. The slow speed of kill may be of particular advantage to the virus, because it results in greatly increased viral yields. However, delays in the death of the host result in more vegetation being consumed by the infected insect. A variety of recombinant viruses have been investigated that have been designed to enhance the efficacy of the virus by reducing the time it takes to kill target insects or by causing the cessation of feeding. These recombinants express insect specific toxins, insect hormones or enzymes, or are deleted for the EGT gene. Significant public resistance was encountered when a plan to test spray an AcMNPV expressing a scorpion toxin gene was announced in 1994 (11) (12).

Another contributing factor to the limited use of baculoviruses for biocontrol is that production of viral insecticides is labor intensive; consequently, their use has been limited to high value crops, particularly those that have become resistant to chemical insecticides or to crops in countries with access to relatively inexpensive labor. An exception appears to be their use against forest insects in North America. However, relative to the size

of forested areas, these applications are also limited. In addition, the use of *Bacillus thuringiensis* preparations is highly competitive compared to baculoviruses because of the simplicity of their growth and formulation. In this chapter, I will review four instances where viruses have been relatively extensively applied in the field. Recent reviews include (13, 14).

An NPV of the velvet bean caterpillar, *Anticarsia gemmatalis*: Application in Brazil – A major setback

The most extensive program employing baculoviruses for insect control was developed in Brazil and to a lesser extent in Paraguay and involves a virus that infects the velvet bean caterpillar (*Anticarsia gemmatalis*), a pest of soybeans (15-17). Virus preparations are applied at 1.5×10^{11} occlusion bodies per hectare (about 20 g or 50 larval equivalents). This program was initiated in the early 1980s, and by 2005, the area treated had expanded to over two million ha, ((10) and references therein). Initially, laboratory production was not found to be economically viable, and virus production was carried out in farmers' fields. Plots of soybeans that were naturally infested with *A. gemmatalis* were sprayed with virus and then the dead larvae were collected 8 -10 days after virus application. Individuals were able to collect about 1.8 kg of larvae/day at a cost in the mid 1990s of about $15. Production varied in the 1990s from enough virus to treat 650,000 to 1.7 million ha/year. By 1999, the production of virus was not sufficient to meet the demand.

Problems occurred with field production. i) The quantity of infected larvae was dependent upon the natural prevalence of the host insect that could vary from year to year; ii) There was a change in the collection procedure - the plants were shaken over drop cloths which led to a poor quality product because the material included other insects, debris, and *A. gemmatalis* that were not in the final stages of the virus infection cycle. The final product was of lower infectivity and also caused problems with application – the debris caused clogging of equipment used for application.

To address these problems a laboratory was established for the growth of larvae on an artificial diet and their infection under controlled conditions. Although slightly more expensive than virus produced under field conditions, it was of much higher quality. The company involved in this project scaled up to employ 45 people and infect 800,000 to 1,000,000 larvae per day resulting in the ability to produce enough virus to treat up to 2 million ha/yr. The infected larvae were processed into a wettable powder that involved milling the infected larvae and formulating them into a mixture that contains kaolin. Kaolin is an aluminosilicate compound first discovered in Kao-lin, China that is commonly used as an inert carrier or filler.

Some of the reasons for the feasibility of this viral control program were caused by specific features of AgMNPV. First, the virus is highly virulent for *A. gemmatalis* and usually only needs to be applied one time. In contrast, chemical insecticides often need to be applied twice. Furthermore, AgMNPV lacks the chitinase and cathepsin genes, so the insects die without 'melting,' and the dead insects can be more readily collected and processed than if they had disintegrated (18) (see Chapter 3). Another factor contributing to the initial success of the program is that soybean plants can endure significant defoliation without a reduction in yield. The virus can also be spread by insect predators and can survive passage through the digestive tract of beetles and Hemiptera (17). Overall, the use of the viruses was 20-30% less expensive than chemical insecticides, and it was estimated that the use of up to 17 million liters of chemical insecticides was been eliminated since the beginning of the program. Limitations included the reluctance of farmers to monitor their fields to determine the optimal timing for virus application and its use in regions that have low mean temperatures, which lengthens the time required to kill the insects. Extended periods of drought also adversely affect the efficacy of the virus preparation, ((10) and references therein).

Recently, a major shift in the method of soybean cultivation resulted in a dramatic decline in the use of the virus (19). This involved a change to a no-till system that involves the application of herbicides prior to planting. The

farmers mixed broad-spectrum insecticides with the herbicides such that all plants and insects were indiscriminately eliminated. At about 2 to 3 weeks after soybean emergence, the fields were again treated with an herbicide-insecticide mixture. This resulted in the reduction of beneficial insects and the emergence of several other insects including mites, *Pseudoplusia includens* (the soybean looper), white flies, and species of *Spodoptera* that previously had been considered secondary pests. Consequently, since they could not be controlled by the AgMNPV preparation, alternative broad spectrum insecticides had to be used. The situation was further complicated by the introduction of soybean rust that required the application of fungicides that reduced the presence of naturally occurring entomopathogenic fungi that had served to naturally control some of the pest species. Collectively these changes led to a reduction in the area treated with AgMNPV to about 300,000 ha/year and resulted in the termination of the laboratory production of virus.

A granulovirus of the codling moth, *Cydia pomonella*: Application in North America and Europe

Whereas the use of AgMNPV has been limited predominantly to one major area in Brazil, a granulovirus of the codling moth *Cydia pomonella* (CpGV) has been used in a number of countries in North America and Europe for the control of the insect on pear and apple crops. CpGV was originally isolated from *C. pomonella* in Mexico in 1963 (20). Because of the development of resistance of codling moth to several chemical insecticides and for a variety of other safety and environmental reasons, the use of CpGV has increased in Europe and North America since 2000. The virus is used on a hundred thousand or more hectares on these continents. Currently, commercial preparations of the virus are available from several different companies and include preparations called Cyd-X and VirosoftCP4 in North America and in Europe include Carpovirusine™ (France), Madex™ and Granupom™ (Switzerland), Granusal™ (Germany), and Virin-CyAP (21) (10). The virus is highly virulent for codling moth with LD50's as low as 1.2-5 occlusion bodies per insect. The codling moth lays eggs on fruit trees, and after hatching, the larvae browse on leaves before entering fruit. They need to feed inside fruit for normal development, which can result in severe damage. Depending on the climate, there can be from one to three generations per season, and to ensure exposure during the brief window from hatching to entry into fruit requires the application of CpGV at least at weekly intervals up to six times in a season.

Recently, resistance to the virus has been described in Europe, with these insects able to tolerate CpGV over 1,000 times higher than previously observed. In laboratory experiments, it was determined that a gene conferring resistance is located on the male (Z) chromosome, and it was found that females with a single Z chromosome could be selected that were almost 100,000 times less susceptible to the CpGV infection (22). Because of the complexity of baculovirus replication it was often assumed that it would be challenging for an insect to develop resistance. However, these results clearly indicate that the alteration of a single or limited number of linked genes can severely compromise the infectivity of these viruses. Although the mechanism of CpGV resistance is not clear, its evolution emphasizes how dependent baculoviruses are on their hosts for carrying out their replication cycle and how a change in a single receptor or other protein, such as would be required for DNA replication, can interfere with virus infectivity.

An NPV of the cotton bollworm, *Helicoverpa armigera*: Application in China

The cotton bollworm, *Helicoverpa armigera,* is a major pest of cotton and other crops, and with the intensive use of chemical insecticides it has developed resistance in many parts of the world. One approach to counteract this resistance has been the use of baculoviruses for control of this insect. In China, HearNPV has been produced for use against the cotton bollworm. In 2014 there were 17 different products from 10 companies that incorporated HearNPV as the insecticidal component of their products and about 968 tons of this product were produced (13). The insects were grown on an artificial diet composed of mainly corn and wheat, and the infected larvae

were processed after removal of lipids into wettable powders or emulsions. Treatment involves spraying fields 3 to 5 times per growing season to control two generations of the cotton bollworm. It was estimated that the virus preparation was used on 200,000 to 300,000 hectares of cotton in 2005. In India, it has been reported that insects are collected by shaking larvae off pea plants onto blankets. HearNPV is then produced by feeding the larvae virus-contaminated chickpea seeds (10).

A recombinant HearNPV is being evaluated in China that expresses a gene encoding an insect-specific toxin (AaIT) from a scorpion found in the Middle East and Africa called *Androctonus australis*. The use of this recombinant baculovirus is limited to experimental plots of about 2 hectares. In this construct, the AaIT gene is inserted at the EGT locus. This causes problems with the production of the recombinant virus in infected insect larvae. Since the toxin is active against larvae, and deletion of the EGT gene reduces the time it takes the virus to kill the insect, the levels of production are significantly affected; under optimal conditions virus yield is less than 50% of wt. However, the yield from cotton plants treated with this virus is about 25% higher than from plants treated with wt virus. Consequently, this recombinant has significant advantages over the wt virus (23).

A variety of other viruses that are being produced in China range from 120 tons of AcMNPV to less than 50 tons of several other viruses in 2005. These were used to control a variety of insects mostly on vegetables and tea. The data described above is from (23) and Xiu-lian Sun (pers. Comm.)(13).

A nudivirus of the coconut palm rhinoceros beetle, *Oryctes rhinoceros*

Although the *Oryctes* virus is not a member of the Baculoviridae, it is of a related lineage and is an interesting example of the advantages of employing viruses for insect control. This section was based on a review by Alois Huger (24). The coconut palm rhinoceros beetle, *Oryctes rhinoceros* was accidentally introduced into a number of Pacific Islands in first half of the 20th century from tropical Asia. They feed on the developing fronds of several types of palm trees including coconut and oil palms. The damage reportedly caused the death of about 50% of the coconut palms in some locations. Attempts to control the insects using chemical insecticides were unsuccessful due to the inaccessibility of the pesticide to the insects.

In 1963 Alois Huger of the Institute for Biological Control in Darmstadt, Germany was hired for 4 months to attempt to isolate pathogens of the beetle. He focused his search on Malaysia where a native indigenous population of the insects were present. He visited oil palm plantations that provided an abundance of all stages of the insect that inhabited rotting palm logs and stumps. This material resulted from older trees that had been cut down as the plantations were replanted on a 30-35 year cycle. Larvae with an apparent disease were identified; they showed a negative geotropism and congregated on the surface of their feeding substrate (buckets of rotting sawdust) and were lethargic, showed a variety of pathological symptoms, and died within 1 to 4 weeks. Extracts from the diseased larvae were fed to healthy larvae resulting in similar symptoms which allowed for the investigation of the disease. It was found to be caused by a rod-shaped virus that replicated in virogenic stroma and was predominantly non-occluded. The virus was initially classified as a member of the Baculoviridae, but decades later viral genome sequence data indicated that it was more similar to members of the Nudiviridae, a viral family in the same lineage as baculoviruses (25).

In 1967 an experimental release of the virus was conducted on two islands in Samoa in which rotting sawdust containing the virus was used to contaminate rotting coconut logs, a major breeding substrate of the beetle. This treatment led to the collapse of the beetle populations on the treated islands and within 18 months diseased larvae were identified on a third island that had not been treated. It was subsequently determined that the adult beetles were also susceptible to the virus and became heavily infected in their midguts. The infected adults could survive for many weeks during which they defecated large amounts of virus into their breeding and feeding sites. The virus not only resulted in the death of infected larvae, but also caused a major reduction in the fecundity of

infected female adult beetles. Subsequently, control of the insects was initiated by the contamination of adult insects. This led to a reduction in tree damage of up to 95%. Recent data suggests that the insect may have developed resistance to the virus (26).

Possible unintended consequences. The Japanese rhinoceros beetle, *Allomyrina dichotoma*, is found in Southeast Asia and is raised commercially for medicinal use, as children's pets, and for entertainment and gambling via staged beetle combat. In 2012 it was reported that *A. dichotoma* larvae on a farm in Korea died en masse. By 2014 similar incidents were reported throughout Korea. Analysis by pcr using primers specific to the *Oryctes rhinoceros* nudivirus (OrNV) gave positive products that were 98% identical to the OrNV genome sequence (27). It was suggested that the virus, which was not previously reported in Korea, had somehow been transported there. In contrast to the *Oryctes rhinoceros* beetle, *A. dichotoma* is not an agricultural pest, but a part of a niche farming economy. The source of this virus has not been determined.

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