Viruses as Entomopathogens and their Role in Coconut Pest Management

Murali Gopal and Alka Gupta
Central Plantation Crops Research Institute, Regional Station, Kayangulam Krishnapuram - 690 533, Kerala.

Introduction

Green revolution has led to intensified agriculture to meet the ever increasing demands for food and fibre but at the same time has resulted in continual loss of natural ecosystems, increased pesticide usage and concomitant pollution of ground water and other environmental degradation. An important aspect of the sustainable agriculture that attempts to minimize negative environmental impact and other deleterious effects due to increased insecticide usage (pesticide resistance, pest resurgence and secondary pest outbreaks due to elimination of natural enemies, etc.) is the Integrated Pest Management (IPM) strategy. In IPM, natural enemies (parasites, predators and pathogens) of pest arthropods and other alternative measures play significant roles in crop protection.

More than 1500 species of pathogens are known to affect arthropods, which include bacteria, viruses, fungi, protozoa and nematodes. Among these, a large variety of entomopathogenic viruses representing over 20 groups are reported from insects.

Viruses and their classification

Viruses are obligate intracellular parasites and the smallest 'living units' varying in size from 20 nm to 300 nm. They consist of a genome (either DNA or RNA) enclosed in a protective protein coat (capsid) and further enclosed in additional layers termed envelopes. The capsid plus the DNA or RNA it enclosed is termed nucleocapsid or virion. In certain cases, the virions are further embedded in protective protein matrices termed occlusion bodies. Viruses replicate inside host cells using the host's protein-synthesizing metabolism and materials in the host cell.

Viruses pathogenic to arthropods are classified based on the nature of their molecular form (single-or double-stranded DNA or RNA) and other characteristics into 16 families : Baculoviridae, Polydnaviridae, Poxviridae, Ascoviridae, Iridoviridae (double-stranded DNA forms), Parvoviridae (single - stranded DNA form), Reoviridae, Birnaviridae (double - stranded RNA forms), Nodaviridae, Picornaviridae, Tetraviridae, Rhabdoviridae, Caliciviridae, Togaviridae, Bunyaviridae, Flaviviridae (single - stranded RNA forms)

Arthropod - pathogenic virus groups

Insect virus associations numbering more than 1270 have been recognised. Baculoviridae and Cytoplasmic Polyhedrosis Viruses (CPV : Reoviridae) are the two commonly observed virus groups.

The cytoplasmic polyhedrosis viruses (CPVs) are reported from 4 insect orders, most commonly from Lepidoptera (201 host species) and Diptera (39 host species). They produce chronic infections and persist as endemic diseases under natural conditions at fairly low rates, thus, having less impact on the host population. Only one CPV of pine caterpillar *Dendrolimus spectabilis* has so far been developed and registered as a viral insecticide.

CPV in coconut pests

In coconut pest management, a highly potent cytoplasmic polyhedrosis virus has been reported against red palm weevil, *Rhynchophorus ferrugineus* F. The red palm weevil is a dreaded pest which damages the crown of the coconut palm and the stem portion close to the crown region, and ultimately the crown of the affected palm topples off. The bole region of the seedlings are also damaged by this pest.

This CPV infects all the life stages of the red palm weevil, including the adult. The diseased grubs of this pest are smaller, yellowish in colour, and show retarded development, less movement and feeding. In the early stages of the CPV disease, the head of the grub becomes enlarged and a constriction appears at the anterior abdominal region, with the hindgut getting completely extroverted. In moribund stage, they are shrunken, leathery with tough and shiny integument. The CPV infection in the last instar stage of the grub results in emergence of malformed adults (beetles) which die within a short span as compared to normal adults, thus suppressing the insect population drastically.

The endopathological symptoms manifested due to CPV disease involves the midgut region becoming opaque, enlarged and milky white in colour and a very fragile midgut line prone to break-off even at the slightest touch. The midgut fluid of such grubs contains thousands of polyhedral inclusion bodies (PIBs) of varying sizes. These PIBs take up Giemsa stain and can be viewed microscopically. The electron microscopic examination show PIBs with hexagonal and pitted appearance containing the spherical virus particles with surface projections characteristic of CPVs.

Baculoviridae

The majority of research and development of viruses as microbial control agents has been devoted to the Baculoviridae group. Over 800 insect species, including Lepidoptera, Hymenoptera, Coleoptera, Diptera and species from several other insect orders have been reported to be infected by baculoviruses. The baculoviruses are unique in that they are entomopahtogenic only and are safe to biosphere including parasites and predators of the host insect. Deleterious effects of baculoviruses on pollinators, predators and adult parasitoids have not been observed in nature. On the other hand, parasitoids and predators can disseminate several baculoviruses without the risk of infection. A large number of baculoviruses can be produced in considerable quantities with relatively little expenditure in terms of effort and cost. Although they infect over 800 species of insects, most of the baculoviruses are quite specific and show a limited host range, attacking a single species of insect or insects within the same genus, the exceptions being the baculoviruses of *Autographa californica* (Speyer) (AcMNPv) and *Anagrapha falcifera* (Kirby), each of which has a host range of 30 or more lepidopteran species.

Occluded Baculovirus

The virus family Baculoviridae is divided into two main groups : the Eubaculovirinae comprising of occluded baculoviruses - Nuclear Polyhedrosis Virus (NPV) and Granulosis Virus (GV), and the Nudibaculovirinae comprising the non-occluded viruses. The virus particles of NPV and GV are enveloped in an extra protein matrix. The protein unit in the case of NPV is polyhedrin and granulin in the case of GV. The viruses in this condition are termed as Polyhedra or Occlusion bodies, which probably provide protection from environmental factors. These proteins account for about 95% of the mass of the polyhedra. In case of NPVs, the virions are usually 1-10μ in size and present either as single (SNPV) or multiple (MNPV) nucleocapsids within the envelope. GVs occur as single enveloped nucleocapsids and are individually occluded within small 0.1- 1.0μ capsule shaped granules (*Fig. 1*).

Infection by these viruses occurs when an insect digests the alkali-soluble polyhedra and the virions are released by the high *p*H of the insect midgut. These viruses gain entry into their hosts through mouth when contaminated (by virus) food is consumed. On entry the NPVs replicated in the nucleus whereas GVs undergo replication both in nuclear and cytoplasmic components of an infected cell. NPVs, in most Lepidoptera, infect many host tissues (fat body, hypodermis, trachea, blood cells, midgut tissue).

Arthropod larvae (such as those of many Lepidoptera) infected with baculoviruses lose their appetite but continue to feed at lower rates until a few days prior to death, which may occur 5-21 days after infection. Before death, some species of infected larvae move to positions high in the plant canopy, a behaviour that facilitates the horizontal transmission of viruses in nature through food contamination. Dead hosts often become flaccid and the integument ruptures, liberating occlusion bodies containing viruses into the environment. Consumption of foliage contaminated by occluded virions by new hosts completes the virus transmission cycle, commonly leading to epizootics.

GV and NPV in coconut pests

There is no report of a granulosis virus (GV) against coconut pests. However, a nuclear polyhedrosis virus

Baculoviruses that have been successfully used as microbial control agents of insect pests

Sl. No.	Virus	Insect Species	Crop	Country	Biocontrol approach	Outcome	Referene
1.	Nuclear polyhedrosis virus (NPV)	*Gilpinia hercyniae* (forest sawfly)	-	Canada	Accidental inoculation	Permanent suppression of the pest	Balch & Bird (1944)
2.	Non-occluded virus	*Oryctes rhinoceros* (Coleopteran pest)	Coconut (*Cocos nucifera*)	India	Deliberate introduction	Suppressed the pest	Zelazny *et al.* (1990), Mohan & Pillai (1993)
3.	NPV	*Anticarsia gemmatalis* (Lepidopteran defoliator)	Soybean (*Glycine* max)	Brazil	Augmentative application	Management of the pest	Moscarid (1983)
4.	NPV	*Wiseana* spp. (Lepidopteran pasture pest)	-	New Zealand	Manipulation of epizootic virus	Natural regulation of the pest	Kalmakoff & Crawford (1982)

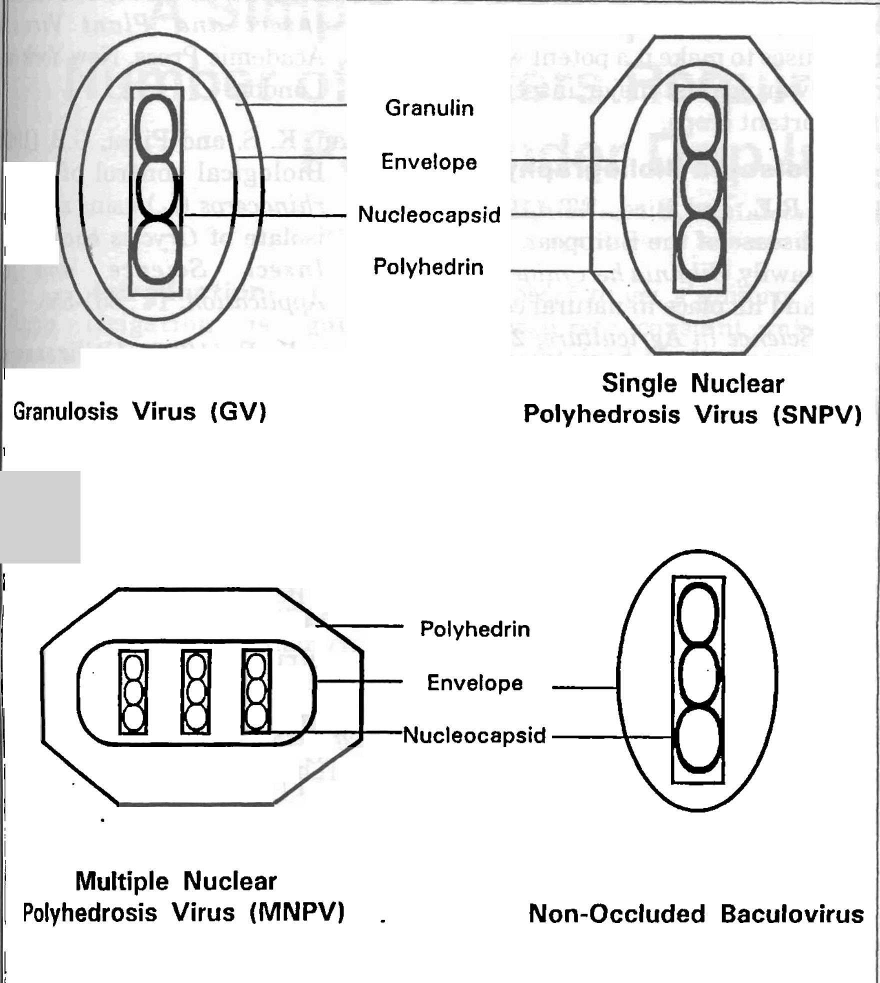

Fig. 1: Occluded (GV, SNPV & MNPV) and non-occluded baculoviruses

(NPV) was described to cause widespread mortality of the black headed caterpillar (*Opsina arenosella* Wlk.; Lepidoptera). The larvae of the *O. arenosella*, which is a serious pest of coconut especially along the East and the West coasts of India, succumb to NPV infection. The infected larvae become sluggish, exhibit loss of appetite, the cuticle appears oily and the body colour turns pink especially on ventral and lateral sides. In later stages of disease development, the cuticle of the larvae becomes very fragile and internal tissues disintegrate into a semi-fluidized mass. The larval death occurs within 3 to 8 days of NPV infection, after which they turn black. The haemolymph and internal tissues of NPV- infected *Opisina* larvae contain a large number of Giemsa-negative polyhedral inclusion bodies (PIBs).

Nuclear viral infection of red palm weevil, an insect pest of coconut palm, has also been reported. Two types of viruses have been detected; one, hexagonal isodiametric with central core and capsid (7.8 - 13.7 nm, attached to nuclear membrane) and the other one 0.30 - 0.33μ in diameter. The viral presence has been confirmed by nuclear hypertrophy, fragmentation of chromatin, vacuolisation in nucleus and cytoplasm in the fore, midgut, hindgut, fat body and nerve tissues of the host.

Non-occluded Baculovirus in coconut pests

The Nudibaculovirinae represents a small group of baculoviruses that have one nucleocapsid per envelope and are not occluded in an extra protein matrix at any stage of their life cycle. The baculovirus of *Oryctes rhinoceros,* a major pest infesting coconut plantations in Kerala and other coconut growing states of India, falls under this group. It consists of rod-shaped nucleocapsid (220-240 x 80-110 nm) surrounded by an envelope.

True to baculoviruses, this virus gains entry into the host through the ingestion of contaminated food. After gaining entry, it reaches the nuclei of midgut epithelium cells of larvae and adults, nuclei of cells of larval fat bodies and the haemolymph where it replicates. Only the grub and adult stages of *Oryctes* are infected by *Baculovirus oryctes.* The infected grubs and adults become lethargic, stop feeding and the midgut is filled with white mucoid fluid. The diseased grubs come to the surface of the feed, the abdomen becomes turgid and glassy with chalky white spots. As the virus multiplies, the fat body disintegrates and the haemolymph content increases. Increase in turgor pressure in abdomen sometimes leads to extrusion of rectum. They die within 15-20 days and do not pupate. As a result of this infection, the adults become short lived (by 60% of normal longevity) and oviposition by females is drastically reduced.

The production and maintenance of this virus is done by propagating it in live grubs/beetles of *Oryctes*. Maintenance of the virus can also be done on *O. rhinoceros* cell culture. Long term storage of virus is possible by cryopreserving the virus-infected grubs at -40^0C.

The perpetuation of this virus in nature is by infected host. It is transmitted mostly during mating, probably when healthy partners contact by mouth the viruses defecated by infected beetles or when healthy and infected beetles feed together. The beetles also pass infection to healthy grubs when they visit breeding sites. The infected beetles excrete viral contaminated faecal matter from 3rd and 9th day after infection into the surroundings. The rate of spread of the virus in the field is estimated to be about 1 km/month.

For the field application of this virus, the simpler, economical and direct method is by release of virus - inoculated beetles @ 15 numbers per hectare. An alternate method of application is inoculating the virus - infected larval tissues into the breeding sites of the pest.

Other than *Oryctes rhinoceros,* this baculovirus also infects other related pests e.g., *Oryctes nasicornis, O. monoceros, O. boas, Scapenes australis grossipunctatus, Papuana uninodis* and *Xylotrupes gideon.*

The release of *Baculovirus oryctes* in South Pacific Islands, Fiji, Mauritius, Seychelles and Papua New Guinea has resulted in drastic reduction in beetle population and subsequent crop damage. In India also, performance of this virus in Minicoy and Andrott Islands of Lakshadweep and Chittilappilly of Thrissur district in Kerala has been highly successful. Apart from coconut, this virus has been found to be a good biocontrol agent of *Oryctes rhinoceros* in oil palm plantations of Kerala.

Conclusion

These few examples prove that viruses have very good potential as microbial control agents of insect pests. Yet, they have been less used in the IPM as compared to the chemical insecticides because of their slow killing action, technically intensive methods of maintenance, application and very low persistence in field among others. Currently, advanced technologies like DNA recombinant technique are being used to address these shortcomings of the viruses to make it a potent weapon in the war against major insect pests of important crops.

Selected Bibliography

Balch, R.E. and Bird, F.T. (1944) A disease of the European spruce sawfly, *Gilpinia hercyniae* (Htg.) and its place in natural control. *Science in Agriculture,* 25 : 65-80.

Dangar, T.K. and Banerjee, A. (1993) Infection of red palm weevil by microbial pathogen. In : *Advances in Coconut Research and Development,* (Eds.) M. K. Nair, H. H. Khan, P. Gopalasundaram and E.V.V. Bhaskara Rao, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, p. 531-533.

Gopinadhan, P.B., Mohandas, N. and Vasudevan, K. P. (1990) Cytoplasmic polyhedrosis virus infecting red palm weevil of coconut. *Current Science,* 59 (11) : 577-580.

Kalmakoff, J. and Crawford, A.M. (1982) Epizootic virus control of *Wiseana* spp. in the pasture environment. In : *Microbial and Viral Pesticides* (Eds.) E. Kurstak, Marcel Dekker, New York, p. 435.

Maramorosch, K. (1977) *The Atlas of Insect and Plant Viruses.* Academic Press, New York and London.

Mohan, K. S. and Pillai, G.B. (1993) Biological control of *Oryctes rhinoceros* (L.) using an Indian isolate of *Oryctes baculovirus. Insect Science and its Application,* 14 : 551-558.

Moscardi, F. (1983) Utilizacao de *Baculoviris anticarsia* para o contrle da lagarta da soya. *Anticarsia gemmatalis. Empresa Brasiliera de Pesquira Agropecuaria, Communicao Tecnico* No. 23. (in Portuguese).

Philip., B. M., Mathai, S. and Jacob, A. (1982) A nuclear polyhedrosis virus of the black headed caterpillar, *Nephantis serinopa* Meyrick (Lepidoptera Gytophasidea). *Current Science* 51 (12) : 611.