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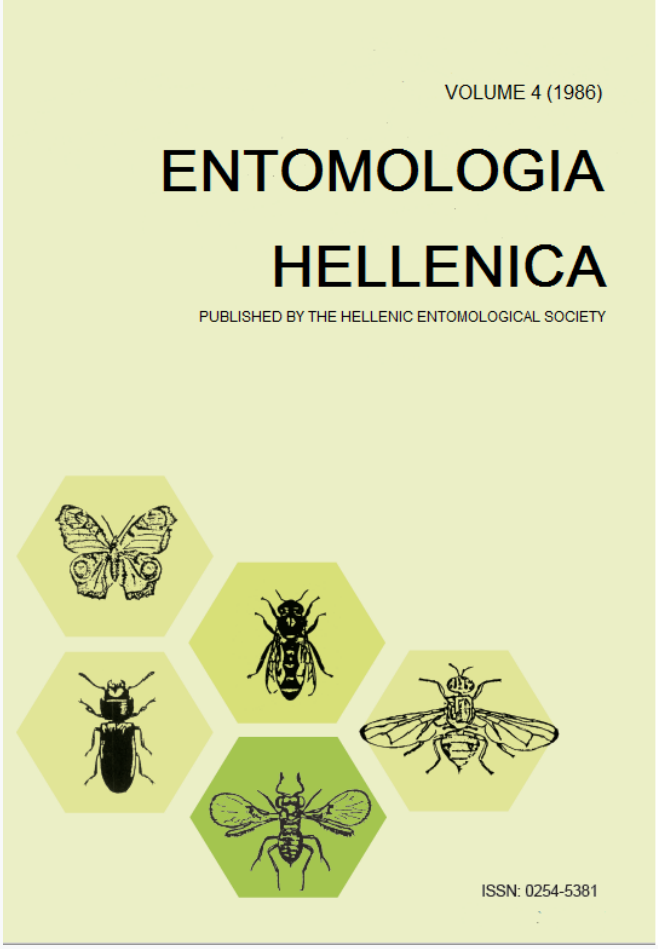

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The Use of Viruses for Controlling Pest Species of Insects[1]

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ABSTRACT

Insect viruses have been used effectively to control pest species of insects. While viruses from several families have been used, members of the Baculoviridae have proven most successful. The successful viruses are mentioned and a brief introduction into insect virus identification is followed by sufficient detail to differentiate between the major groups of viruses which have been used as field control agents. References are minimised to recent key articles on all the families of viruses found in insects.

Introduction

Virus diseases have been documented in a large number of species of insects. The class Insecta forms in excess of 75% of animal species and relatively few have been examined in detail for pathogens. Without doubt there will be many more reports of viruses present in natural and laboratory maintained populations of insects. Obviously the insect species which have been examined are primarily those which affect man, his animals or crops, but we will only consider some of the viruses which cause severe diseases in insect pest species.

The families of viruses which infect insects are shown in Table 1. Key articles are cited in Table 1 for those who wish to examine a particular virus group in detail. Some of the families are apparently exclusive to insects, the most notable of these being the baculoviruses (more recently also found in crustaceans). Some insect viruses or groups of viruses are related to mammalian viruses by having similar genomes, replicative mechanisms and structures, and can be classified in the same family. For example, the cytoplasmic polyhedrosis virus group is a major genus within the Reoviridae where the other genera infect mammals (Orbiviruses, Reoviruses and Rotaviruses) and plants (Phytoreoviruses and Fijiviruses). Some families of viruses have single or few reported insect representatives such as the Rhabdoviruses (Sigma virus), the Birnaviruses (Drosophila X virus) and the Caliciviruses with a virus from the navel orange worm *(Amyelois transitella)*.

Identification of Insect Viruses

When a diseased insect or population of insects is found in the field it could be attributed to many possible causes including environmental conditions, predatory attack, and pathogens such as bacteria, fungi or viruses. These causes may be synergistic; for example, environmental stresses such as excess cold or rainfall followed by fungal or bacterial attack or apparently simultaneous attack by several bacteria and viruses. In this article the viral situation will be considered and even in this case there are many examples when more than one virus type has been isolated from a single population.

Unlike many mammalian diseases, it is extremely difficult to "clinically" diagnose a dis-

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TABLE 1. Viruses pathogenic for insects.

	Virus types*	Nucleic acid	Virus particle morphology
1.	Baculoviridae	dsDNA	Bacilliform
	Subgroup (a) Nuclear polyhedrosis virus (NPV) Subgroup (b) Granulosis virus (GV) Subgroup (c) Oryctes virus		
2.	Polydnaviruses	dsDNA	Ovoid
3.	Ascoviruses	dsDNA	Allantoid
	Poxviridae		
4.	Entomopoxviruses	dsDNA	Brickshaped or ovoid
	Iridoviridae	dsDNA	Isometric
5.	Iridoviruses		
	Parvoviridae	ssDNA	Isometric
6.	Densoviruses		
	Reoviridae	dsRNA 10 segments	Isometric
7.	Cytoplasmic polyhedrosis virus (CPV) Reoviruses		
8.	Birnaviruses	dsRNA 2 segments	
	Picornaviridae		
9.	Unclassified group	ssRNA	Isometric
10.	Nudaurelia ß family	ssRNA	Isometric
11.	Nodaviridae	ssRNA 2 segments	Isometric
12.	Caliciviridae	ssRNA	Isometric
13.	Rhabdoviridae	ssRNA	«Bullet» shaped

* The subgroups a and b baculoviruses, the entomopoxviruses and the cytoplasmic polyhedrosis viruses are occluded within proteinaceous structures. Key references for the different virus groups are (1) Faulkner 1981; Kelly 1982; (2) Stolz et al. 1984; (3) Federici 1983; (4) Arif 1984; (5) Hall 1985; (6) Kawase 1985; Siegel et al. 1985; (7) Payne & Mertens 1983; (8) Dobos et al. 1979; (9, 10) Moore and Tinsley 1982; (11) Moore et al. 1985; (12) Hillman et al. 1982; (13) Teninges et al. 1980, Richard-Molard et al. 1984.

ease of an insect and be able to say with any confidence that it is caused by a specific virus. It is necessary to examine extracts of the insects using techniques to define the infectious agent. These will include staining and light microscopy to look for the larger polyhedral viruses, differential centrifugation to clarify and purify the viral components and determination of physical parameters such as buoyant density. Using the electronmicroscope it is possible to differentiate between many viruses on the basis of morphology. However, conclusive proof can only be obtained by examining the form and size of the nucleic acids and proteins on agarose and polyacrylamide gels.

Initial identification of insect viruses can be performed in some cases by immunological methods including double diffusion, enzyme linked immunosorbent assay, immunoprecipitation or radioimmunoassay. The majority of these methods when performed on crude extracts of insect material require at least one other procedure as confirmatory evidence for the presence of a specific type of virus (for example, the serological reagents could be reacting with the minor species in a mixture of viruses).

Classically, insect viruses are divided into two groups, those which are occluded (polyhedrosis viruses) and those which are not. The occluded viruses have virus particles encapsulated in a polyhedron or inclusion body which is a large proteinaceous structure made primarily of one protein. Most baculoviruses, cytoplasmic polyhedrosis viruses and entomopoxviruses (see Table 1) are all present in occlusion bodies. The situation is somewhat complicated by a proportion of these viruses occurring as nonoccluded viruses, but with all infections a number of polyhedra are apparent. The nonccluded viruses do not have these specialized structures. Due to the occurrence of large (1-15μm) polyhedral structures the occluded viruses were the first recognized in insects and the baculoviruses and CPVs have received considerable attention

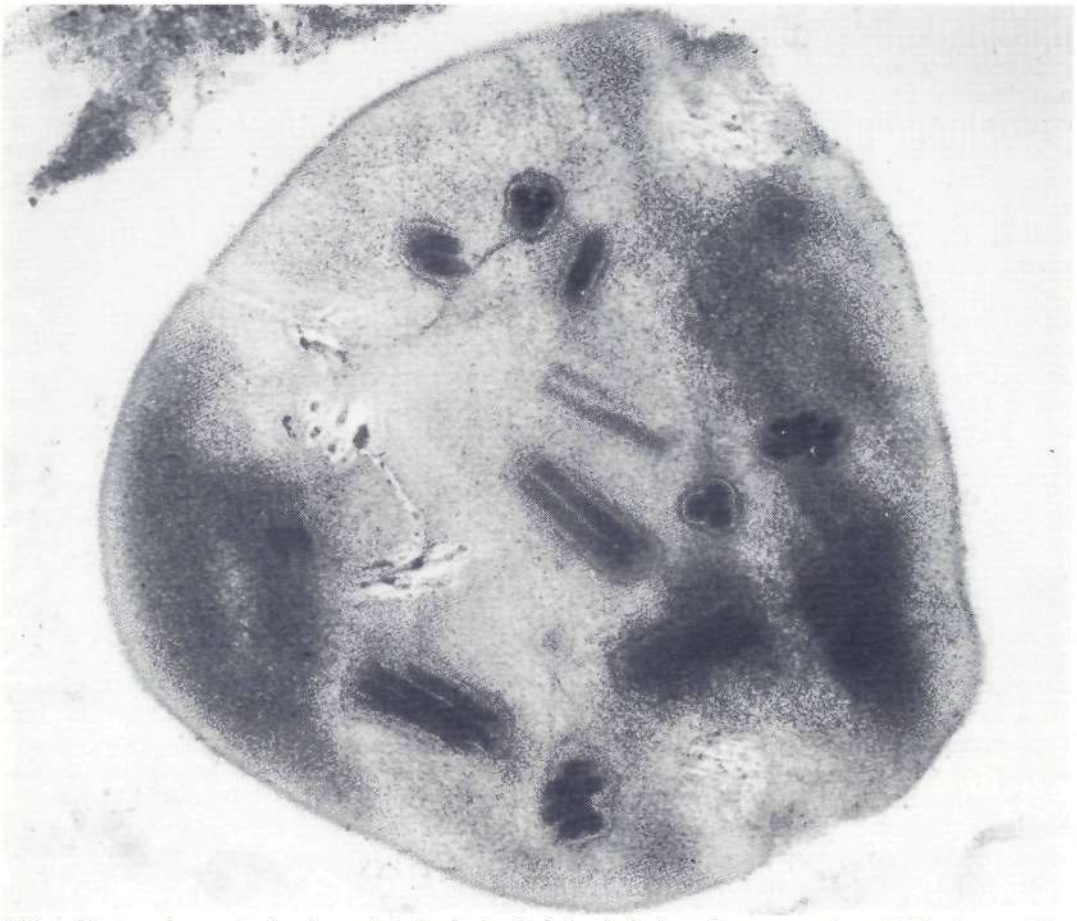

FIG. 1. Electronmicrograph of nuclear polyhedrosis virus inclusion body from *Gonometa podocarpi:* The virus particles are easily distinguished with one to several nucleocapsids. Mg × 65,000.

because they are potentially useful control agents. In the absence of any mammalian virus which produces polyhedra, and the physiochemical differences between for example the baculoviruses and known mammalian viruses it is more acceptable to use these viruses as biological control agents (after stringent safety testing).

Baculoviruses

The polyhedral bodies are necessary to ensure the long term survival of the virus particles outside the host as there are considerable interruptions in the availability of insect hosts. The baculoviruses include several types:

(1) The subgroup (a) nuclear polyhedrosis viruses which have polyhedra which contain many virus particles (see Fig. 1). The virus particles may contain one or several nucleocapsids.

(2) The subgroup (b) granulosis viruses where one virus particle is found within each proteinaceous structure, termed a granulin in the case of these viruses.

(3) The subgroup (c) nonoccluded baculoviruses, where the virus from *Oryctes rhinoceros* is the type member. These viruses do not synthesize polyhedral proteins and are hence nonoccluded.

When polyhedra are subjected to alkaline conditions above pH 9.5, dissolution of these structures results, releasing the infectious virus particles. An alkaline protease is present which assists in the degradation of the polyhedron. The alkaline pH and the protease activity reflect the conditions found in the insect gut when the polyhedra are ingested by

the insect, the released virus particles then infect the gut cells.

Several baculoviruses are available commercially for the control of insects. These include Elcar for controlling *Heliothis* spp. (specifically *H. zea*, the cotton bollworm), Gypchek for *Lymantria dispar* (the gypsy moth), Virox for *Neodiprion sertifer* (pine sawfly) and TM Biocontrol-l for *Orgyia pseudotsugata* (Douglas fir tussock moth).

The singly enveloped nuclear polyhedrosis virus for *Heliothis* spp. was developed for use on cotton pests because these insects had become resistant to a range of chemical pesticides (see Ignoffo and Couch 1981, for a review of the use of this virus). Generally, biological control methods are not so applicable to the pests of annual food crops because harvesting results in the removal of much of the viable control agent. The longevity of the virus is mainly dependent on its presence in soil, and its appearance in a less virulent form in "overwintering" stages of the host and in secondary hosts (see Evans and Harrap 1982, for a review on the factors which affect insect virus persistence in the environment).

As baculovirus infection results in the production of a massive number of polyhedra, which enter the environment as faecal deposits or from the corpses of rotting insects many stable infective viruses are available for either immediate reinfection by the (faecal) oral route or for survival for relatively long periods in the environment. In the case of the nuclear polyhedrosis virus of *N. sertifer*, polyhedra can persist attached to coniferous foliage or the bark of trees. As with much of the field control effort with insect viruses, *N. sertifer* nuclear polyhedrosis virus is applied at the larval stage and preferably at an early instar because the young insects are normally more susceptible and it is obviously desirable to infect the insect before it has time to do much damage to the foliage. *N. sertifer* larvae are more susceptible to attack by biological control agents because they are gregarious, existing in colonies of relatively large numbers. Hence, if one larva is infected, the rest become infected by the oral route (Cunningham and Entwistle 1981). Nuclear polyhedrosis virus applied to the douglas-fir tussock moth has also resulted in successful control. Several other viruses have been used in field tests to control forest pests such as the nuclear polyhedrosis viruses of *Panolis flammea*, (pine beauty moth), and *Choristoneura fumiferana*, (spruce budworm). Even with the control limitations imposed by cropping, several nuclear polyhedrosis viruses and granulosis viruses have been used in agriculture on annual crops. Much of the work has concentrated on nuclear polyhedrosis virus of *Trichoplusia ni* (cabbage looper), *Mamestra brassicae* (cabbage moth) and granulosis viruses of *Cydia pomonella* (codling moth) and *Phthorimaea operculella* (potato tuber moth).

The granulosis virus control of the codling moth is particularly of interest because of the difficulties encountered due to the life cycle of the insect. Newly hatched larvae penetrate young apples and for successful control of this insect it must become infected between hatching and penetrating the fruit. Hence, frequent spray applications are made, dependent on the speed of virus inactivation and the generation time of the moths.

In the case of *Oryctes rhinoceros* (Rhinoceros beetle) which causes severe damage of oil and coconut palms, adults were released after infection with the naturally occurring nonoccluded (type C) baculovirus; this resulted in contamination of the feeding and mating sites, the death of larvae and the production of fewer eggs by infected females.

Cytoplasmic Polyhedrosis Viruses

The second major group of viruses which have been utilized for field control of pest species of insects is the cytoplasmic polyhedrosis viruses. Unlike the other members of the Reoviridae these viruses do not have a double outer membrane layer and are occluded into polyhedral structures. These viruses are distinguished from nuclear polyhedrosis viruses as the polyhedra are formed in the cytoplasm of midgut epithelial cells rather than the nucleus, and also by the absence of an outer polyhedral membrane. Electron microscopic examination of cytoplasmic polyhedrosis virus polyhedra shows the presence of pits in the outer layer which are the size of virus particles (see Fig. 2). As with nuclear polyhedrosis virus infections large numbers of "nonoccluded" viruses can be found which are indistinguishable from occluded particles on release. Virions are icosahedral with spikes at each of the 12 vertices. RNA extraction of all cytoplasmic polyhedrosis viruses has de-

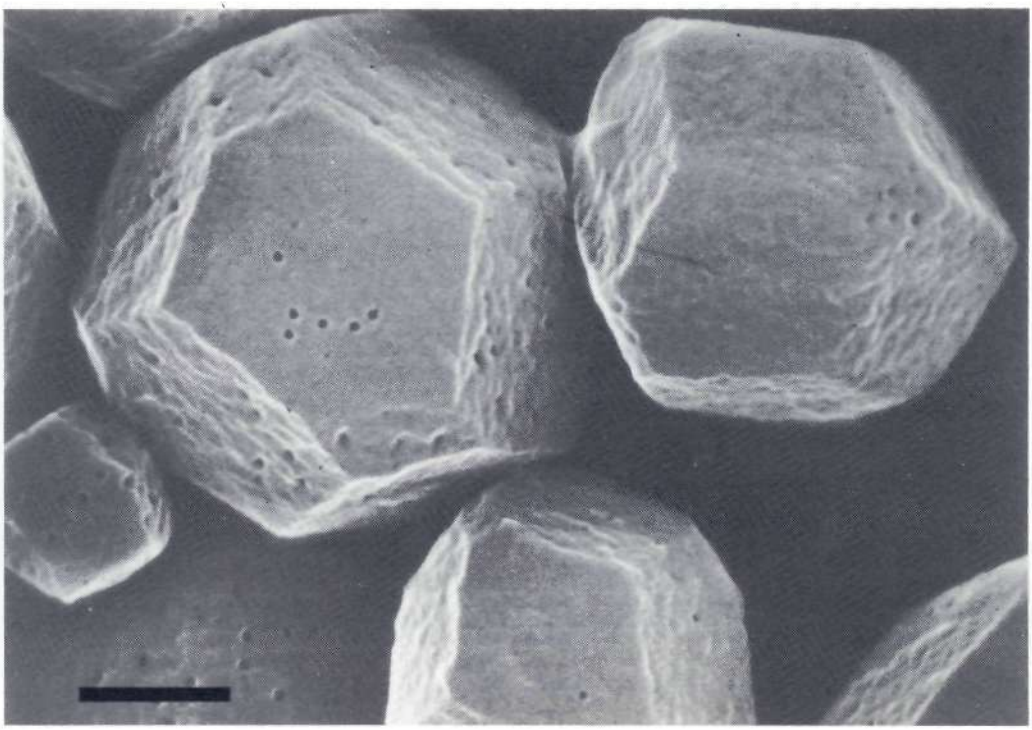

FIG. 2. Scanning electronmicrograph of polyhedra of type 2 cytoplasmic polyhedrosis virus from *Inachis io*. The pits on the surface result from detachment of virus particles. Bar = 1μm.

monstrated the presence of 10 strands of dsRNA with mol. wts. of approximately 0.3 - 2.5 x 10^6 and Payne and Rivers (1976) initially subdivided the different isolates on the basis of major differences in the RNA profiles. Twelve types have been described (Payne and Rivers 1976, Payne et al. 1977).

Cytoplasmic polyhedrosis viruses are not considered to be as effective as field control agents as baculoviruses for various reasons (see Payne 1982). However, the pine processionary caterpillar moth, *Thaumetopoea pityocampa*, was successfully controlled in France by aerial application of a cytoplasmic polyhedrosis virus (Grison et al. 1959). The pine caterpillar, *Dendrolimus spectabilis* has also been controlled by a cytoplasmic polyhedrosis virus and this virus is commercially available in Japan (Katagiri 1981).

Non-occluded Viruses

In addition to the occluded viruses a number of other viruses have been demonstrated to have severe effects on insect populations. Cricket paralysis virus (see Fig. 3) causes paralysis in *Teleogryllus* species and is the most studied of the RNA viruses (primarily because it was the first one demonstrated to replicate in insect tissue culture cells, see Moore et al. 1985). *Nudaurelia cythera capensis* (the pine emperor moth) is controlled naturally in the field by a small RNA virus which is the type member of the Nudaurelia family of viruses. Two other viruses from the family affect major lepidopteran pests, *Darna trima* which defoliates coconut and oil palms in south east Asia and *Thosea assigna* which is a pest of oil palms in Malaysia (see Moore and Tinsley 1982, for a review on the small RNA viruses of insects). Spraying a natural mixture of small RNA viruses including the *Nudaurelia* β-like virus gave control of *Darna trima* in Sarawak. Another small RNA virus from *Gonometa podocarpi* controlled the larval stage of the insect which was causing defoliation of exotic pines in Uganda.

Several of the non-occluded viruses, particularly the picornaviruses (e.g. *Drosophila* C virus), appear to exist as inapparent infections. The virus is passed from generation to generation without having a major effect on

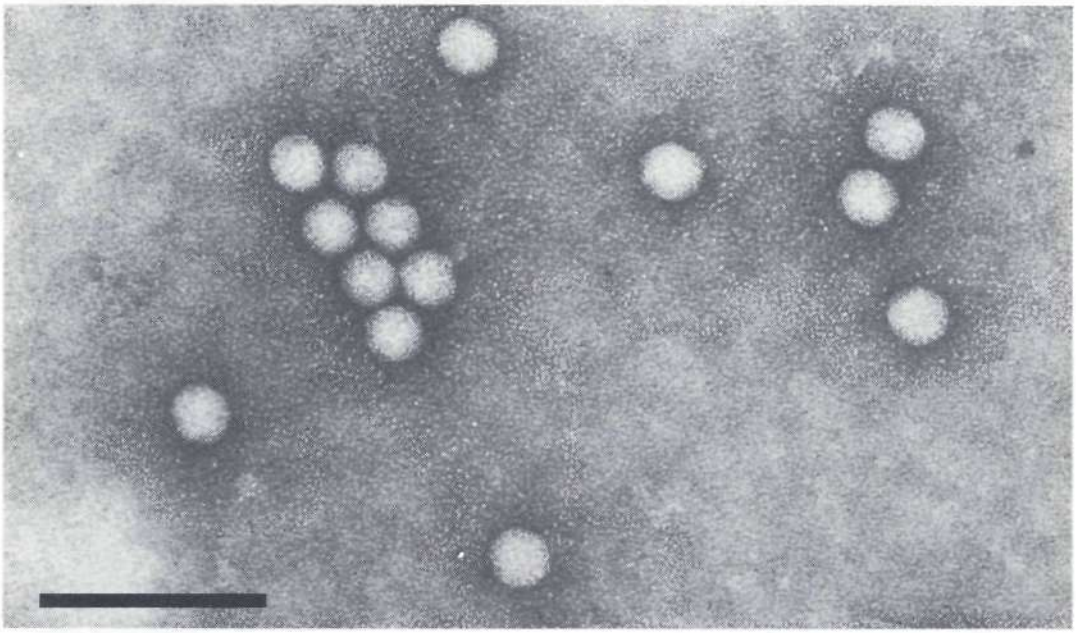

FIG. 3. Electronmicrograph of cricket paralysis virus purified from *Drosophila melanogaster* cells. Bar = 100 nm.

the population. This may be the "natural infection" and it is possible that the lethal infections which periodically occur are attributable to viruses infecting unfamiliar hosts. It is also possible that lethal infections are attributable in some cases to a combination of stresses on the population. However, viruses which cause inapparent infections should not be discarded as potential control agents. They may weaken a given population making it more susceptible to environmental conditions or other agents, or they may be successful in controlling another insect species.

Production of Viruses for Use as Control Agents

It is relatively easy to produce viruses in the laboratory in insect cultures maintained on natural or synthetic diet. However, before initiating large scale production of any virus as a control agent several questions should be asked. The first and most important is whether the virus will affect the host in an acceptable time and manner, so accurate LD_{50} determinations under appropriate conditions should be made. If the virus kills the host or reduces its viability in an acceptable manner the host specificity of virus must be demonstrated in detail. It is obviously important to investigate if the virus affects non-target insects with particular reference to useful species such as honey bees, silkworms, or insects involved in pollination. It is also important to investigate if the intended control agent is a single virus type. The virus should preferably be plaque purified in a tissue culture system before large scale production in insects. If small amounts of a second virus are present it could interfere with the effects of the major species on target or useful insects.

It is valuable to investigate if the virus is best applied by ground or aerial spray, or used in combination with a chemical or physical attractant. Another important aspect is the longevity of the virus in the field and whether additives should be incorporated in the application fluid to protect the virus from inactivation by ultraviolet light. It may also be necessary to add emulsifiers to alter the spraying and contact properties of the solution in which the virus is suspended. Once the efficiency of the virus preparation is proven by use in small scale field control experiments, it has to go through a thorough and well documented series of safety tests (Harrap 1982).

However, even if the virus proves to be a safe, useful field control agent for a pest insect species other areas should be considered, for example, could the virus be better used in

conjunction with other controlling agents whether chemicals, fungal or bacterial. As with all agents, it is also necessary to continually monitor the effects of the pesticide in the field, to determine whether it controls the succeeding generations of insects, or if the virulence decreases.

Conclusions

The baculoviruses and cytoplasmic polyhedrosis viruses have been demonstrated to be effective control agents for several species of pest insects. They appear to be neither toxic nor infective for wildlife and man. They have the further advantages of being self-propagating in the host insect and in some cases extremely specific. While relatively few viruses are currently marketed as control agents, many have been examined in relatively small scale trials. Viruses have the definite advantage over chemicals in that they are not cumulative poisons and insects do not appear to become resistant to them.

Greater research impetus is needed to find more viruses specific for pest species and more financial support is necessary for industry to take the viruses through the lengthy and expensive safety testing procedures. More work is required to investigate if combinations of viruses are more effective and to see if integrated control can be employed using combinations of biological and chemical control agents.

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KEY WORDS: Virus, Pest insects, Control, Baculovirus, Occluded and non-occluded virus, Review

Χρησιμοποίηση Ιών για τον Έλεγχο Βλαβερών Ειδών Εντόμων

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ΠΕΡΙΛΗΨΗ

Υπάρχουν αποτελεσματικές μέθοδοι για την καταπολέμηση διαφόρων βλαβερών ειδών εντόμων, που περιλαμβάνουν ιούς. Έχουν χρησιμοποιηθεί μέχρι τώρα ενάντια σε έντομα σε εκτροφές ιοί που ανήκουν σε τρεις μεγάλες ομάδες, οι Μπακουλοϊοί (Baculoviruses), οι ιοί της κυτοπλασματικής πολυέδρωσης (Cytoplasmic polyhedrosis viruses) και οι μικροί RNA ιοί. Οι Μπακουλοϊοί και οι ιοί της κυτοπλασματικής πολυέδρωσης ανακαλύφθηκαν σχετικά πρόσφατα και είναι μοναδικοί για τα έντομα, από την άποψη ότι χαρακτηρίζονται από τον εγκλωβισμό ενός ποσοστού των ιοσωματίων σε πολύεδρα (μεγάλες πρωτεϊνικής φύσης δομές που συνίστανται κύρια από μια δομική πρωτεΐνη που κωδικοποιείται από το γονιδίωμα του ιού). Τα πολυεδρικά αυτά «σωμάτια έγκλισης» όπως ονομάζονται, καθιστούν τα εγκλωβισμένα ιοσωμάτια περισσότερο ανθεκτικά στις περιβαλλοντικές συνθήκες και εξασφαλίζουν τη μολυσματικότητα του ιού κατά τη διάρκεια του ασυνεχούς κύκλου ζωής των εντόμων. Τα βιολογικά μέσα ελέγχου και καταπολέμησης των εντόμων, όπως είναι οι ιοί, είναι ασφαλή για το περιβάλλον από την άποψη ότι δεν δηλητηριάζουν τον άνθρωπο και το υπόλοιπο ζωικό και φυτικό Βασίλειο. Επιπλέον, είναι πιο εξειδικευμένα μέσα, και συχνά κατευθύνονται ενάντια σε ένα μόνο είδος εντόμου χωρίς να επηρεάζουν άλλους οργανισμούς στο οικοσύστημα. Η επίδραση της ιϊκής μόλυνσης τείνει να διατηρείται για μεγαλύτερο χρονικό διάστημα από ό,τι άλλες μορφές καταπολέμησης, και ιδιαίτερα αν τα μέσα που χρησιμοποιήθηκαν είναι Μπακουλοϊοί ή ιοί της κυτοπλασματικής πολυέδρωσης. Περιγράφονται διάφορες περιπτώσεις χρησιμοποίησης ιών εντόμων για τον αποτελεσματικό έλεγχο βλαβερών ειδών, καθώς επίσης και μέθοδοι απομόνωσης, χαρακτηρισμού και χρησιμοποίησής τους.