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Viruses as Pest-Control Agents

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Introduction

Pressures from a variety of sources are causing man to investigate alternatives to the chemical pesticides which have been used so widely during the past few decades. Pressures are brought to bear by environmentalists concerned about the effects of pesticides on wildlife, by pest-control experts concerned about the effects of these pesticides on parasites and predators of the pests and about the increasing resistance of the pests to the pesticides, by consumers concerned about toxic residues in food, and by public health officials concerned about human poisoning. In Sri Lanka, for example, more people die of pesticide poisoning than of malaria (Matthews, 1983). Furthermore, research, development and production costs for chemical pesticides have soared, making them expensive in the developed nations, while in the developing nations, if pesticides are used at all, farmers select the least expensive—which are usually the most toxic. 'Biological' control strategies, including the use of pathogens of pests, attempt to circumvent most of these problems.

Viruses have been used to control mites (Reed, 1981) and rabbits (Fenner, 1983) but this review is concerned principally with the insect-pathogenic baculoviruses (BVs). Insects are hosts to a wide variety of viruses, including picornaviruses, parvoviruses and poxviruses. Each of these groups also has representatives infecting vertebrate animals. Attention has been focused on the BVs as pesticidal agents because of their lack of similarity to any viruses of hosts other than invertebrates. There is some logic in this approach, but other groups of insect-pathogenic viruses should not be ignored. It may be that the host spectra of other virus groups, such as the cytoplasmic polyhedrosis viruses and the iridescent viruses, are restricted to invertebrates even though viruses with similar morphologies and biochemical characteristics infect vertebrates (and higher plants in the case of iridescent viruses). In fact, a cytoplasmic polyhedrosis virus is used in Japan against the pine caterpillar, *Dendrolimus*

Abbreviations: BV, baculovirus; DNA, deoxyribonucleic acid; ELISA, enzyme-linked immunosorbent assay; FP, few polyhedra; GV, granulosis virus; IB, inclusion body; IPM, integrated pest management; LD_{50}, median lethal dose; LT_{50}, median lethal time; MNPV, multiple nucleocapsids per virion envelope; MP, many polyhedra; NPV, nuclear polyhedrosis virus; REN, restriction endonuclease; RNA, ribonucleic acid; SDS-PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; SNPV, single nucleocapsid per virion envelope; UV, ultra-violet.

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spectabilis (Aizawa, 1976), and an iridescent virus has been tested against leatherjackets (*Tipula* spp. larvae), although with disappointing results (Carter, 1978).

Insect viruses are in use, or are being considered for use, in forestry, horticulture and agriculture, including grassland. Entwistle (1983) listed 31 lepidopteran, 6 hymenopteran and one coleopteran pest species for which control with BVs has been demonstrated to be feasible or highly likely. There is little or no current effort to apply viruses for control of disease vectors or of stored product and timber pests.

If a virus is to be considered seriously as a pest-control agent then detailed knowledge of the virus, its host, and their interactions with the environment must be amassed. Information is required on the structural and biochemical characteristics of the virus, its host spectrum, and median lethal doses (LD_{50}s) and median lethal times (LT_{50}s) for different stages of the host(s). The habits and life cycle of the host, and the mechanisms whereby the virus persists and spreads in the field (epizootiology) must be understood. Techniques for mass production and purification of the virus must be developed and it must be shown to be safe for man and other non-target organisms. This chapter considers all these aspects and discusses the advantages and disadvantages of using insect-pathogenic viruses as pesticides. Industrial aspects are discussed only briefly as they will be considered in detail in a subsequent volume. Recent reviews on the use of viruses as pest-control agents include those of Falcon (1982), Payne (1982) and Entwistle (1983).

The baculoviruses

Only an outline of the structure and replication of BVs is given here. For more detailed accounts the reviews of Harrap and Payne (1979), Granados (1980a) and Kelly (1982) should be consulted.

The BVs have been classified into three subgroups according to whether or not the virions become embedded (occluded) in inclusion bodies (IBs), and, if so, on the size and shape of the IB (Matthews, R.E.F., 1982). Details of the subgroups are presented in *Figure 1* and *Table 1*. The rod-shaped virions are enveloped. Those of the granulosis viruses (GVs) become occluded in capsule-shaped IBs (granules), whereas the nuclear polyhedrosis virus (NPV) IBs (polyhedra) are polyhedral, cuboidal, or 'orange segment-shaped', depending on the virus. The NPVs are subdivided into those in which each occluded virion has a single nucleocapsid per virion envelope (SNPVs) and those in which the occluded virions have multiple nucleocapsids per virion envelope (MNPVs).

STRUCTURE

The virion

BV virions have been found to contain up to 35 proteins (Vlak, 1979), some of which are glycosylated (Dobos and Cochran, 1980) and/or phosphorylated (Tweeten, Bulla and Consigli, 1980). The nucleocapsid consists of a protein capsid containing DNA and further proteins (*Figure 1*). Its dimensions fall

Table 1. Baculovirus subgroups.

Subgroup	Inclusion body dimensions	Number of virions per inclusion body	Single or multiple nucleocapsids per virion envelope	Hosts
Nuclear polyhedrosis viruses	0·8–15 μm diameter	Many		
1. MNPVs			Multiple (1–5 usually; up to 39)	Lepidoptera
2. SNPVs			Single	Lepidoptera, Diptera, Hymenoptera, Trichoptera, Coleoptera, Neuroptera, Crustacea
Granulosis viruses	approx. 200 × 500 nm	1, usually	Single	Lepidoptera
Non-occluded baculoviruses	No inclusion bodies formed		Single	Coleoptera, Diptera (possibly) Mites, Crustacea

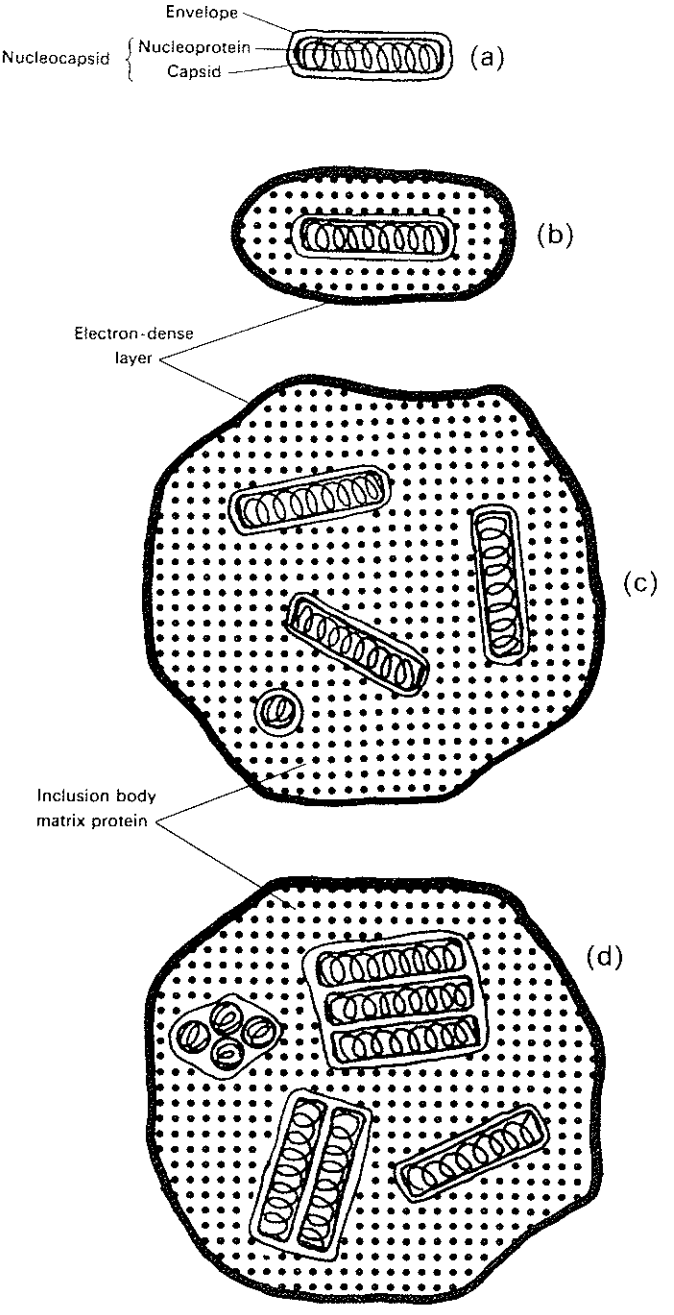

Figure 1. Baculovirus subgroups. (a) Non-occluded baculovirus virion. (b) Granulosis virus inclusion body. (c) Nuclear polyhedrosis virus inclusion body; singly enveloped nucleocapsids (SNPV). (d) Nuclear polyhedrosis virus inclusion body; multiple nucleocapsids per virion envelope (MNPV).

within the ranges 40–140 nm × 250–400 nm. The capsid is constructed from helically arranged subunits (Burley *et al.*, 1982), and structures described as claws and nipples (Kawanishi and Paschke, 1970) or caps (Federici, 1980) are present at its ends.

The double-stranded DNA molecule is a closed circle and is supercoiled. Most molecular weight estimates for BV DNAs fall between 70×10^6 and 120×10^6. Associated with the DNA is a highly basic protein (Tweeten, Bulla and Consigli, 1981) which may play a part in its condensation (Burley *et al.*, 1982).

Many of the larger virion proteins are associated with the lipid-containing membrane which forms the virion envelope. The virions of occluded BVs occur in two forms, each with a distinct envelope: the form which becomes occluded derives its envelope from membrane synthesized within the nucleus, while another form acquires its envelope by budding from the plasma membrane. The latter form normally has only one nucleocapsid per envelope, even if the virus is a MNPV, although Longworth and Singh (1980) observed that a few budded virions of *Epiphyas postvittana* MNPV had two nucleocapsids per virion. The occluded virions are specialized for infection of the host midgut cells, while the budded virions spread the infection to other cells and can readily infect susceptible cell cultures.

Some of the envelope proteins of occluded and budded forms of the same virus are distinct, while others are related (Volkman, 1983). At one end of a budded virion the envelope bears a number of spikes (Summers and Volkman, 1976), which are probably glycoproteins.

The polyamines spermidine and putrescine have been found in some NPVs. In *Heliothis zea* SNPV all of the spermidine and most of the putrescine was shown to be associated with the virion envelope (Elliott and Kelly, 1979).

The inclusion body

IBs are formed by the cytoplasmic polyhedrosis viruses and poxviruses of insects as well as by the occluded BVs. They afford protection to the virions outside the host, often for considerable periods between generations of larvae. Retention of occluded virus infectivity is far superior to that of viruses which do not form IBs, and is a further reason why interest has centred on the occluded viruses as microbial control agents.

The IB matrix is a paracrystalline lattice of protein subunits laid down to form an extremely stable structure which survives putrefaction of the dead host, but is broken down at low and high pH values. Reducing agents enhance the rate of IB dissolution in alkali (Croizier and Meynadier, 1972) and are essential for the dissolution of IBs of poxviruses and of the SNPV of *Tipula paludosa* at pH 10·5 (Bergoin, Guelpa and Meynadier, 1975).

The protein subunits are constructed from monomers, some of which have been reported to be glycosylated and phosphorylated (Kelly, 1981a). Proposals as to how the subunits are formed from the monomers include ionic, hydrophobic and disulphide bonding (Eppstein and Thoma, 1977).

There appears to be a high degree of similarity between the IB monomer proteins of different BVs. Their molecular weights all fall within the range

25 000–33 000. Several serological investigations with antisera have demonstrated relationships within and between the IB proteins of GVs, SNPVs and MNPVs, and these have been confirmed recently using monoclonal antibodies (Roberts and Naser, 1982a; Hohmann and Faulkner, 1983). The latter authors found stronger reactions within BV subgroups than between subgroups. The amino acid sequences of a few IB proteins have been determined and confirm that there is a high degree of similarity between them, especially between lepidopteran NPVs (Rohrmann *et al.*, 1981; Rohrmann, 1982).

Around each IB is a layer of material which appears electron-dense when sections are viewed in the electron microscope. It appears to be more resistant than the IB matrix to alkaline dissolution (Kawanishi, Egawa and Summers, 1972; Green, 1981) and may be composed of carbohydrate (Minion, Coons and Broome, 1979).

IBs from infected insects contain alkaline protease activity. This is displayed when IBs dissolve in alkali (Yamafuji, Yoshihara and Hirayama, 1957) and enhances their rate of dissolution (Summers and Smith, 1975). No such enzyme activity has been detected in IBs from infected cell cultures.

INFECTION OF THE HOST

Most infections are initiated by the ingestion of infective virus. The virions of occluded viruses are released by dissolution of the IBs in the alkali of the midgut. Gut enzymes (Faust and Adams, 1966) and the IB protease may also have roles. Granados and Lawler (1981) found that few *Autographa californica* MNPV IBs remained intact after 15 minutes in the larval midgut (pH 10·4) of the cabbage looper, *Trichoplusia ni*.

The virions must survive the harsh conditions of the midgut while they traverse the peritrophic membrane and attach to the microvilli of midgut cells. The virion envelope fuses with the microvillus membrane, releasing the nucleocapsid(s) into the cell (Granados, 1978).

The SNPVs of the most Diptera and Hymenoptera replicate only in the midgut cells, and IBs are shed into the gut lumen by lysis of infected cells. In the Lepidoptera, however, infection of the midgut is only the preliminary to infection of other tissues. Enveloped nucleocapsids develop and IB protein polymerization may occur in midgut cells, but virions are rarely occluded. Instead they bud into the haemocoel (Harrap, 1970) and are carried in the haemolymph to other susceptible tissues. It has also been suggested (Granados and Lawler, 1981) that some inoculum nucleocapsids may pass straight through the gut cells and bud into the haemocoel.

REPLICATION

BVs replicate in the nucleus, within which the DNA is released from NPV nucleocapsids, while GV nucleocapsids release their DNA into the nucleus via nuclear pores (Granados, 1980a). The infected nucleus hypertrophies and becomes the dominant feature of the cell. A 'virogenic stroma' is formed and nucleocapsids develop at its periphery. Nucleocapsids produced early enter the

cytoplasm either by budding through the nuclear envelope (Injac *et al.*, 1971) or via ruptures in it (Adams, Goodwin and Wilcox, 1977). They then leave the cell by budding through a portion of modified plasma membrane which becomes the virion envelope (Hunter, Hoffmann and Collier, 1975). These virions spread the infection to other cells. Later in occluded BV infections most nucleocapsids are retained in the nucleus where they acquire envelopes and become occluded. Occlusion of naked nucleocapsids has never been observed, which suggests that there is an IB protein receptor on the virion envelope.

IBs develop randomly throughout the nucleus, except those of two dipteran SNPVs which develop in intimate association with the inner nuclear membrane (Smith and Xeros, 1954; Stoltz, Pavan and Da Cunha, 1973). The number of IBs produced per cell may vary from a few to several hundred, depending in part on the IB size. The yield per insect depends on many factors, including species and instar. Evans, Lomer and Kelly (1981) found maximum yields of $2{\cdot}7 \times 10^7$ IBs per first-instar larva and $3{\cdot}4 \times 10^9$ IBs per fifth-instar larva for *Mamestra brassicae* MNPV. IBs have been reported to constitute up to 40% of the insect dry weight (Bucher and Turnock, 1983).

Some progress has been made recently in understanding the biochemical events involved in BV replication. Kelly and Lescott (1981) identified four phases of virus protein synthesis in *Spodoptera frugiperda* cell cultures infected with *T. ni* MNPV. The phases were induced in a cascade fashion, with synthesis of one phase blocked if the proteins of the previous phase were rendered non-functional. The early proteins include enzymes such as thymidine kinase (Kelly, 1981b), while the virus structural proteins appear later. Synthesis of the later proteins is probably dependent on virus DNA synthesis, which reaches a high rate.

Nearly all of the late messenger RNA is virus-specific, with approximately 25% of that in *A. californica* MNPV-infected cells specific for IB protein (Adang and Miller, 1982). The control of IB formation is undoubtedly complex; studies with *A. californica* MNPV mutants led Potter and Miller (1980) to suggest that about half of the genome might be involved. Another small protein (molecular weight 10000) is produced late and in large quantities in *A. californica* MNPV-infected cells. It is present in the virion as a minor component, but its function is not known (Smith, Vlak and Summers, 1983).

Insect-cell culture

A brief account of insect-cell culture techniques is relevant because of their value in studies of BV replication (page 380), genetics (page 385) and safety testing (page 388). Furthermore, there are hopes that viruses used as insecticides might be mass produced in cell cultures (page 400). Recent reviews of insect-cell culture include those of Stockdale and Priston (1981), Vaughn and Dougherty (1981) and Grace (1982).

Increasing numbers of insect cell lines and culture media are becoming available (Hink, 1976, 1980). Most of the cell lines are from lepidopteran and dipteran insects; lines from several insect orders, including the Hymenoptera, have not yet been developed.

MNPVs, SNPVs and non-occluded BVs have been replicated in cell cultures.

All attempts to replicate GVs in cell lines have failed so far, but Vago and Bergoin (1963) and Rubinstein, Lawler and Granados (1982) have reported GV replication in primary cell cultures. Replication was incomplete in the latter case. As one of the preferred sites of GV replication is the fat body it will be of interest to see if a GV will replicate in any of the cell lines derived from fat bodies which are now becoming available (Mitsuhashi, 1981).

Susceptible cell cultures are readily infected with budded virions, so the haemolymph of an infected insect or medium from an infected cell culture provides effective inoculum. Virions released from IBs have much lower infectivity for cell cultures.

One of the most widely used cell lines is one derived by Hink (1970) from the ovaries of *T. ni* adults and designated TN-368. It has been used for plaque assays of *A. californica* MNPV (Hink and Strauss, 1977) and *Galleria mellonella* MNPV (Fraser and Hink, 1982). A plaque assay of *H. zea* SNPV in an *H. zea* cell line was described by Yamada and Maramorosch (1981).

A virus which will produce plaques in cell culture can be cloned by picking from single plaques, as carried out by Lee and Miller (1978) for *A. californica* MNPV in a *Spodoptera frugiperda* cell line.

A cell culture, like the whole organism, can harbour inapparent virus infections (Granados, Nguyen and Cato, 1978; Plus, 1978; Heine, Kelly and Avery, 1980). Plus (1980) stressed the importance of initiating cell lines from insects reared from surface-sterilized eggs as a precaution against virus contamination.

Baculovirus characterization and identification

ANALYSIS OF PROTEINS BY SODIUM DODECYL SULPHATE–POLYACRYLAMIDE GEL ELECTROPHORESIS

The technique of sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) permits the number of virus proteins to be determined and their molecular weights to be estimated. It provides useful information, but suffers from a number of limitations and should not be used as the sole technique in virus identification (Allaway and Payne, 1983).

SEROLOGY

Serological methods are used to compare different viruses, and to diagnose infection in insects, especially in epizootiological studies. They are also used in safety testing (page 388) where they provide a means of detecting virus or virus components in non-target organisms and of detecting anti-viral antibodies in vertebrates exposed to the virus. Apparently the IB protein, the virion envelope and the nucleocapsid of an occluded BV each bears distinct antigenic determinants.

Prominent among several techniques which have been used is immunodiffusion, which is useful for investigating antigenic relationships, although it lacks sensitivity. The sensitive technique of enzyme-linked immunosorbent assay (ELISA) is becoming widely used. McCarthy and Henchal (1983) used an

antiserum against nucleocapsids in an ELISA to detect *A. californica* MNPV virions in larvae and in cell cultures. Brown, Allen and Bignell (1982), investigating the relationships between four MNPVs of *Spodoptera* spp., used an indirect ELISA with enzyme-labelled protein A of *Staphylococcus aureus* in place of enzyme-labelled anti-immunoglobulin.

Monoclonal antibodies are increasing the specificity of serological techniques. Roberts and Naser (1982b) developed hybridomas secreting monoclonal antibodies against the IB protein and against a major virion protein of *A. californica* MNPV. These antibodies were used in several serological methods, and have recently been used in a protein-blotting technique incorporating ELISA (Naser and Miltenburger, 1983). Hohmann and Faulkner (1983) reported the application of a similar technique to investigate BV relationships. Volkman and Falcon (1982) used a monoclonal antibody against the IB protein of *T. ni* SNPV in an ELISA to diagnose infection in larvae. They found that host tissue caused interference, but concluded that the test was sensitive enough to be useful.

RESTRICTION ENDONUCLEASE ANALYSIS OF DNA

For definitive characterization and unequivocal identification of a BV it is preferable to analyse the genome rather than phenotypic characters. One of the techniques that discriminates best between double-stranded DNA viruses is restriction endonuclease (REN) analysis of their nucleic acids. Smith and Summers (1979) could differentiate five *A. californica* MNPV isolates by this technique, whereas the SDS–PAGE protein profiles of the isolates were identical.

BIOASSAYS

Precise bioassay techniques yield important information about the virus–host relationship. This information is vital for selecting virus strains with high infectivity and for estimating suitable rates for field application. Many factors can affect the dose–response relationship and/or the LD_{50} of an insect virus and each of these must be standardized. Larval instar (page 396), larval weight and/or age within instar (Burgerjon *et al.*, 1981; Evans, 1983) diet composition, IB purification technique (Baugher and Yendol, 1981) and incubation temperature (Boucias, Johnson and Allen, 1980) should all be carefully controlled.

Techniques in which a larva consumes only a portion of virus-inoculated diet are less preferable to those in which the whole of the dose is ingested on a leaf disc (Evans, 1981), a small piece of diet (Nordin, 1976), or in a small drop (Klein, 1978; Hughes and Wood, 1981). Laing and Jaques (1980) described a bioassay technique for larvae of boring species such as the codling moth, *Cydia pomonella.*

Estimates of LT_{50}s may also be useful, especially for predicting how rapidly insects will be killed in the field.

HOST RANGE

BV host ranges have been widely investigated but many of the results require

confirmation, as viruses which replicated in inoculated hosts were not always identified, often because suitable techniques were not available when these experiments were carried out. Some of the cases of virus replication could have been due to latent virus activation (*see below*) rather than to cross-transmission. In many studies only gross effects of infection (e.g. IB formation, host death) were looked for, although a virus might infect a host sublethally or only some virus functions might be expressed without IB formation. Furthermore, an insect resistant to infection by ingestion of IBs might be susceptible if injected with budded virions.

With these provisos in mind it can be stated tentatively that the MNPVs are the least host-specific of the occluded BVs. *A. californica* MNPV has the widest known host range, infection having been reported in more than 30 insect species and in cell cultures from at least 13 species. Replication of *H. zea* SNPV, on the other hand, appears to be restricted to members of the genus *Heliothis* (Ignoffo and Couch, 1981). Some GVs have been transmitted to other species, e.g. *C. pomonella* GV to five closely related species (Huber, 1982), and *Heliothis armigera* GV to four other species including *T. ni* and two *Spodoptera* species (Hamm, 1982).

When selecting a virus for possible use against more than one pest species it is important to determine the dose–mortality relationship (page 383) for each host. A virus is not likely to control an insect if the LD_{50} is extremely high, as for *Agrotis segetum* GV in *Agrotis exclamationis* larvae in which the LD_{50} for neonate larvae was found to be $1{\cdot}2 \times 10^6$ IBs compared with $1{\cdot}1 \times 10^4$ IBs for the homologous host (Allaway and Payne, 1984).

LATENCY

There have been many reports of insects harbouring 'latent' viruses, especially BVs, but no firm conclusion can be drawn from many of them. The best-substantiated reports concern the development of a homologous NPV in an insect fed with IBs of a heterologous NPV, with both viruses being characterized (Longworth and Cunningham, 1968; Maleki-Milani, 1978; Jurkovíčová, 1979). McKinley *et al.* (1981) found that activation of a latent virus was more common than cross-infection after feeding four NPVs to heterologous hosts. Two aspects of their results are particularly interesting: first, there was a straight-line relationship between dose and mortality, i.e. there was no threshold dose of heterologous virus above which activation of homologous virus occurred; second, it appeared that each of the insects in their cultures carried a latent virus.

Because of the phenomenon of latency it is vital that all insect and cell-culture stocks used for virus studies are checked as closely as possible for the presence of latent viruses.

Baculovirus classification and nomenclature

The BV subgroups were described on page 376, and the reader will have gathered that an individual virus is identified by the name of the insect from which it was isolated, e.g. *Gilpinia hercyniae* SNPV, *Pieris rapae* GV. Some insects, e.g. *T. ni*

and the Douglas fir tussock moth, *Orgyia pseudotsugata*, are host to both a SNPV and a MNPV; regrettably some authors do not specify which type of virus they have worked with.

The system of naming a BV after an insect host is far from satisfactory because many, if not most, of the BVs can infect several hosts. The wide host range of *A. californica* MNPV has been discussed (page 384), and DNA REN analyses indicate that this virus, *T. ni* and *G. mellonella* MNPVs (Smith and Summers, 1979) and an NPV from *Diparopsis watersi* (Croizier *et al.*, 1980) are very closely related. In fact many of the REN pattern differences between these viruses were no greater than the differences between strains of *A. californica* MNPV.

Sometimes a virus is found to be more infective for another host, e.g. *Pieris brassicae* GV is more infective for *P. rapae* than for the 'natural' host (Payne, Tatchell and Williams, 1981), and *M. brassicae* MNPV is more infective for *Plusia gamma* than for the 'natural' host (Allaway and Payne, 1984). Clearly, a more logical approach to BV classification and nomenclature is required.

Baculovirus genetics

There appears to be a multiplicity of genotypes for each of the BVs. A virus isolated from a single infected larva may contain a variety of genomes, as demonstrated by the regular presence of submolar fragments of DNA after REN digestion (e.g. Smith and Summers, 1978; McIntosh and Ignoffo, 1983). Even when no submolar fragments can be detected in REN analysis, a small proportion of the genomes may display variability which can be detected in plaque-purified strains (Smith and Summers, 1980).

There may be differences between virus isolates from members of the same host species collected from different geographical areas, e.g. isolates of *Spodoptera littoralis* MNPV (Kislev and Edelman, 1982), *Neodiprion sertifer* SNPV (Brown, 1982) and *Lacanobia oleracea* GV (Crook, Brown and Foster, 1982) differed in their DNA REN patterns. Heterogeneity in the genome of a single 'virus' is also reflected in variability of phenotypic characters. Isolates may differ serologically, e.g. *A. segetum* MNPV (Allaway and Payne, 1983), in their SDS–PAGE protein patterns, e.g. *N. sertifer* SNPV (Brown, 1982), and in biological characteristics of crucial importance in the use of these agents for pest control. Isolates of *Oryctes rhinoceros* non-occluded BV (Zelazny, 1979), *C. pomonella* GV (Harvey and Volkman, 1983) and *A. segetum* MNPV (Allaway and Payne, 1983) have been shown to differ in LD_{50} for their hosts.

For the reasons just outlined it is preferable that cloned virus strains be used in all investigations. Viruses which produce plaques in cell culture can be cloned from single plaques. For those viruses for which no plaque system is available, the next best approach is to inject groups of insects with serial dilutions of budded virions, and to select for virus isolation a single infected insect from a group injected with a dose smaller than the LD_{50}. Green (1981) used the latter approach with *T. paludosa* SNPV.

A. californica MNPV has been adopted for study by a number of laboratories and rapid progress is being made in mapping the genome of its dominant variant. Physical maps have been derived using RENs (Miller and Dawes, 1979;

Vlak, 1980; Cochran and Faulkner, 1983). *Eco*RI digestion yields 24 fragments, 21 of which have been cloned by Lübbert *et al.* (1981). It has been agreed that the map should start at *Eco*RI fragment I, which includes the IB protein gene. Smith and Summers (1982) found that DNAs from several NPVs, GVs and a non-occluded BV had sequences homologous with this fragment.

The locations on the physical map of the genes for several functions, including IB protein, have been found by marker rescue and by using Southern and Northern blotting techniques. The copy-DNA technique has been used to determine the relative amounts of virus messenger-RNA species in the infected cell, and to identify the proteins for which they code (Adang and Miller, 1982; Smith, Vlak and Summers, 1982; Erlandson and Carstens, 1983).

Recombination between MNPV genomes has been demonstrated. Croizier, Godse and Vlak (1980) inoculated *G. mellonella* larvae with MNPVs from *G. mellonella* and *A. californica*, and isolated recombinants. Smith and Summers (1980) plaque-purified recombinants between *A. californica* MNPV and *Rachiplusia ou* MNPV from wild-type *R. ou* MNPV, and suggested that recombination may be important in the evolution of BVs.

The genomes of BVs can now be manipulated using the techniques of genetic engineering. It may soon be possible to construct new virus strains with improved characteristics as microbial control agents.

Epizootiology

A common objective in pest control with a virus is the establishment of an epizootic in a pest population from which the virus is absent or in which it is only enzootic. In order to achieve this it is important that the mechanisms whereby the virus spreads from host to host within a generation and between generations are understood. Some knowledge of how well the virus persists in the field is also necessary.

Insects which feed at plant surfaces become infected with occluded viruses principally by ingesting IBs present on the plant, deposited there from the faeces or the cadavers of infected insects. In order to ensure virus persistence, large quantities of virus are produced, of which only a tiny proportion may be utilized as inoculum. During a SNPV epizootic in the European spruce sawfly, *G. hercyniae*, in Wales it was estimated that more than 10^{14} IBs/hectare were produced, of which only 0·00025% was utilized the following year (Evans and Harrap, 1982).

Virus may be disseminated by the movement of infected larvae, e.g. NPV-infected larvae of the cabbage moth, *M. brassicae* can move several metres in cabbage plots (Evans and Allaway, 1983). NPV-infected larvae of some species, e.g. the gypsy moth, *Lymantria dispar*, (Doane, 1970) and *M. brassicae* (Evans and Allaway, 1983) tend to climb to the tops of plants before they die, thus ensuring maximum contamination of the plants with their virus load. In many lepidopteran species the BV-killed cadaver hangs from the host plant while putrefaction occurs; then the skin bursts, shedding the liquefied contents together with the virus IBs. Soil-dwelling, plant-feeding insects, such as *Tipula* spp. larvae, are less likely to contaminate their food source with infective doses of virus.

The main mode of transmission for two viruses of *Tipula* spp. appears to be by cannibalism (Carter, 1973a, b; Green, 1981).

IBs may also be deposited on plants in the faeces of predators, or they may be transferred from the soil by rain-splash or by the activities of animals.

TRANSMISSION TO THE NEXT GENERATION

In a permanent ecosystem, such as a forest, IBs produced in one generation of larvae may persist on foliage until the next generation has hatched, as Entwistle and Adams (1977) showed for *G. hercyniae* SNPV. Virus may also contaminate the egg surfaces, and this may be ingested by the hatching larvae, as Doane (1975) demonstrated for *L. dispar* NPV.

In an annual crop, on the other hand, virus is transferred to the plants from a reservoir, usually the soil. There have been several investigations into the survival in soil of viruses of brassica pests. David and Gardiner (1967) reported good survival of *P. brassicae* GV in soil for at least two years, and Jaques (1969) found large amounts of an NPV of *T. ni* in soil 231 days after application with little or no evidence of leaching of IBs. Evans (1982), however, found a 98% loss of *M. brassicae* NPV IBs after 52 weeks: nevertheless, with sufficient IBs initially, enough could survive to infect the next generation.

Some larvae which receive small doses of virus, and/or which become infected in a late instar, may survive to produce infected adults which may disperse the virus and transmit it to their progeny. Entwistle (1976) considered that this was an important dispersal mechanism during an epizootic of *G. hercyniae* SNPV.

It has been claimed that some viruses are transmitted within the egg, but this has not yet been unequivocally demonstrated. It has been shown, however, that infected adults can contaminate the egg surface. Hamm and Young (1974) demonstrated transmission of *H. zea* NPV to the next generation in this way.

ROLES OF PARASITES AND PREDATORS

Hymenopteran parasites of insects can act as virus vectors when females oviposit in infected insects and subsequently in uninfected insects. The infective material probably consists of budded virions. Transmission by this mechanism has been shown for several viruses, including *P. rapae* GV (David, 1965), *Heliothis virescens* NPV (Irabagon and Brooks, 1974) and *L. dispar* NPV (Raimo, Reardon and Podgwaite, 1977).

Predators may disperse virus after feeding on infected insects. The following are a few examples of cases in which an infective BV, often in significant amounts, has been demonstrated in the faeces of predators: insects predatory upon *Heliothis punctiger* (Beekman, 1980) and *M. brassicae* (Evans and Allaway, 1983); birds predatory upon *G. hercyniae* (Entwistle, Adams and Evans, 1978) and *Wiseana* spp. (Kalmakoff and Crawford, 1982); and mammals and birds predatory upon *L. dispar* (Lautenschlager and Podgwaite, 1979).

Parasites and predators therefore have important roles in the transmission and dispersal of viruses, in addition to their more direct roles in regulating

insect numbers. Integrated pest management (IPM) practices should therefore aim at maximum conservation of these animals.

EPIZOOTICS

The normal situation for most virus diseases is an enzootic, occasionally becoming epizootic when the host population density increases. Doane (1976) has described how an NPV epizootic develops in an *L. dispar* population, resulting in a spectacular reduction in population size, which is then likely to remain small for a number of years because of the high level of virus in the environment. Only when this declines is there likely to be a repeat of the cycle of resurgence in insect numbers followed by another epizootic. Briese (1981) proposed that climate, too, might influence the development of GV epizootics in the potato moth, *Phthorimaea operculella.*

Entwistle *et al.* (1983) described the patterns of virus dispersal in *G. hercyniae* SNPV epizootics. The spread of the disease from an initial focus became wave-like and then became random. These authors suggested that other insect viruses, e.g. *O. rhinoceros* non-occluded BV, might follow similar patterns of spread.

Safety

It has been argued (Burges, Croizier and Huber, 1980) that BVs are inherently safe for use as pesticides because man has been exposed to them throughout his evolution and no adverse effects are known. The presence of BVs can be demonstrated on marketed vegetables, some of which are eaten raw. There are, however, a number of potential hazards associated with the mass production and mass application of BVs, and these should be evaluated as fully as possible. It is better to use a pesticide with the confidence that it has passed a series of stringent safety tests than to risk an accident which could set back microbial control for decades.

The viruses which have been most exhaustively tested for safety to date are those registered for use in the US. In a large series of tests on *H. zea* SNPV no adverse effects have been found, except for possible enhancement of simian virus 40-transformation of human amnion cells (McIntosh and Maramorosch, 1973).

POTENTIAL HAZARDS

The virus, and other materials in the formulation, should be tested for infectivity, toxicity, carcinogenicity, teratogenicity and allergenicity in non-target organisms.

A change in the host specificity of a virus might occur by mutation, or by recombination with another virus or with cellular DNA. BV genomes resemble those of papovaviruses, many of which are oncogenic, in that they are both circular double-stranded DNA molecules. Tests for hybridization between BV and vertebrate virus DNAs, and between BV and cell DNAs, would provide an indication of the likelihood of recombination events.

The safety of humans is the prime concern, and it must be remembered that some highly susceptible individuals, i.e. those with hereditary immunodeficiency, those with acquired immune deficiency syndrome, and those receiving immunosuppressant therapy, could be exposed to virus-containing sprays and dusts. Persons involved in virus production and field application receive the greatest exposure, especially when the virus is disseminated as a spray.

Perhaps the most likely hazard is an allergic response in the skin or respiratory system. Repeated inhalation might lead to a pulmonary condition similar to farmers' lung disease. One worker involved in *H. zea* SNPV-production is reported to have developed an allergy (Rogoff, 1975).

The welfare of other organisms, including domestic animals, wild mammals, birds, fishes and beneficial insects, must also be safeguarded.

TESTS ON VERTEBRATE ANIMALS

Animals have been inoculated with BV IBs, virions and DNA via a variety of routes. In the vast majority of these tests, e.g. after feeding *H. zea* SNPV IBs to pregnant rats (Ignoffo, Anderson and Woodard, 1973) and after inoculating *O. rhinoceros* non-occluded BV into mice (Gourreau, Kaiser and Monsarrat, 1982), no harmful effects were found. *M. brassicae* NPV IBs and *A. californica* NPV virions were fed to rodents and no chromosomal aberrations were detected (Miltenburger, 1980).

There are two reports of adverse effects in BV-inoculated pigs: Gourreau *et al.* (1979) found an increased rate of liver lesions in pigs inoculated intraperitoneally with *O. rhinoceros* non-occluded BV; and Döller, Gröner and Straub (1983) found slight temperature increases in piglets fed *M. brassicae* NPV IBs.

G. Döller and co-workers have suggested that an antibody response in an animal is suggestive of virus replication, and have been unable to detect antibodies to IBs and virions in mammals exposed by feeding and inhalation (Döller and Huber, 1983; Döller, Gröner and Straub, 1983). Carey and Harrap (1980), however, found that some rats exposed to *Spodoptera* spp. NPVs developed antibodies to the virions and/or the IB protein and antibody responses have occurred in mice fed IBs (D. L. Knudson, in discussion after Granados, 1980b).

Workers involved in *H. zea* SNPV production (Ignoffo and Couch, 1981) and in field trials with *N. sertifer* SNPV (Entwistle *et al.*, 1978) have been monitored and no antibodies against BV components have been found in their sera.

Care is necessary when interpreting results of serum tests as a number of non-specific reactions have been detected between mammalian sera and IB proteins (Döller, 1980, 1981).

There is evidence both for the survival of BV IBs intact in the mammalian gut, and for their breakdown. Carey and Harrap (1980) recovered infective IBs 21 days after feeding to rats, while Döller, Gröner and Straub (1983) found evidence of IB breakdown in the piglet gut, but were unable to detect infectious virus in the organs.

To test for adverse effects on wildlife the approach of Lautenschlager, Rothenbacher and Podgwaite (1978) could be emulated. These authors

monitored a variety of parameters in five species of caged and free-living mammals in a woodland after aerial application of *L. dispar* MNPV; they found no adverse effects. Döller and Enzmann (1982) showed that fish can mount a good antibody response to IB protein, and proposed that tests for immune responses in fish could form part of an environmental monitoring programme.

TESTS ON VERTEBRATE-CELL CULTURES

BVs have been inoculated into a wide variety of vertebrate-cell cultures and in the majority of cases no cytopathic effect occurred and no evidence of virus replication could be found, e.g. *H. zea* SNPV in primate cells (Ignoffo and Rafajko, 1972), *A. californica* MNPV in three mammalian cell lines (Miltenburger, 1980) and *O. rhinoceros* non-occluded BV in mammalian and fish cells (Gourreau, Kaiser and Montsarrat, 1981). Lack of IB production or other cytopathic effect should not be construed as lack of virus replication, but sensitive tests for a range of virus functions should be performed.

BV virions are readily taken up by vertebrate cells in culture. Granados (1980b) reported uptake of *A. californica* MNPV virions into cytoplasmic vacuoles in HeLa and fathead minnow cells, and similar observations were made by Volkman and Goldsmith (1983) and Miltenburger and Reimann (1980). The latter authors (Reimann and Miltenburger, 1983) also found evidence of some nucleocapsids breaking down in the vacuoles, and of others budding out of the cell. They could not detect virus in the cell nuclei, but Tjia, Zu Altenschildesche and Doerfler (1983), using a DNA hybridization technique, found DNA of *A. californica* MNPV in the nuclei of inoculated mammalian cells for at least 24 hours, after which it was rapidly lost. The limit for DNA detection by this technique is one viral genome per 5–10 cells (Miltenburger, 1980), so it is possible that it might have persisted undetected in a few cells. No evidence of transcription of the virus genome could be found.

McIntosh, Maramorosch and Riscoe (1979) found that *A. californica* MNPV virions were taken into cytoplasmic vacuoles in a viper cell line. There was no evidence of virus replication, but the cells grew more slowly, and there was a large increase in the number of C-type particles present in that cell line.

There have been a few reports of BV replication in mammalian cells. The first of these was by Himeno *et al.* (1967) who announced that IBs had developed in human cells inoculated with *Bombyx mori* NPV DNA. Aleshina *et al.* (1973a) subsequently reported replication of *B. mori* NPV in mouse fibroblasts. McIntosh and Shamy (1980) reported evidence of *A. californica* MNPV replication in a Chinese hamster cell line, but no evidence of replication was found by Volkman and Goldsmith (1983) in the same virus-cell system, or by Reimann and Miltenburger (1983) in another Chinese hamster cell line. One further report of a BV-induced change in mammalian cells is of an increase in nuclear size after inoculation with *L. dispar* NPV (Aleshina *et al.*, 1973b).

OTHER COMPONENTS OF BACULOVIRUS PREPARATIONS

Potential hazards from other materials present in a virus formulation must also

be assessed. Insect fragments, insect diet and contaminant micro-organisms may be present, depending on the production method. Chemicals may be added to protect the virus from ultra-violet (UV)-light, to enhance adhesion to foliage or to stimulate larval feeding, and some viruses are applied in oil suspensions.

One cause for concern is the possible presence of contaminant viruses. Two small RNA viruses were found in a preparation of *Darna trima* GV (Harrap and Tinsley, 1978), and a small RNA virus has been found in *A. californica* MNPV preparations (Morris, Hess and Pinnock, 1979; Vail *et al.*, 1983). The latter virus has affinities with the mammalian caliciviruses, and is infective for *T. ni* larvae, in which small doses can initiate inapparent infections. *T. ni* larvae are used for *A. californica* MNPV production.

The risks posed by contaminant viruses are still largely unknown, but one small RNA virus (Nodamura virus) isolated from insects is lethal to mice when injected by various routes (Scherer, Verna and Richter, 1968). Until the risks can be shown to be negligible it would seem prudent for any virus which is to be applied as a spray to undergo a purification procedure sufficient, at least, to remove contaminant virions.

Most mass-produced insect virus preparations, however, consist of ground, lyophilized virus-infected larvae, and therefore contain insect material and contaminating micro-organisms. Podgwaite, Bruen and Shapiro (1983) found approximately 10^8–10^9 viable bacteria and fungi per gram of 'Gypchek' (*L. dispar* MNPV). Many of the organisms that they found are opportunistic human pathogens. Padhi and Maramorosch (1983) determined viable bacterial counts in commercial preparations of *H. zea* SNPV. They found 10^5 bacteria per gram in 'Elcar', whereas 'Viron/H' (now discontinued) contained 10^8 bacteria per gram, including *Bacillus cereus* which was pathogenic to silkworm larvae.

Dubois (1976) demonstrated that bacterial contaminants can be destroyed chemically. In the UK, field trials have been carried out with highly purified preparations of *N. sertifer* SNPV (Cunningham and Entwistle, 1981), *P. brassicae* GV (Tatchell and Payne, 1984) and *C. pomonella* GV (Glen and Payne, 1984).

'Gypchek' production also involves the hazard posed by the allergenic, urticarious setae of *L. dispar* larvae. Personnel are protected by filter masks, and a method has been devised for removing the setae during processing (Shapiro *et al.*, 1981).

Other components of BV preparations (e.g. UV-protectants, oils) should be tested for possible hazard, especially for carcinogenicity by inhalation.

REGISTRATION REQUIREMENTS AND GUIDE-LINES FOR SAFETY TESTING

Harrap (1982) has given a comprehensive account of registration requirements for viral (and other microbial) insecticides, and of the guide-lines produced by several national and international bodies for safety testing them. In the UK the controlling body is the Pesticides Safety Precautions Scheme of the Ministry of Agriculture, Fisheries and Food (Papworth, 1980), while in the US it is the Environmental Protection Agency (Rogoff, 1980). Many developing nations lack the facilities and resources for safety testing. Virus preparations which might

be of value to those nations could be safety tested in laboratories in the developed nations as a contribution to their overseas aid programmes.

Too few BVs have been exhaustively safety tested to allow conclusions to be drawn about the safety of BVs in general, but the current impression is that these viruses appear to be safe for field use. However, the evidence that IBs can be dissolved in the mammalian gut and that virions can be taken into mammalian cells, together with the reports of replication in mammalian cells and of adverse effects in mammals, mean that several BVs will need to pass stringent safety tests before the group in general receives a blanket seal of approval.

Strategies for pest control with viruses

INTRODUCTION OF VIRUS

Many pests have been introduced into new areas of the world as a result of man's activities. It has been estimated that 30% of the most serious pests in the US are of foreign origin. The pests are often introduced without all of their natural enemies, including viruses. There have been several cases where a virus has subsequently been introduced, either deliberately or by accident, and has provided effective control of the pest. Two examples concern sawflies introduced into North American forests from Europe. In each case the subsequent release of an NPV from Europe initiated epizootics and controlled pests (Bird, 1953; Bird and Elgee, 1957).

This strategy was also applied to the non-occluded BV of the coconut palm rhinoceros beetle, *Oryctes rhinoceros*, which was discovered in Malaysia (Huger, 1966), but was apparently absent from the Pacific islands where *O. rhinoceros* causes serious damage to palms (pages 393 and 396).

SUPPLEMENTATION OF EXISTING DISEASE

Where a virus is present in an insect population, increasing the amount of virus in the environment may lead to a greater proportion of insects becoming infected. One virus application may be sufficient to reduce numbers of a pest to an economically acceptable level, especially in a forest. Cunningham and Entwistle (1981) stated that a single NPV application to young trees is likely to protect them from sawfly damage for their lifetime. In an agricultural situation it may be necessary to use a virus more like a chemical insecticide, with adequate protection provided only by several applications during the lifetime of the crop.

MANIPULATION OF EXISTING DISEASE

In some situations it is possible to increase the level of virus disease in a pest population by the adoption of certain management practices. An example concerns *Wiseana cervinata* which damages pasture in New Zealand. An NPV is widespread and can control this pest, but cultivation of the land buries the virus reservoir beyond the range of the larvae. Kalmakoff and Crawford (1982)

therefore recommended oversowing damaged areas of pasture without cultivation. They also recommended the regular movement of stock over pastures to spread the virus.

Techniques for virus dissemination

RELEASE OF INFECTED/CONTAMINATED INSECTS

This dissemination technique has special attraction for viruses which survive poorly outside the host, e.g. *O. rhinoceros* non-occluded BV, which has been introduced into a number of South Pacific islands by releasing infected beetles (Bedford, 1981). Only the mid-gut cells are susceptible in the adult, which may survive for many weeks. The infection has a debilitative effect, however: the beetles stop boring into palms and females stop egg-laying. Monsarrat and Veyrunes (1976) estimated that an infected adult excretes about 300 ng virus per day. Some of this virus is transmitted during mating, and some serves as a source of infection for larvae, in which the infection rapidly becomes systemic and causes death.

Some insect viruses, e.g. *P. brassicae* GV (Tatchell, 1981), are transmitted to the progeny if the ovipositor of the female is contaminated, but this technique has not yet been widely applied to virus dissemination in the field.

SPRAYING

Most viruses are applied in aqueous sprays using equipment developed for spraying chemical insecticides. Morris (1980) and Smith and Bouse (1981) have argued for a research programme to design equipment specifically for the application of viruses and other microbes.

Equipment producing small droplets is preferred. Virus application in droplets with diameters of 100–150 μm usually results in higher insect mortality than in larger droplets (Smith and Bouse, 1981). Entwistle *et al.* (1978) used a micro-droplet machine producing droplets with a mean diameter of 50 μm. Reed and Springett (1971) suggested that *P. operculella* GV might best be disseminated as a mist as the IBs would be more likely to enter the stomata, thereby becoming more accessible to the larvae within the leaves.

Virus dissemination in charged droplets from an electrostatic sprayer means that a larger proportion of IBs adhere to the leaves, especially the undersides which are the sides often favoured by insects, and which provide some protection from sunlight for the virus. A disadvantage of electrostatic sprayers is poorer spray penetration into the plant canopy (Matthews, G.A., 1982).

A modification of spray application was carried out by Hamm and Hare (1982) who introduced NPVs of *H. zea* and *S. frugiperda* on to corn via an overhead irrigation system. Instead of spraying the crop, Young and Yearian (1980) sprayed the soil with an NPV of the soybean looper, *Pseudoplusia includens*, at soybean planting time.

If a virus spreads rapidly, then blanket spraying may be unnecessary. This is the situation with the SNPV of the red-headed pine sawfly, *Neodiprion lecontei*,

for which Cunningham (1982) has proposed spot introductions or 'zebra stripe spraying' from aircraft. Spot introductions into glasshouses of the GV of the tomato moth, *Lacanobia oleracea*, were suggested by Crook, Brown and Foster (1982).

BAITS

The application of insecticides in baits has the advantage that less insecticidal material is required, and the disadvantage of increased costs of field application. Baits are especially valuable if insects which have a burrowing or mining habit can be encouraged to spend longer at the plant surface and ingest larger doses of insecticidal material.

Most research into the application of viruses in baits has involved *H. zea* SNPV and baits based on cottonseed and soybean (page 395). Johnson and Lewis (1982) used wheat bran baits to apply two MNPVs to corn.

DIPPING SEEDLINGS

Ignoffo *et al.* (1980) suggested that IBs could be introduced on to cabbages by dipping them in an IB suspension at the time of transplanting.

Viruses undergoing trials and/or in use

Details of viruses registered for use in various countries are given in *Table 2*, and some of them are discussed more fully below.

1. *Heliothis zea* SNPV has been marketed for almost a decade in the US for the control of *H. zea* and *H. virescens* on cotton (Ignoffo and Couch, 1981). Some workers, e.g. Shieh and Bohmfalk (1980), have found it to be an effective

Table 2. Viruses registered for use.

Virus	Used on	Country	Product name
Heliothis zea SNPV	Cotton and other crops	US	Elcar
	Cotton, sorghum	Australia	
Orgyia pseudotsugata MNPV	Fir trees	US	TM Biocontrol-1
		Canada (temporary registration)	Virtuss
Lymantria dispar MNPV	Deciduous trees	US	Gypchek
		USSR	Virin-ENSh
Autographa californica MNPV	Several crops	US (experimental use permit)	SAN 404
Neodiprion sertifer SNPV	Pine trees	US	Neochek S
		USSR	Virin-Diprion
		Finland	none
Neodiprion lecontei SNPV	Pine trees	Canada (temporary registration)	Lecontvirus
Dendrolimus spectabilis cytoplasmic polyhedrosis virus	Pine trees	Japan	Matsukemin

insecticide, while others, e.g. Pfrimmer (1979), have obtained variable and sometimes disappointing results.

Much effort has been expended in attempts to achieve more consistent results. Some of this effort has involved the development of baits, and two in particular have been tested: 'Coax' based on cottonseed and 'Gustol' based on soybean. Many workers (e.g. Hostetter *et al.*, 1982, and Potter and Watson, 1983a) have shown in laboratory and field tests that applying the virus in a bait increases larval mortality. Some of the increased mortality may not be virus-induced, however, as treatment of cotton with 'Coax' alone results in increased mortality (Henry, 1982). This has been attributed to larvae spending longer at the surface before tunnelling into the bolls, thereby extending their exposure to parasites and predators.

Smith, Hostetter and Ignoffo (1978, 1979) compared different formulations, application rates, types of spray nozzle and nozzle pressures. They found that the efficiency of application was affected by nozzle type and droplet size.

H. zea SNPV can also control *Heliothis* spp. on other crops. Ignoffo *et al.* (1978) found that it reduced *H. zea* populations on soybeans by 92–100%, and Smith and Hostetter (1982) reported better control of *H. zea* on soybean and cabbage than on cotton. In Australia *H. zea* SNPV is undergoing tests for its ability to protect navybeans from *Heliothis* spp. (R. E. Teakle, personal communication).

2. *Autographa californica* MNPV, originally isolated from the alfalfa looper, is considered to have a potential commercial value because of its wide host range. It has been reported that it can control *T. ni* as effectively as chemicals on cabbage (Hostetter *et al.*, 1979) and lettuce (Vail, Seay and Debolt, 1980), and it is being assessed as an alternative to *Orgyia pseudotsugata* MNPV for the control of the Douglas-fir tussock moth, *O. pseudotsugata*. Although the latter virus can control its host effectively, the high cost of its production and its limited market mean that there is no commercial interest in it (Martignoni, Steltzer and Iwai, 1982).

3. *Neodiprion sertifer* SNPV has been extensively tested against its host, the European pine sawfly, in Eastern and Western Europe and in North America (Cunningham and Entwistle, 1981). Entwistle *et al.* (in press) have induced high larval mortality in pine forests in Scotland with applications of 5×10^9 to 2×10^{10} IBs/hectare. These quantities of virus can be produced in 20–50 larvae. This remarkable efficiency is attributed to the high larval susceptibility to this virus and to its rapid spread.

4. *Neodiprion lecontei* SNPV has shown promise in trials in Canada. Its host has been controlled with applications of 5×10^9 to 8×10^9 IBs/hectare (Cunningham, 1982).

5. *Galleria mellonella* MNPV was shown by Dougherty, Cantwell and Kuchinski (1982) to control wax moth larvae effectively in bee-hives. A non-hazardous insecticide is especially important for this pest of honeycomb.

6. *Panolis flammea* NPV has shown very promising results against its host, the pine beauty moth, which is a pest of lodgepole pines in Scotland (P. F. Entwistle, personal communication).

7. *Heliothis armigera* SNPV was shown to provide control of its host on sorghum in Botswana by Roome (1975) and is still under investigation in that country where *H. armigera* is a pest of many crops (Flattery, 1983).

8. *Choristoneura occidentalis* NPV and GV have shown promise for the control of their host, the western spruce budworm on Douglas fir. The impact of the NPV on the population size was still detectable one year after spraying (Shepherd, Gray and Cunningham, 1982), and a GV application rate of only 25 'larval equivalents'/acre resulted in 56% mortality (Cunningham, Kaupp and McPhee, 1983).

9. *Pieris brassicae* GV has been demonstrated by a number of workers, including Kelsey (1958), to provide control of larvae of the small cabbage white butterfly, *Pieris rapae*. Tatchell and Payne (1984) recently found that a spray containing 10^8 IBs/ml reduced the larval population by more than 90%. The virus is rapidly inactivated in the field, however, and regular spraying would be necessary to maintain satisfactory control.

10. *Cydia pomonella* GV has been tested in many countries for control of the codling moth in orchards. Huber and Dickler (1977) reported that four sprays resulted in good control, but there was no persistence of the disease into the next season. Much virus is probably removed from the orchard on the surface of the apples. Trials carried out by Glen and Payne (1984) led them to conclude that the use of *C. pomonella* GV effectively reduces the more severe forms of fruit damage, but the quantities of virus required to control less severe forms of damage would probably be uneconomic.

11. *Oryctes rhinoceros* non-occluded BV has been introduced into a number of South Pacific islands. In Tonga it was still infecting 84% of the beetle population after seven years (Young and Longworth, 1981). Control of the rhinoceros beetle has led to a revival of the copra industry in Western Samoa (Marschall and Ioane, 1982).

Timing of field applications

The timing of field applications of virus can be crucial in determining the level of pest control achieved. Significant pest damage is not usually noticed until the larvae are in the later instars, when larger doses of virus are necessary to infect them. This, coupled with the fact that most insect viruses kill their hosts more slowly than chemical insecticides, means that for many pests the virus must be applied before crop damage appears. Pest forecasting systems can be used to indicate when pest numbers are approaching damaging levels.

Increases in LD_{50} of 10^4-fold to 10^6-fold from early to late larval instars have been found for a number of lepidopteran BVs, including *P. brassicae* GV

(Payne, Tatchell and Williams, 1981) and *Mamestra configurata* MNPV (Bucher and Turnock, 1983), and LT_{50}s are often longer in later instars. In some cases the increased LD_{50} may be offset by the increased food consumption of larger larvae, as in *H. armigera* where the first three larval instars have a similar probability of becoming infected with *H. zea* SNPV in the field (R. E. Teakle and J. M. Jensen, personal communication).

Increases in resistance to NPVs in the later instars of sawflies appear to be small compared with those in the Lepidoptera. For *N. sertifer* and *G. hercyniae* the increases in LD_{50} are about tenfold to fiftyfold from the first to the fifth larval instar (Entwistle, Adams and Evans, unpublished work cited in Evans and Harrap, 1982; Entwistle *et al.*, in press). This means that these pests can be controlled if virus is applied after the first instar. Infection of the larvae when they are larger means that more virus is produced and is available to infect the next generation. This approach is more applicable in stable ecosystems, such as forests and pastures where some pest damage can be tolerated, than in annual crops.

Virus persistence in the field

A rapid loss of infective virus from plant surfaces can usually be detected after field application, which could be a physical loss of IBs from plants and/or a loss of infectivity in virus on the plants.

There have been many studies of rates of infectivity loss, but, as pointed out by Richards and Payne (1982), most of them have started with amounts of virus giving 100% mortality in bioassays and have not therefore achieved their objectives. These authors outlined a sound experimental approach which they applied to measure survival of infectivity of a *Pieris* sp. GV on cabbage in the UK. They found that the half-life varied from 0·35 day in June to 1·0 day in October. They showed, using ^{32}P-labelled IBs, that the IBs had not been lost from the cabbages.

If a virus can be protected from inactivation then smaller amounts need be applied and/or the timing of application becomes less critical. The extent to which virus inactivation can make timing of application critical was shown by Potter and Watson (1983b). If they sprayed *H. zea* SNPV against *H. virescens* just after the eggs were laid, 15% of the larvae died, whereas if they sprayed just before the eggs hatched, 80% died.

INACTIVATION BY ULTRA-VIOLET LIGHT

The main factor causing infectivity loss in the field appears to be the UV component of sunlight. Attempts are made to protect some IB preparations by adding a UV-absorbing substance. A polyflavinoid marketed as 'Shade' has been used with *H. zea* SNPV, and increased virus persistence and/or mortality of larvae has resulted. 'Shade' is incorporated into *L. dispar* MNPV preparations (Lewis, 1981), and has been shown to act as a UV-protectant for this virus in a laboratory test, although more protection was afforded by the feeding stimulant 'Coax' (Shapiro, Poh Agin and Bell, 1983).

INACTIVATION BY COTTON LEAF SECRETIONS

Cotton leaf secretions have a high pH due to substances secreted by epidermal glands (Elleman and Entwistle, 1982), and there is some evidence that IBs on the leaf surface can be affected (Andrews and Sikorowski, 1973). Richards (MSc thesis cited in Richards and Payne, 1982) found that an unbuffered suspension of *S. littoralis* NPV was completely inactivated 6 days after application to cotton leaves, whereas infectivity was preserved for much longer if the virus was applied in a phosphate buffer, pH 7. Some workers in the US have applied *H. zea* SNPV to cotton in buffered suspensions, but results have varied in different areas. Further investigations are necessary to determine whether there are advantages to be gained by applying viruses to cotton in buffered suspensions.

Virus production

Viral insecticides are currently produced in the host insect which is either collected in the field or reared in an insectary. For some species more than 10^8 larvae per year are produced. High standards of hygiene are vital to reduce the risk of infection by pathogens which could decimate the insect stocks and contaminate the product.

The production of *H. zea* SNPV ('Elcar') has been described by Ignoffo and Anderson (1979) and Ignoffo and Couch (1981). *H. zea* larvae are reared on a semi-synthetic diet in the wells of plastic trays. Each larva yields about $3{\cdot}5 \times 10^9$ IBs which are extracted, purified and spray dried. The final product contains 99·6% inert ingredients and is stored at −20°C. 'Elcar' is produced in the US by Sandoz Inc. who also produce smaller quantities of *A. californica* MNPV and an NPV of *T. ni* for experimental purposes. Both of these viruses are produced in *T. ni* larvae and the IB preparations are spray-dried (Yearian and Young, 1982).

A process for the mass production of *L. dispar* MNPV ('Gypchek') has been described by Shapiro *et al.* (1981) and Shapiro (1982), in which the IB yield represents a 5600-fold increase over the inoculum.

For the production of sawfly viruses, either field-collected larvae are infected and then maintained on host plant material, or infected larvae are collected in the field (Cunningham and Entwistle, 1981).

Production of viruses in insects is labour-intensive and therefore costly in the developed nations. The most time-consuming stage in 'Elcar' production is the introduction of larvae into the trays, while in 'Gypchek' production it is the removal of the infected larvae from their containers.

OPTIMIZING PRODUCTION

The host insect

The insect species from which a virus was isolated may not be the most susceptible (page 385). Use of a more susceptible host for virus production would mean a smaller inoculum requirement.

Alternative hosts might also be considered for insect species which have a long life cycle, which are small and produce a low yield, or which have allergenic and urticarious setae. Shapiro *et al.* (1982) suggested that *O. pseudotsugata* MNPV might be produced in the saltmarsh caterpillar, *Estigmene acrea*, which is more easily reared than the homologous host. It is important to check that virus produced in an alternative host does not have reduced virulence for the original host.

Insect diet

Diet may affect the growth rate of an insect, its susceptibility to virus infection, and the virus yield. Synthetic or semi-synthetic diets are used for most insects, and cost is an important factor. A diet rich in wheat germ was found to be the most cost effective for *L. dispar* MNPV production, although higher IB yields could be obtained using other, more expensive, diets (Shapiro, Bell and Owens, 1981). Shapiro (1982) found a substitute for agar which was 40% cheaper than agar and resulted in improved growth of *L. dispar* larvae with higher IB yields.

Glen and Payne (1984) increased the yield of *C. pomonella* GV by incorporating into the diet a juvenile hormone analogue (methoprene) which resulted in larger larvae.

Insect stage and virus dosage

The lower IB doses necessary to infect younger larvae must be balanced against the early deaths of these larvae with smaller IB yields. The optimum dosage must be determined. If it is too low, many larvae will not become infected, whereas if it is too high, inoculum will be wasted and larval growth will be retarded resulting in suboptimal yields.

Incubation environment

Temperature affects the rate of insect growth, the rate of virus replication and the virus yield. The optimum temperature for each of these may not be the same and it is necessary to determine the optimum for yield. Relative humidity and photoperiod must also be maintained at their optima.

Preservation of virus infectivity

Conditions which destroy infectivity (e.g. increased temperature, extremes of pH) must be avoided during harvesting, purification, formulation and storage of virus.

QUALITY CONTROL

Each batch of virus must be carefully bioassayed (page 383) and tested for the presence of harmful contaminants, especially human pathogens. Morris, Vail and Collier (1981) suggested that quality control procedures should include tests for contaminants such as small RNA viruses.

Future prospects

USE OF VIRAL INSECTICIDES IN INTEGRATED PEST MANAGEMENT

The relatively narrow host spectra of insect viruses may be environmentally attractive, but mean that markets for viral insecticides are restricted and that a virus alone is unlikely to afford protection against all the pests in a particular ecosystem. For example, if *C. pomonella* GV is used for codling moth control in orchards, other lepidopteran pests, especially tortrix moths, may resurge. On the other hand, use of the virus has the advantage that parasites and predators of the fruit-tree red spider mite, *Panonychus ulmi*, are not killed, so damaging numbers of this pest are not reached, which may occur if an organophosphorus insecticide is used to control codling moth (Glen *et al.*, 1984).

A virus may form a useful component of an IPM programme in which pests are controlled by husbandry practices, chemicals and biological agents. The most widely used microbial control agent is *Bacillus thuringiensis*, most strains of which have wide spectra of activity against lepidopteran insects. In fact, the existence of this microbial insecticide is one of the factors limiting the development of viral insecticides, although for some pests the two might be used together. *B. thuringiensis*, together with *P. rapae* GV and *A. californica* MNPV, have been reported to control *P. rapae* and *T. ni* on cabbage almost as effectively as chemical insecticides (Sears, Jaques and Laing, 1983).

IPM on cotton might include the use of *H. zea* SNPV and *B. thuringiensis* or chlordimeform against *Heliothis* spp., *A. californica* MNPV and *B. thuringiensis* against the cotton leaf-perforator, *Bucculatrix thurberiella* (Bell and Romine, 1982), and diflubenzuron against the cotton boll weevil, *Anthonomus grandis* (Bull *et al.*, 1979).

VIRUS PRODUCTION IN CELL CULTURE

Several laboratories are attempting to develop reliable and economic cell-culture systems for the mass production of insect viruses as alternatives to production in insects, which has a number of associated problems (pages 398–399), and because a high-purity product is more feasible from cell cultures. Much progress has been made, but several problems remain to be solved. An outline of insect-cell culture techniques was given on pages 381–382. The possible application of those techniques to virus production will now be discussed.

Production systems

Insect cells can be grown in fermenters of the type used for vaccine production and Vaughn (1981) has suggested that the slack periods of such plants could be used for insect-virus production. When *A. californica* MNPV was produced in TN-368 cells in fermenters 2–3 litres in volume it was found (Hink and Strauss, 1980) that more vigorous aeration was required than in small volumes, and this resulted in foaming and cell damage. Antifoam was added and the concen-

tration of methylcellulose, already present to inhibit cell clumping, was increased to protect the cells.

Attempts have been made to avoid the stresses imposed on cells in traditional fermenters by using alternative systems. Miltenburger and David (1980) blew air through silicone rubber tubing coiled inside a fermenter. Oxygen diffused through the silicone rubber into the medium. Hilwig and Alapatt (1981) and Vaughn and Dougherty (1981) have worked on roller bottle systems, but Stockdale and Priston (1981) believe that they are too bulky and labour-intensive for adoption by industry. Vaughn and Dougherty (1981) are also developing a 'perfusion culture system': this consists of vessels containing coils which provide a large surface area for cell attachment; pH and oxygen concentration are adjusted outside the vessel. Pollard and Khosrovi (1978) presented a design for a continuous-flow tubular fermenter.

Optimizing production

Some of the factors which can affect IB yield were investigated by Gardiner, Priston and Stockdale (1976) for *A. californica* MNPV in TN-368 cells. They found an optimum temperature of 27°C, an optimum pH range of 5·5–6·5 and an optimum osmotic pressure range of 250–500 milliosmoles. For the same virus–cell system Hink (1982) reported production of 10^8 IBs/ml medium and suggested that this must be increased twentyfold before the system becomes economic.

The IB yield can be affected by the growth phase of the cells at the time of virus inoculation (Lynn and Hink, 1978), and by their concentration. The cell concentration giving maximum IB yield per ml of medium was higher than that at which the maximum number of IBs per cell was produced (Hink, Strauss and Ramoska, 1977; Stockdale and Gardiner, 1977). The latter authors suggested that the reduced IB production at higher cell densities might be due to depletion of a vital precursor. Wood, Johnston and Burand (1982) reported a 98% reduction in virus production in high-density attached cultures compared with low-density cultures. Inhibition of virus production did not occur unless there was cell-to-cell contact. Further investigations are necessary into the mechanisms of, and ways of overcoming, inhibition of IB production at high cell concentrations.

Virus strains and cell strains should be selected to give a high-yielding system. Cells should be cloned and the clones screened for desirable properties, e.g. more rapid growth rate (McIntosh and Rechtoris, 1974). The quality of IBs produced in a cell line must also be checked. Lynn and Hink (1980) found that *A. californica* MNPV IBs produced in cells from four insect species were less infective for *T. ni* larvae than IBs produced in *T. ni* cells.

Most insect-cell culture media are expensive, principally because most of them contain foetal bovine serum. Dougherty, Cantwell and Kuchinski (1982) calculated that, for *G. mellonella* MNPV production in cell culture, half the cost, including labour, was for serum. Serum-free media are now being developed and have been reported for a *S. frugiperda* cell line with replication of *A. californica* MNPV (Wilkie, Stockdale and Pirt, 1980), an *L. dispar* cell line with

replication of *L. dispar* NPV (Goodwin and Adams, 1980) and for several other cell lines (Mitsuhashi, 1982). Weiss *et al.* (1981) reduced the cost of their medium for *S. frugiperda* cells by omitting antibiotics. They encountered no contamination problems.

Changes in virus on passage

Several studies, e.g. Faulkner and Henderson (1972), have demonstrated that IBs produced during the first few passages in cell culture are as infective as IBs produced in insects. Upon repeated passage, however, the quality and yield of IBs have been found to decline. Hirumi, Hirumi and McIntosh (1975) reported that passage of *A. californica* MNPV in *T. ni* cells led to the production of aberrant virions and a reduction in IB yield. After 40 passages the yield had dropped a hundredfold, with IBs developing in only 4% or less of the cells (McIntosh, Shamy and Ilsley, 1979). MacKinnon *et al.* (1974) found a reduction in average yield of *T. ni* MNPV IBs from 28 per cell initially to 2·5 per cell after 50 passages, with extensive production of abnormal capsids. Knudson and Harrap (1976) found that passage of *S. frugiperda* NPV in *S. frugiperda* cells led to the production of IBs containing few or no virions, and Yamada, Sherman and Maramorosch (1982) reported reduced yields of *H. zea* SNPV IBs after 20 passages in *H. zea* cells.

Hink and Strauss (1976) described two plaque morphologies after passage of *A. californica* MNPV *in vitro*. In one type of plaque there were between 81 and 352 IBs per nucleus, while in the other there were only 2–13 IBs per nucleus. The plaque types were named many-polyhedra (MP) and few-polyhedra (FP) plaques respectively. The FP plaques became increasingly dominant on passage. IBs from MP plaques contained normal virions (multiple nucleocapsids per virion) and were much more infective for *T. ni* larvae than IBs from FP plaques, which either contained only a few virions (each with only a single nucleocapsid per virion) or appeared devoid of virions. Similar phenomena have been reported for *T. ni* MNPV (Potter, Faulkner and MacKinnon, 1976), *G. mellonella* MNPV (Fraser and Hink, 1982) and for *H. zea* SNPV (M. J. Fraser and W. J. McCarthy, unpublished, in Fraser, Smith and Summers, 1983).

Most FP forms are genetically stable. They have a selective advantage *in vitro* as FP-infected cells produce higher titres of budded virions than MP-infected cells (Potter, Jaques and Faulkner, 1978). Wood (1980) suggested that FP forms might be deletion mutants of MP forms, but several FP forms have been found to contain insertions of host DNA (Miller and Miller, 1982; Fraser, Smith and Summers, 1983).

One way of avoiding the FP form becoming dominant in cell cultures would be to return regularly for inoculum to haemolymph from insects infected by ingestion of IBs. Insect haemolymph, however, is unlikely to supply the quantities of inoculum that an industrial-scale process would demand, so some means will have to be found of preventing the development of FP and other aberrant virus forms *in vitro*.

VIRUS PURIFICATION

The debate concerning the degree of purification necessary before a virus is sprayed in the field has not yet been resolved. Of primary concern are the potential hazards posed by the presence of contaminants (pages 390–391). Other considerations are the costs involved and the possible effects of purification on virus infectivity and persistence.

There have been several reports (e.g. Magnoler, 1968; Carner, Hudson and Barnett, 1979; Evans and Harrap, 1982) of laboratory and field tests in which purified IB preparations had lower infectivity and/or poorer environmental persistence than IB preparations contaminated with insect fragments, gut contents and micro-organisms. It is well known that proteins can protect viruses from inactivation, so the contaminants may afford some protection, especially from UV light. *N. sertifer* SNPV, however, controls its host effectively when applied as a highly purified IB preparation (Entwistle *et al.*, in press). It is interesting that *L. dispar* larvae were deterred from feeding on foliage contaminated with decayed cadavers or extracts from healthy larvae, but were not deterred by foliage treated with purified NPV IBs (Capinera, Kirouac and Barbosa, 1976).

The most efficient way of purifying IBs is by some form of gradient centrifugation, but this can make the final product prohibitively expensive in the developed nations, let alone the developing nations. In the future, if viruses are produced in cell cultures they should be free from contaminating micro-organisms and minimal purification should be necessary. In the meantime, tests should be carried out to evaluate the hazards posed by contaminants in virus preparations produced in insects, which should be subjected to rigorous quality control procedures before field application.

NEW VIRUS STRAINS

Ideal attributes in a viral pesticide are high infectivity and high virulence for a broad range of pest species, rapid replication with high yields, and good field persistence with high resistance to UV inactivation. No known virus is endowed with all of these attributes, but progress towards the development of such an agent should be possible by two approaches, i.e. by searching in nature for new virus strains and by the genetic manipulation of existing isolates.

There can be no doubt that the number of insect virus strains isolated to date is only a tiny fraction of the total in nature. Virus strains with desirable properties will undoubtedly be found among future isolates.

A virus might be genetically improved by selecting for desired traits, or by using the techniques of genetic engineering. The former approach was used by Brassel and Benz (1979) who selected a strain of *C. pomonella* GV which was 5·6 times more resistant to UV light than the original isolate, and remained infective for twice as long in the field. Wood *et al.* (1981) induced mutations in *A. californica* MNPV, then isolated a mutant with increased virulence for *T. ni* larvae, demonstrated by a significantly reduced LT_{50}. Among several possibilities for genetically engineering virus strains is the suggestion by Miller, Lingg

and Bulla (1983) that the gene for an insect-specific toxin might be incorporated into the virus genome to kill the host more rapidly.

PATENTS

A search by Stockdale (in press) revealed that 13 patents had been filed for processes or formulations involving insect viruses. It is not possible to patent the viruses, however, and this is one of the reasons why viral pesticides have not been developed more rapidly. There are some hopes that this situation may change and that it may become possible to patent an organism if it is the product of a biotechnological process (Crespi, 1980) or if it has undergone genetic manipulation (Kayton, 1983). If these hopes are realized then there will be more incentive for commercial concerns to invest in microbial pesticide development.

DEVELOPMENT OF PEST RESISTANCE

There are not yet any reports of selection of an insect strain with high resistance to a virus, as occurred in the rabbit to myxoma virus (Fenner, 1983). It is a possible outcome, however, if a virus is widely used over a long period. Genetic variability, upon which selection could operate, has been demonstrated in a number of insect species, e.g. varying levels of susceptibility to a GV in the Indian meal moth, *Plodia interpunctella* (Hunter and Hoffmann, 1973) and to an NPV in the light brown apple moth, *Epiphyas postvittana* (Briese *et al.*, 1980).

Some workers have attempted to select for virus resistance. Ignoffo and Allen (1972) failed to select for increased resistance to an NPV in *H. zea* after inoculating 25 generations of larvae with doses at, or greater than, the LD_{50} and breeding from the survivors. Briese and Mende (1983), however, selected for resistance to a GV in *P. operculella* within six generations of insects from the wild, but they were able to select for only a slight increase in resistance in a laboratory strain which was already highly resistant.

CONCLUSION

Advances in BV research are providing greater insight into the viruses themselves, e.g. their genetics, and into their interactions with their hosts, e.g. their epizootiology. This information means that the viruses can be used as pesticides on a more rational basis.

A number of insect viruses are currently used as pesticides and the potential of others has been demonstrated. Entwistle (1983) is optimistic that BVs will become the principal means of regulating lepidopteran and sawfly pests of forests. Viral pesticides can have an important role in Third World countries if they can be produced locally and if it can be shown that they are safe to disseminate in an unpurified or semi-purified state.

The potential for viral insecticides in the agriculture and horticulture of the Developed World is more limited at present. Consumers demand fruit and vegetables free from blemishes, so growers look for products which provide a

quick and virtually complete kill of pests. Often this cannot be achieved with viruses, so until there is a change in consumer attitude, chemicals are likely to remain the main tools for pest control. As the integrated pest management approach gains ground, however, viruses of pests should have increasingly useful roles.

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