Institute of Virology and Environmental Microbiology

Scientific Report 1996–97

Centre for Ecology and Hydrology

Natural Environment Research Council

The IVEM mission

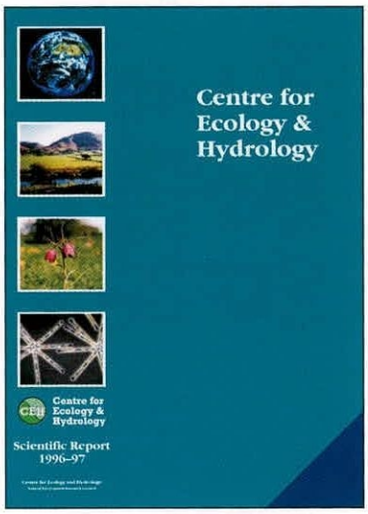
- To advance the science of environmental microbiology (including virology) through high-quality internationally recognised research leading to a better understanding of the functional roles and structure of micro-organisms in the environment and the interactions of micro-organisms with their natural hosts.

- To investigate, through monitoring, experimentation and modelling, natural changes in the environments of micro-organisms and to assess past, present and future effects of man's impact on them.

- To secure, expand and provide data relevant to environmental microbiology (including virology) to further scientific research and provide the basis for advice on environmental conservation and sustainable development to governments and industry.

- To promote the use of the Institute's research facilities, expertise and data to provide research training and education of the highest quality, and to enhance the United Kingdom's industrial competitiveness, research basis and quality of life.

CEH REPORT RANGE 1996–97



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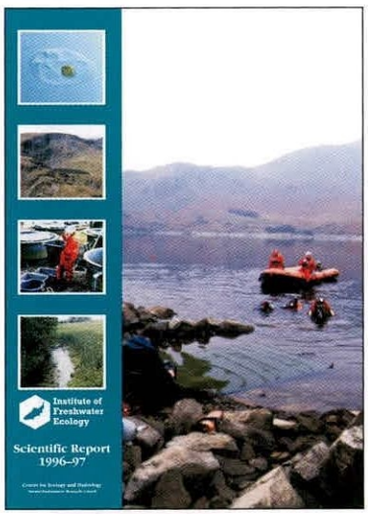

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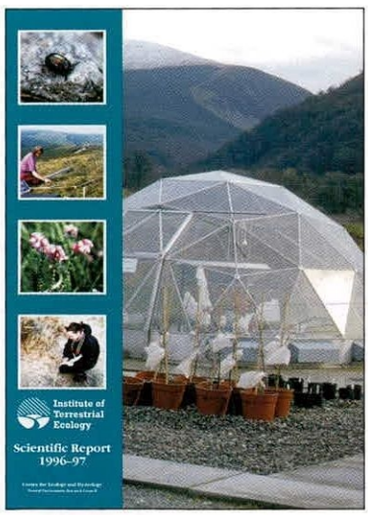

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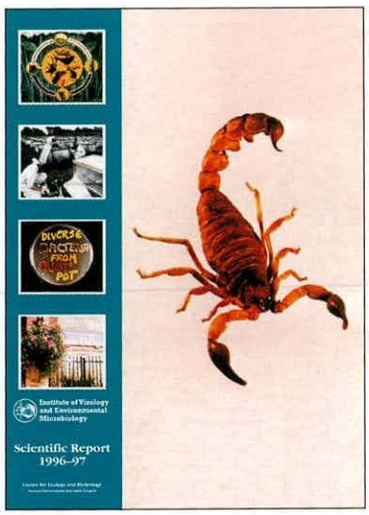


Institute of Virology and Environmental Microbiology

http://www.nox.ac.uk/

Scientific Report of the Institute of Virology and Environmental Microbiology (IVEM)

1996–1997

Centre for Ecology and Hydrology

Natural Environment Research Council

Introduction by IVEM Director

Another successful year saw IVEM move forward with two major initiatives. The first of these culminated in the establishment of a new company, Oxford Vacs Ltd, set up to exploit the intellectual property deriving from a novel family of tick saliva proteins. These histamine-capture proteins were discovered by Dr Guido Paesen, working on a project to investigate why ticks are such efficient transmitters of microbial pathogens. The Company is a 50-50 venture between Vacs of Life plc and the Natural Environment Research Council (NERC) (the legal entity representing IVEM). This exciting development will allow us to determine whether the tick proteins can be used as biopharmaceuticals for treating various diseases.

A second new initiative was the establishment of the Oxford Centre for Environmental Biotechnology (OCEB). The Centre, of which IVEM is a part, involves the University of Oxford's Departments of Engineering and Plant Sciences. The newly appointed Director of OCEB, Professor Chris Knowles, is recognised internationally for his work on bioremediation, particularly in treating cyanide and nitrile pollution. The Centre provides a conduit for applying IVEM's expertise in microbial ecology and molecular biology to tackling waste and pollution. OCEB also offers exciting opportunities for other CEH Institutes.

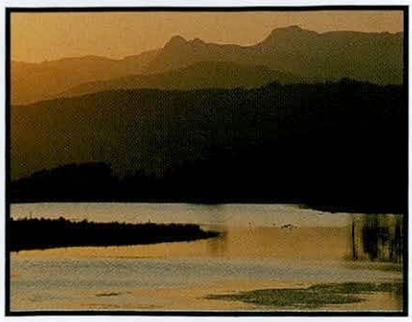
One of IVEM's many strengths continues to be in the risk assessment of genetically manipulated micro-organisms. Field trials have continued with the engineered baculovirus containing a scorpion toxin gene. The important questions of virus persistence and spread to non-target species are being addressed under carefully controlled, but natural, conditions. These are sensitive issues about which there is understandable public concern. We held an Open Meeting, inviting members of the public and press to meet scientists and view display boards. There were some lively, wide-ranging discussions. Several visitors to the meeting expressed their appreciation of the efforts to communicate our science to the public. Our thanks go to Sheila Anderson and her team at NERC Swindon for their invaluable help in staging this meeting.

As part of CEH, the Institute took on an additional Integrating Project with the Institute of Terrestrial Ecology at Monks Wood. The project, on monitoring gene activity during periods of environmental change, harnesses IVEM's expertise in molecular biology. The Institute is now involved in seven Integrating Projects with other CEH Institutes.

Scientists at IVEM have continued to win accolades. Our congratulations go to Polly Roy who was awarded a Professorship of the University of Oxford, to Steve Sait, Andy Lilley, Rosie Hails and Klaus Kurtenbach for their Fellowships and Lectureships, and to Claire Hill for her prize-winning presentation to the Oxford University Postgraduate Symposium.

This scientific report complements the Annual Report of CEH, 1996-97.

Professor P A Nuttall



CEH is undertaking ten NERC Core Strategic Programmes which provide a science base that underpins both national and international requirements in the terrestrial and freshwater sciences. The ten component programmes cover a wide range of topics. They are also dynamic and can be changed to incorporate new and emerging environmental issues.

CEH Core Strategic Programme

1: Soils and Soil–Vegetation Interactions

This programme is designed to improve our understanding and ability to model key soil processes controlling the transformations of materials within soils and the flux of water through the soil–vegetation–atmosphere continuum.

2: Land Use Science

This is aimed at promoting an integrated approach to land use science that is applicable to the wide range of user community requirements. The programme's themes will be developed to provide the basis for large-scale, long-term analytical studies of major land use change.

3: The Urban Environment

This relatively new programme aims to extend the interdisciplinary knowledge base and to understand the key environmental patterns and processes in urban situations and particularly change due to human activities. This knowledge is required to plan more sustainable urban environments.

4: Freshwater Resources

Increasing demands on freshwater resources have resulted in the need for a scientific basis for the effective strategic and sustainable management of freshwater resources. This programme will address this by integrating CEH research in the areas of water quantity, water quality, and the ecological aspects of freshwater systems.

5: Biodiversity

Aimed at improving our understanding of microbiological and biological resources at a range of spatial scales. The research considers the underlying processes and resulting functions, and directs knowledge to the sustainable management of biodiversity.

6: Pest and Disease Control and Risk Assessment for GMOs

The primary aim of this programme is to undertake research in the provision of novel pest and disease control strategies whilst addressing any possible risk to the environment. The use of molecular biology is essential to maintain a novel and progressive approach to the themes of pest control and animal disease control.

7: Pollution

This programme is aimed at developing a better understanding of generic processes such as atmospheric transport, fluxes of pollutants and the fate of pollutants, in order to predict more accurately the likely impacts on environments and organisms.

8: Environmental Risks and Extreme Events

This research programme will develop understanding of how environmental extremes affect mankind and the natural environment, developing quantitative, predictive tools to describe these effects, and contributing to mitigating measures.

9: Global Change

This programme will help to reduce uncertainty in the magnitude of global change and its impacts. The research is focused on improving the accuracy of global change predictions through measurement programmes, the development of scaling-up methods and models, and the identification of ecosystem responses.

10: Integrating Generic Science

Programme 10 has been designed to provide a research framework for those areas of CEH science which underpin the nine other programmes (eg providing the data and technological support), as well as conducting its own fundamental research.

The following section of this Scientific Report describes research which is currently being carried out in six of the ten programmes by the Institute of Virology and Environmental Microbiology. Further details of the projects and issues that make up each of the ten Core Strategic Programmes are listed in Appendix 3 of the CEH Annual Report.

in collaboration with Professor Prasad, Baylor College of Medicine, Texas. X-ray crystallography data of VP7 are currently being used to interpret the reconstructions and thereby locate the amino acids involved in the protein-antibody interaction.

Orbiviral structures

By studying the functions and interactions between proteins of dsRNA viruses in the *Orbivirus* genus, we are increasing our understanding of biodiversity in relation to the structure/function and evolution.

Bluetongue virus (BTV) which infects sheep and African horsesickness virus (AHSV) are transmitted by insect (gnat) vectors. By contrast, orbiviruses classified in the Kemerovo group, such as Broadhaven virus (BRDV), are only transmitted by ticks. The distribution of these arthropod vectors and the spread of orbiviruses are dependent upon environmental factors, e.g. temperature and humidity.

In organisation, the orbivirus particle, with seven structural proteins, is architecturally complex although the assembly of the seven proteins is highly specific. Comparison of the sequences of the core proteins demonstrates that despite the pathobiological properties and host range of these distinct orbiviruses, extreme conservation is evident within the capsid genes. Sequence analyses also suggest that the similarity levels between virus serogroups depict the structure and function of the individual capsid proteins. The data indicate that the evolution of the capsid genes of gnat-transmitted orbiviruses is strongly influenced by functional and structural constraints. This is evident when comparing the structures of the major capsid protein VP7 (Fig. 5).

We are extending our studies to the proteins responsible for the transcription and replication of orbiviruses. Recent work has shown BTV VP6 to be a dsRNA helicase (Fig. 6), the first time such activity has been associated with a particular protein of any dsRNA virus. Little sequence homology exists between BTV VP6 and other viral helicases, and the protein performs functions analogous to those of certain prokaryotic and eukaryotic helicases. In view of this it might therefore represent a new class of viral helicases not previously identified to date with a novel evolutionary history. We are using sequence data from other orbiviruses combined with structural and mutational analyses to map the active sites of this enzyme.

As predicted from sequence homology, the structure of BRDV, a tick-borne orbivirus, resembles that of the insect-transmitted BTV with the notable exception of one of the outer shell proteins. In collaboration with Dr Elizabeth Hewat and Guy Schoehn at the Institute of Structural Biology in Grenoble, France, the 3D structure of BRDV has now been resolved by cryo-EM. The cores of BRDV and BTV are identical at medium resolution; they have a diameter of 710 Å and the VP7 trimers are arranged on a T=13 icosahedral lattice. The outer shell proteins, VP5 of BRDV and BTV, have roughly the same molecular weight while VP4 of BRDV is only half the molecular weight of the corresponding VP2 of BTV. This size difference allows unambiguous determination of the identity of the triskelion shape as trimers of VP4 of BRDV (VP2 of BTV). The VP4 of BRDV sits on the VP7 trimers and projects outwards 40 Å, giving the capsid an overall diameter of 790 Å (Fig. 7a). This contrasts with VP2 of BTV, which projects outwards 95 Å to give the capsid a diameter of 900 Å (Fig. 7b). The difference in accessibility of the outer shell proteins of BRDV and BTV correlates

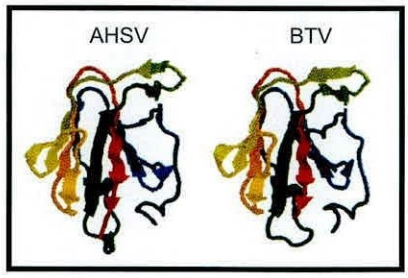


Fig. 5. The close relationships of orbiviruses is demonstrated by identical 3D crystallographic folding of major capsid protein VP7

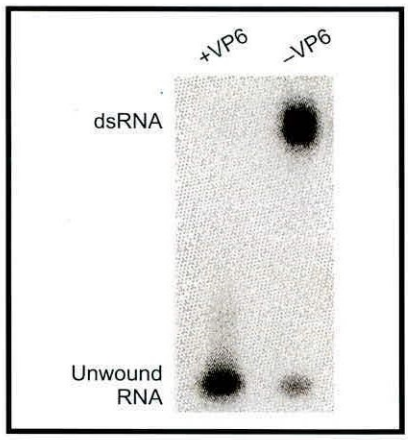


Fig. 6. BTV VP6 exhibits dsRNA helicase activity

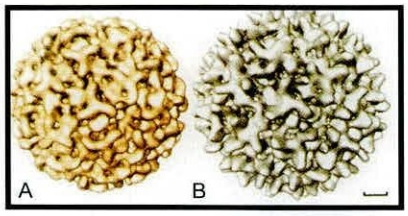


Fig. 7. Structural comparison of the two outer shell proteins of BRDV (A) and BTV (B) using cryo-EM at 39 Å resolution

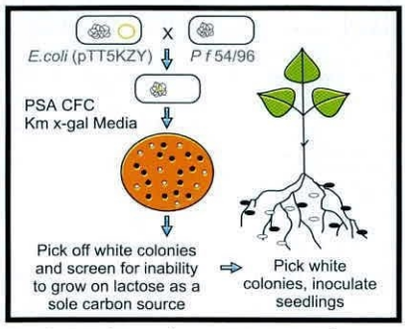


Fig. 8. In-planta characterisation of mutants

Fig. 9. Fluorescent pseudomonad mutant (A) and its genetic complement (B)

with the difference in antigenic properties of these viral proteins. Thus structural comparisons are helping us to explain functional differences, thereby unravelling the nature and significance of virus biodiversity.

Regulation of ecosystem function

Molecular promoter probe vectors constructed in disarmed transposons allow the selection of mutants that only express genes in the natural habitat. By utilising the reporter genes for lactose utilisation and β-galactosidase expression (*lacZY*) selective methods have been developed that permit the detection and localisation of individual cells in the plant rhizosphere (Fig. 8). In a further development, antibody to an introduced outer membrane expressed protein (lactose permease) has been prepared that facilitates detection by immunochemical methods and flow cytometry. By determining which bacterial genes are active (induced) in the natural environment it will be possible to evaluate their function and begin to understand the biochemical processes that occur within the natural microbial community. Furthermore, once characterised, such environmentally regulated promoters may be utilised for the differential expression of reporter genes such as *lacZY* (as above) and fluorescent (gfp) or bioluminescent markers (*lux*, *luc)* for direct *in situ* detection. The application of imaging systems is of particular value in determining the temporal and spatial distribution of specific populations and for assessing cellular activity and function.

The application of mutagenesis techniques has also identified other regulated genes. Fig. 9a illustrates the selection of a fluorescent pseudomonad isolate that produces a strong orange pigment as a result of the mutation of the genetic loci usually associated with repression of expression under laboratory conditions. When this pigment is constitutively expressed, as in the mutant F1, the bacteria are more competitive antagonists of fungi than either the wild type isolate or, as shown in Fig. 9b, the F1 mutant complemented with a chromosomal fragment carrying the repressor. Further investigations are needed to identify the pigment and its relevance to the microbial ecology and fitness of the bacteria in the environment. Already the results illustrate the relevance of molecular signalling and biological antagonists in niche exploitation.

Role of microbial biodiversity in regulating function in a freshwater ecosystem, Priest Pot, Cumbria

Work with simplified terrestrial ecosystems suggests that increased species richness increases productivity. When addressing this concept on land, there are many technical difficulties and consequential uncertainties attributable to ecosystem size, space and time factors. A more promising approach is to investigate microbial 'species' in an aquatic system, in collaboration with the Institute of Freshwater Ecology, Windermere.

As with all real ecosystems, aquatic communities are in flux. Seasonal change in microbial diversity is often obvious at the surface of lakes (as with algal blooms). At deeper levels in the water there are similarly rapid turnovers in microbial populations in step with (and often having driven) dramatic changes in amounts of dissolved oxygen, nitrogen, etc.

To investigate these variations, we are addressing productivity in a one hectare pond known as Priest Pot where the dominant biota are

microbiological. Many challenges must be addressed before the intimate interrelations of microbial abundance and nutriment cycling can be resolved. In particular, there is a lack of convenient means for recognising microbial 'species' not least because many morphological units actually comprise intimately interacting consortia from widely different taxonomic backgrounds. During the past year we investigated the occurrence of cryptic (lysogenic) viruses in bacteria isolated from lake water and sought to measure diversity among the bacteria using morphological and compositional criteria (microbial-incorporated fatty acid methyl ester profiles; FAME) (Fig. 10).

Bacterial collection

Using water samples supplied by colleagues in IFE Windermere, 450 bacterial isolates were obtained, mainly on tryptic soy broth agar, from sediment and water under the ice in January, or from water taken at 12 different depths in June. Twenty-five random colonies were subcultured from those which grew from each depth sampled. Seventeen different types of bacteria were recognised based on pigmentation and colony morphology. Numbers and diversity of isolates cultured from a standard amount of sample varied with time of year and among samples taken on the same day but at different depths.

Some colony types were more common in or indeed were isolated only from a single sample. Thus, purple pigmented colonies grew only from the sediment sample.

Incidence of lysogeny

The presence of cryptic viruses in bacterial cultures was inferred after elicitation with mitomycin C. Virus-like particles were recognised by electron microscopy in 7 of 36 cultures which had failed to grow after mitomycin C treatment (Fig. 11).

Bacteria differentiation/ identification by MIDI-FAME

Thirty-six isolates have been processed through FAME and 11 of these were assigned to genus and species by reference to the database of characteristics within the current FAME system (Table 1). An additional eight isolates were identified at genus level and with a lesser degree of certainty at species level (index numbers <0.5). The other cultures were not identified using this method.

Algae and cyanobacteria differentiation by MIDI-FAME

Axenic cultures of *Oscillatoria amoena* CCAP 1459/39, *Euglena gracilis* CCAP 1224/5Z, *Asterionella formasa* CCAP 1005/5 and *Scenedesmus subspicatus* CCAP 276/20, *Nephroclamys subsolitoria* CCAP 252/1, *Chlorella vulgaris* CCAP 211/12, and *Chlorella* sp (ex *Paramesium bursaria* Pbi), *Ankistrodesmus* (ex Priest Pot) could be distinguished (Fig. 12).

Fig. 10. Dr Mary Lou Edwards, investigating the occurrence of cryptic (lysogenic) viruses in bacteria isolated from lake water

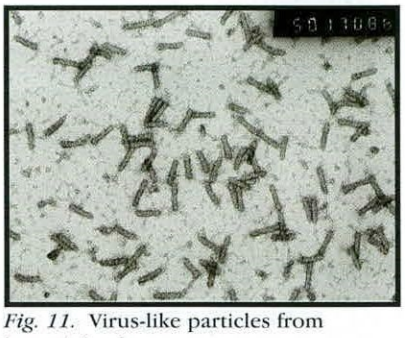


Fig. 11. Virus-like particles from bacterial culture

Fig. 12. Five different bacteria isolated for Priest Pot

Table 1. Representative FAME identifications of Priest Pot bacteria to show: (i) range of genera/species (S.I. <0.5), (ii) repeat isolation of 'same' species

Genus	Species	Similarity Index	Isolate
Bacillus	*mycoides*	0.688	18
Bacillus	*mycoides*	0.722	19
Bacillus	*mycoides*	0.776	22
Bacillus	*mycoides*	0.788	63
Bacillus	*subtilis*	0.652	51
Bacillus	*subtilis*	0.550	62
Pseudomonas	*chlororaphis*	0.681	4
Pseudomonas	*chlororaphis*	0.815	34
Pseudomonas	*coronafaciens*	0.820	14
Pseudomonas	*syringae*	0.765	16
Serratia	*proteamaculans*	0.590	9

Pest and disease control and risk assessment for GMOs

CEH Core Strategic Programme 6

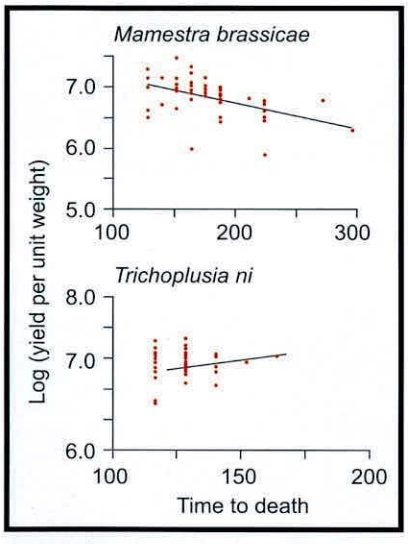


Fig 13. The relationship between yield per unit weight (of baculovirus) and speed of kill for a semi-permissive (*Mamestra brassicae*) and permissive (*Trichoplusia ni*) host

Ecology and biocontrol of baculoviruses

Baculoviruses occur commonly in a wide range of insect species and appear to play a role in the regulation of insect populations. One broad objective for baculovirus research concerns how the insect-baculovirus interaction may be manipulated to enhance control of insect pests. This manipulation may be very direct (e.g. by genetic modification to increase the speed of kill) or more indirect (e.g. exploiting existing variation of natural baculoviruses, or manipulating the habitat to enhance their persistence). A greater understanding of the behaviour of insect baculoviruses in natural populations is required to develop such control strategies. The emphasis in the last year has been to develop molecular tools in the laboratory which will allow us to study the dynamics of baculoviruses in more detail, and also to study their dynamics in semi-permissive hosts: that is, those hosts which are not highly susceptible to the virus.

Conventional wisdom predicts that when genes are either added or deleted from the genome of a baculovirus, the fitness of that virus would be adversely affected. However, the nature of this fitness cost is unknown. The dynamics of wildtype and engineered baculoviruses are being investigated at the level of the individual insect, by the development of diagnostic tools which are both sensitive and reproducible. This laboratory-based study will provide baseline data on the behaviour of genetically modified baculoviruses (GMBVs) in mixed infections of wildtype and engineered viruses at the individual level. These data and developed diagnostic tools will then allow the design of experiments to investigate the fate of mixtures at the population level.

Not only are more data required on novel (modified) baculoviruses, but also on natural baculoviruses in novel (semi-permissive) hosts: i.e. those hosts which require a much greater dose to sustain, and die of, an infection. Most baculoviruses are highly pathogenic for only a small number of hosts, but several species may fall into the semi-permissive category, and it is the behaviour of baculoviruses in these hosts which

will determine their potential to invade and perturb natural communities.

Detailed laboratory studies have shown that the relationship between yield per unit weight, and speed with which the host was killed, is fundamentally different in nature for a model permissive and semi-permissive species (Fig. 13). For permissive hosts which take longer to die, the yield of baculovirus appears to be increasing faster than the host body weight - however, this trend is reversed in semi-permissive hosts. These relationships are intriguing. If this proves to be a general pattern, it could provide a more rigorous and quantitative means by which hosts may be categorised as permissive or semi-permissive.

Previous work on the behaviour of GMBVs has illustrated that those insects dying of the modified virus are paralysed before death and are therefore likely to fall off the plant. This interrupts the normal behaviour pattern of an infected insect, which frequently climbs to the tip of foliage in the latter stages of infection. Such behaviour places the cadaver in a fortuitous place for the liberation of infectious virus particles on to foliage beneath. Susceptible insects feeding on this foliage will then ingest the virus particles and consequently become infected themselves (Fig. 14). Thus, modified viruses are more likely to be dispersed to the soil, whereas wildtype viruses are likely to be distributed on the leaves. Consequently, soil-dwellers such as *Agrotis segetum* (Fig. 15) may be more likely to sustain a second round of infection if controlled by modified viruses producing toxins which cause paralysis, whilst foliar feeders (e.g. *Mamestra brassicae*) may be more effectively controlled by wildtype viruses. A field trial containing the factorial combinations of foliar feeder/soil-dweller and modified/wildtype viruses was conducted to investigate this issue. The results showed that soil-dwellers do sustain higher infection rates with modified viruses whereas foliar feeders sustain higher infection rates with wildtype viruses (Fig. 16). This trial was conducted with two semi-permissive hosts. The issues raised here are not just those of control but also of risk assessment. Features which allow a baculovirus to persist for two or more rounds of infection will make it suitable as a control agent with longer-term action. Such features also pose questions concerning persistence and invasion of non-target Lepidopteran populations.

Host range of genetically modified baculoviruses

Baculovirus insecticides cause disease in a limited number of insect species, giving them a major advantage over chemical agents, which are indiscriminate in their mode of action. The host range of baculovirus insecticides appears to be regulated by specific virus genes, as well as the innate susceptibility of a given insect species to virus infection. The study of a number of different baculoviruses has identified at least two major types of host range genes, both with roles in preventing apoptosis, or programmed cell death, in virus-infected insect cells. Some baculoviruses, such as *Autographa californica* nuclear polyhedrosis virus (AcNPV), contain the p35 gene which permits replication in a number of different insect cell lines in culture. In other insect cells, viruses lacking the p35 gene induce apoptosis and only produce low levels of infectious virus progeny. Other baculoviruses lack the p35 gene, but prevent apoptosis by expressing the unrelated iap

Fig. 14. Damage by foliar feeder

Fig. 15. Agrotis segetum

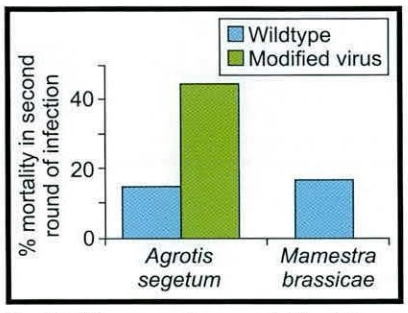


Fig. 16. The percentage mortality at the second sample for a soil-dweller (*Agrotis segetum*) and foliar feeder (*Mamestra brassicae*) from a wildtype baculovirus and a modified form of that virus producing a toxin that causes paralysis before death

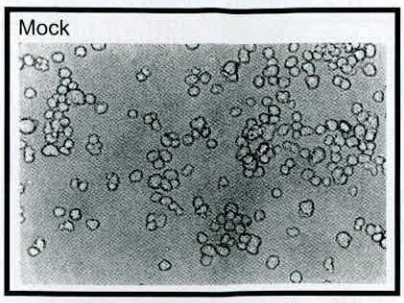


Fig. 17a. Spodoptera frugiperda cells prior to virus infection

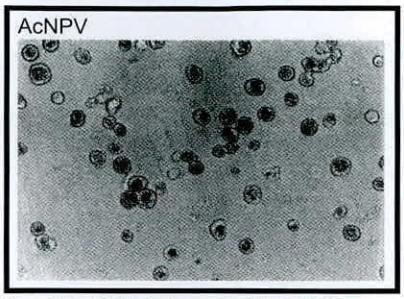


Fig. 17b. Cells infected with AcNPV displaying the characteristic occlusion bodies or polyhedra late in infection

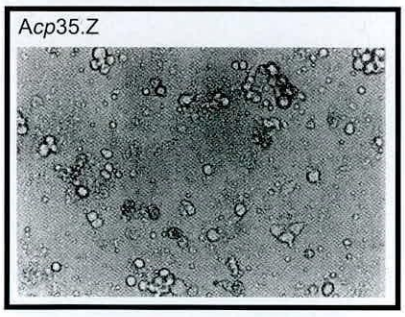


Fig. 17c. Acp35.Z-infected cells, where the anti-apoptotic gene (p35) is inactive, showing the characteristic symptoms of programmed cell death (production of many small vesicles by blebbing from the plasma membrane)

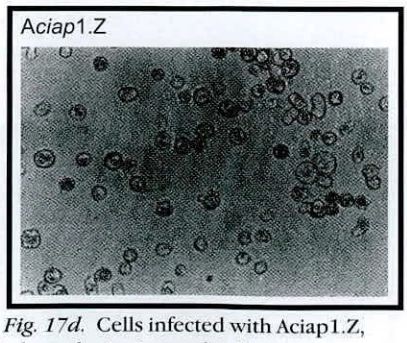


Fig. 17d. Cells infected with Aciap1.Z, where the iap1 gene has been interrupted, demonstrate normal occlusion body production

(inhibitor of apoptosis) gene. Our understanding of the roles of p35 and iap genes is complicated by the fact that AcNPV also contains genes (iap1 and iap2) which are very similar to the iaps found in other baculoviruses. These genes, however, do not inhibit apoptosis in *Spodoptera frugiperda* cells infected with AcNPV recombinant viruses lacking the p35 gene (Fig. 17). We proposed that the AcNPV iap1 and iap2 genes might be important for the replication of this virus in other insect species. The hypothesis was tested by constructing a number of recombinant viruses lacking iap1, iap2, or both virus genes. These viruses were then used to challenge a range of insect cell lines maintained in culture to determine the outcome of virus infection. The removal of the AcNPV p35 gene did not affect virus replication in *Trichoplusia ni*, *Panolis flammea* or *Mamestra brassicae* cells. In *Lymantria dispar* cells, apoptosis was induced even though AcNPV is not thought to replicate in this species. The AcNPV recombinants lacking either the iap1 or iap2 genes did not display altered phenotypes in comparison with the wildtype virus. Even a virus lacking both the p35 and iap1 genes replicated in a similar manner to the p35 gene deletion mutant. These results were obtained using virus-infected cell cultures. Although we have not identified a role(s) for the AcNPV iap genes in cell culture, the whole animal contains a variety of different cell types. Virus genes may have a tissue-specific function which can only be discerned when monitoring virus replication in the insect larva.

Predicting the ecological impacts of pest- and disease-resistant genetically modified crops

Genetic modification (GM) for pest or pathogen tolerance offers new methods of increasing crop yields and reducing chemical inputs. Fibre crops such as cotton genetically modified to resist insect herbivores are widely planted for commercial use in the USA or in Australia. More recently, potato leaf roll luteovirus-tolerant potatoes and potyvirus-tolerant squash (a marrow-like vegetable) have entered the marketplace and a diverse range of virus-tolerant GM crops are presently under test as 'authorised' releases into the environment.

There is concern, however, that genetically modified (GM) crops with pest or disease resistance may also have undesirable impacts on the environment. If pathogens or insects control the growth rate of wild plants that can hybridise with crops, the release of GM resistant crop varieties could enhance the fitness of progeny plants - perhaps enhancing their nuisance status. To provide information for risk assessments prior to commercial use of GM pest and disease crops in the UK, CEH has several projects which aim to increase our understanding of the role of insects and diseases in the dynamics of feral crops and wild crop relatives.

Insect and virus resistance in wild cabbage

To assess the potential consequences of the spread of GM insect resistance, staff at IVEM and ITE Furzebrook are studying insect damage to wild cabbage on the Dorset coast (Fig. 18). A large proportion of wild cabbage plants were found to contain one or more viruses; four different viruses were prevalent - turnip mosaic potyvirus (TuMV), cauliflower mosaic caulimovirus, turnip yellow mosaic tymovirus (TYMV) and beet western yellows luteovirus. To assess their impact we raised wild cabbage seedlings in a glasshouse, and

manually inoculated them with either TuMV, TYMV or sterile water (Fig. 19). Two months after inoculation, the cabbages were planted in the field within metres of the sites of maternal origin and adjoining a large natural population of wild cabbage. Plants infected with TYMV died at a higher rate than the water-inoculated controls, whereas plants with TuMV seemed to survive better than the controls. Furthermore, the plants infected with TYMV were smaller, had fewer leaves and flowered less frequently.

Understanding and controlling tick-transmitted diseases

The distinct clinical manifestations of the important human disease, Lyme borreliosis, appear to be caused by different genospecies of *Borrelia burgdorferi* spirochaetes. In North America, arthritis is common where *B. burgdorferi sensu stricto* is prevalent whereas, in Euroasia, neuroborreliosis and acrodermatitis, associated respectively with *B. garinii* and *B. afzelii*, are typical manifestations. A newly recognised European genospecies, *B. valaisiana*, is apparently non-pathogenic for humans. Thus an important question in the epidemiology of Lyme disease is what are the ecological factors that determine the geographical distribution and prevalence of different Lyme disease spirochaetes.

In a collaborative project with the Department of Zoology, University of Oxford, and the Game Conservancy, the genetic diversity of Lyme disease spirochaetes was assessed at a field site in Dorset where pheasants (*Phasianus colchicus*) predominate (Fig. 20). A new sensitive polymerase chain reaction (PCR)-based genotyping method was used to assess the genetic diversity of *B. burgdorferi* in rodents, pheasants, and ticks (*Ixodes ricinus*) that transmit the spirochaete (Fig. 21).

In questing ticks, three genospecies of *B. burgdorferi* were detected, with the highest prevalence found for *B. garinii and B. valaisiana*. *B. burgdorferi s.s* was rare (<1%) in all tick stages. *B. afzelii* was not detected in any of the samples. Of engorged nymphs collected from pheasants, >50% were infected with *B. garinii* and/or *B. valaisiana*. In contrast, rodents carried infections of *B. burgdorferi s.s* and/or *B. garinii* in internal organs, but only *B. burgdorferi s.s* was transmitted from infected rodents to uninfected ticks feeding on them. Thus in a field site where three different genotypes of *B. burgdorferi* are circulating, two genotypes were transmitted by pheasants to ticks whereas the third genotype was transmitted from rodents to ticks. The data indicate that different genospecies of *B. burgdorferi* can be maintained in nature by distinct transmission cycles involving the same vector tick species but different vertebrate host species. Wildlife management, by manipulating vertebrate populations, may influence the distribution and prevalence of different *B. burgdorferi* genotypes thereby affecting the relative risk of different clinical forms of Lyme borreliosis.

Persistence of louping ill virus in the environment

The encephalitic disease caused by louping ill (LI) virus is seriously threatening the red grouse (*Lagopus scoticus;* Fig. 22) population on the Scottish, Welsh and English moorlands. Molecular biological studies of louping ill epidemiology on the Lochindorb Estate in Scotland have now revealed that the blue mountain hare (*Lepus timidus*) contributes significantly to the persistence of this virus disease by acting as a host for infected ticks

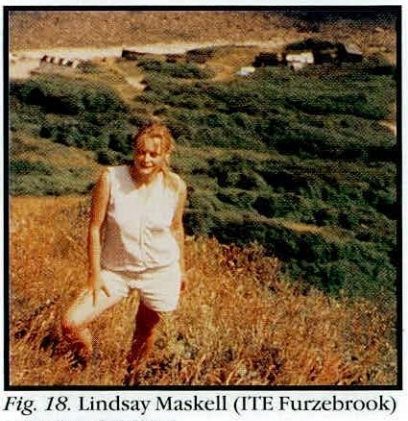


Fig. 18. Lindsay Maskell (ITE Furzebrook) at Dorset field site

Fig. 19. Dr Mary-Lou Edwards, assessing wild cabbage seedlings infected with virus

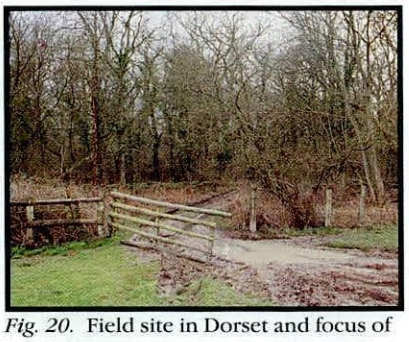


Fig. 20. Field site in Dorset and focus of Lyme disease

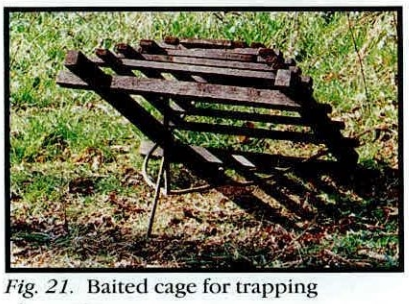


Fig. 21. Baited cage for trapping pheasants

Fig. 22. Lagopus scoticus

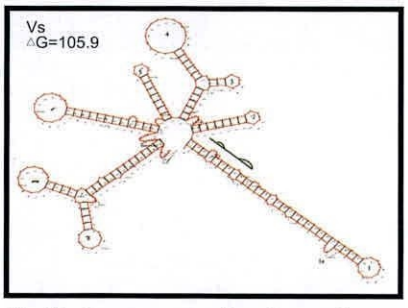


Fig. 23. Computer-predicted secondary structure for the conserved part of the 3' UTRs of the TBE complex viruses. The position of conserved sequence presenting as a complementary inverted repeat within the 5'UTR, is depicted by the solid line

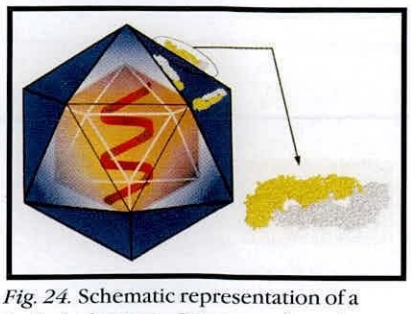


Fig. 24. Schematic representation of a typical arbovirus. Structure of envelope protein shown by arrow

which transmit the virus to uninfected ticks as they co-feed on the hare. Louping ill virus has been studied in detail at IVEM and we have developed an infectious clone of a related tick-borne virus that we call Vasilchenko (Vs) virus. We have now undertaken a molecular study of this virus in an attempt to understand the factors that determine the virulence of these viruses.

The RNA genome of LI and related viruses consists of a 10.5kb sequence which encodes three structural and seven non-structural proteins that are reproduced during the viral replication cycle. In addition, each terminal region of the RNA molecule contains an untranslated region (UTR) of approximately 0.5 to 1kb which does not encode proteins. Recent research indicates that the secondary structure of these UTRs is important in determining the rates of nucleic acid replication and protein translation through the formation of replication complexes within virus-infected cells. Comparative alignments and computer analysis of the UTR sequences of related viruses enables predictions of their secondary structure. Our analyses have shown that the UTRs consist of stem and loop structures (Fig. 23); detailed analysis shows that in general the loop structures are conserved whereas the stem structures may be variable in length. Thus the overall secondary structures of viruses with significantly different sequences remain very similar.

Fine-detail analysis has revealed critical sites where mutation may have profound effects on virus virulence and we believe this can form the basis of a project to custom design tick-borne viruses that do not produce disease in vertebrates. These genetically engineered viruses (Fig. 24) with modified replication and tropicity characteristics will serve as useful biological tools for studies of virus transmissibility in ticks and persistence in the environment.