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Pollution

CEH Core Strategic programme 7

Fig.18. Virgin and used metal cutting fluid

Fig. 19. Bioreactor

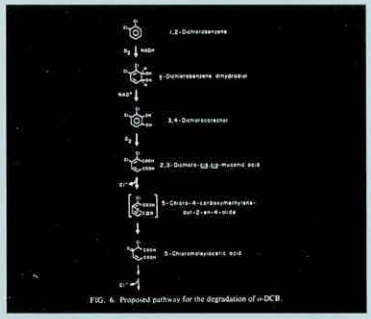


Fig. 20. Proposed pathway for the degradation of 1,2 - DCB

Bacterial degradation of metal cutting fluids

Engineering workshops produce large quantities of metal cutting fluids (MCFs) that are, environmentally hazardous.

In collaboration with IBS Viridian and the University of Kent we are developing bacterial inocula that can detoxify used MCF's (Fig. 18). This has been achieved by improving our understanding of the microbial ecology of MCF degradation, specifically the interaction of the chemical components of the fluids and the bacteria involved in the their degradation.

Using small bioreactors a consortium, composed of six coryneform (mostly *Arthrobacter*), is currently being tested (Fig. 19) for its ability to degrade spent MCF's.

Impact and degradation of dichlorobenzene in soil

Large quantities of organic pollutants are chemically stable and can only be effectively degraded in the environment by micro-organisms. To exploit the metabolic potential of microbes, more information about the impact of pollutants on their communities, and the events that lead to increased mineralisation of contaminants, is required. In an integrated project with ITE (Monks Wood), the microbial communities of soils exposed to dichlorobenzene (DCB) are being investigated to determine why the rate of DCB degradation in soils containing root material is significantly greater than that detected in bulk soils (Fig. 20).

Using phenotypic and genotypic methods (FAME, BIOLOG and RFLP), no significant difference in the taxa composition was observed between soil conditions. Enhanced rates of DCB degradation were due to the presence of root material stimulating the activity of the degraders, and not to the selection of distinct sub-populations.

In further studies, the impact of DCB introduction on the soil microbial community is now being examined.

Global change

CEH Core Strategic programme 9

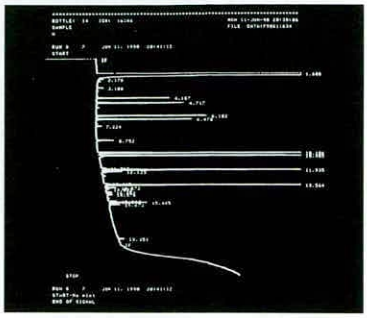


Fig. 21. FAME profile of a methyl oxidiser

Enormous diversity exists in microbial methane oxidisers of soil.

Microbial basis of methane oxidation in soil

Some soils contain large and active microbial communities that oxidise the greenhouse gas, methane. This activity is of great importance since it is estimated that methane may be responsible for 15% of global warming. The type of soil and, in particular, land use has a highly significant bearing on the rate and activity of methane oxidation. Some soils are indeed net producers of methane, thus contributing to climate change.

In collaboration with ITE Merlewood and IFE Windermere, we are currently investigating soil factors that influence the activity and diversity of the bacterial methane oxidisers which are being identified by fatty acid methyl ester (FAME) analysis (Fig. 21). In addition the phenotypic characteristics of methane oxidisers grown in culture are being investigated by gas chromatography. This has revealed that methane oxidisers are a very heterogeneous group which share few phenotypic characteristics. For instance, of the methane oxidisers investigated to date, only one fatty acid of the thirteen detected is common to all taxa. This enormous heterogeneity within the group is problematic. Nevertheless, using complementary molecular techniques to assess diversity *in situ,* we aim to assess the soil factors that determine the nature of methane oxidisers, their diversity and their rate of activity.

$$CH_4 \rightarrow CH_3 \rightarrow HCHO \rightarrow HCOOH \rightarrow CO_2$$

Fig. 22 Methane oxidisers achieve the recycling of carbon dioxide in the environment

Integrating generic science - biotechnology

CEH Core Strategic programme 10

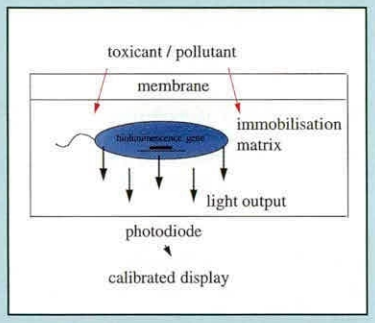


Fig. 23. Diagrammatic representation of real time biosensor design.

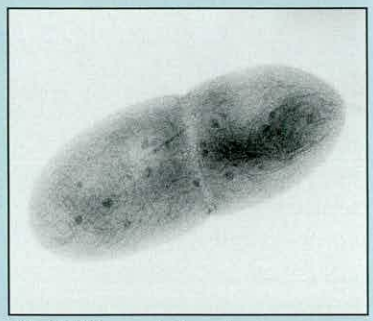


Fig. 24 Transmission electron micrograph of a fluorescent pseudomonad isolated from the phytosphere.

Tracey Timms-Wilson, Student

Development of biosensors of pollution and toxicity.

Studies over the past 12 months have improved our understanding of microbial ecology in relation to colonisation, survival, adaptation, succession and gene transfer between bacterial populations in the natural environment, at the cellular level in relation to adaptation (phenotypic and genotypic variation) and molecular perception of stress caused by local change or pollution. The genetic basis of such responses by individuals, populations and communities can be exploited. One particular example of this is in the development of biosensors (Fig. 23).

Biosensors have enormous commercial potential in environmental science as indicators of pollution, in process control and a myriad of other applications. Current DTI LINK funding awarded to IVEM/Napier University/ Edinburgh Instruments Ltd/British Steel /East of Scotland Water has allowed the development of a project named BIOMATE [**Bio**sensors for **M**ulti-determinand **A**ssessment of **T**oxic **E**nvironments].

The aim of BIOMATE is to develop, through two stages, highly sensitive *in situ* sensing devices that allow time-resolved detection of bacterial bioluminescence. By combining research into state-of-the-art electronics and synthesising polymer immobilising materials on-line, real-time detectors for pollution events can be developed. Toxic inputs to waste water treatment impact on public health and cause serious economic losses. BIOMATE will provide rapid measures of toxicity and allow immediate intervention to prevent contamination from the products of industrial processes, and provide suitable portable devices for use in the open environment. IVEM will contribute expertise in the isolation of novel bacterial isolates collected from the environment (Fig. 24) and their genetic modification for the regulated expression of bioluminescence genes. These reporter genes will be based on genes isolated from light emitting bacteria and fire flies.

Recombinant technologies for virus protein analysis

Hepatitis C virus (HCV) is one of three newly identified viral causes of hepatitis and up to 1% of the worlds population may be infected by it. HCV is therefore an important target for effective chemotherapy or vaccine strategies aimed at preventing infection.

However, there is, currently, no efficient tissue culture system for HCV making the study of the virus and the isolation of viral components difficult. Recombinant baculoviruses offer an attractive alternative for the expression of HCV gene products and an analysis of their inherent function. An example of this technology in use is the expression of the HCV E proteins (E1 ~30KDa; E2 ~ 70kda) which, during synthesis in the insect cell, become associated as an E1/E2 heterodimer mimicking the form present on the virion surface (Fig. 25).

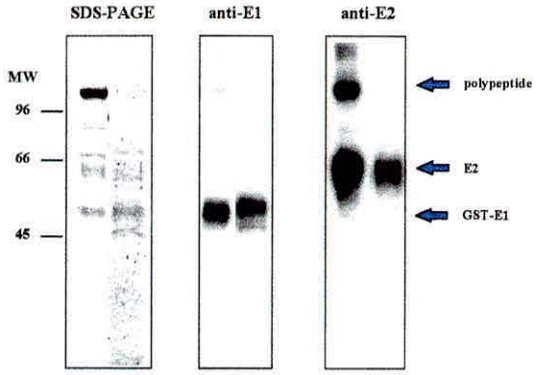


Fig. 25. Expression of tagged HCV E proteins showing complex formation after affinity purification. The panels show stained gel (left), E1 blot (centre) and E2 blot right). In each panel the leftmost track is a GST-E1-E2 and the rightmost a GST-E1 + E2 co-infection. In both, purification of GST-E1 domain leads to co-purification of E2.

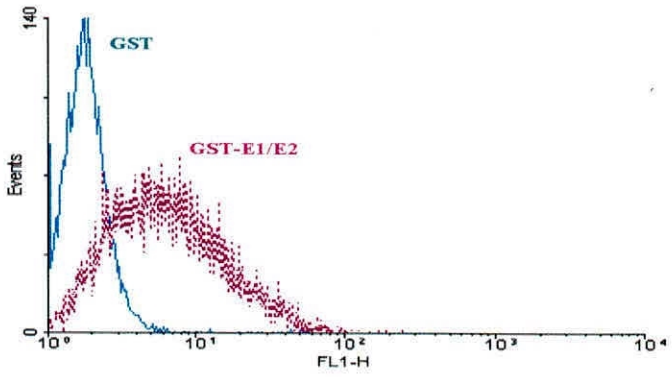


Fig. 26. Cell surface binding by purified HCV GST-E protein compared to GST only. Increased fluorescence was only observed following incubation with the E complex.

Previously, IVEM scientists developed a specific expression vector (pAcSG2T) for the use of an affinity tag (glutathine-S-transferase, GST) in the production of complex glycoproteins from insect cells using recombinant baculoviruses. A series of constructs have been prepared expressing E1, E2 or E1-E2 as fusion proteins with GST to allow easy purification of the E protein complex. The purified HCV E proteins prepared as above have proven their bioactivity in binding to the cell surface as a mimic of virion binding, the first step in the infection process. E complex but not purified GST used as a control bound to cells in a flow cytometry assay using specific antibody for E2 or GST (Fig. 26). These reagents and assays will provide for an interesting and purposeful study of the role of the HCV envelope proteins in cell binding and should contribute to the development of effective therapies.

PRIVEM

Consultancies

Steve Howard

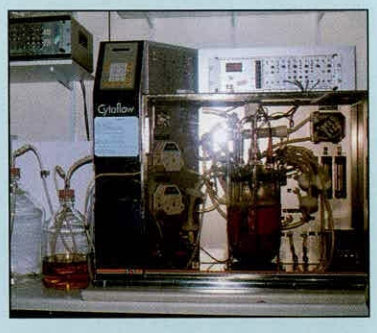


Fig.27. Production of recombinant virus protein using a continuous culture process

The development of the polymerase chain reaction (PCR) at the end of the last decade had enormous impact on molecular biology. Suddenly it was possible to reproduce, in the laboratory, significant amounts of nucleic acid from extremely small quantities of starting material. Overnight, the impossible became possible! PCR has now been applied to wide varieties of disciplines in biological sciences.

One of many ways to exploit this innovative technology is through environmental, medical or veterinary diagnostics. Many small companies have been formed over the past few years to produce diagnostics based on biotechnology. In essence, the PCR is used to amplify the nucleic acid from a microorganism i.e., a protozoon, bacterium or virus. The amplified nucleic acid is then introduced into a model bacterium or virus, such as *E. coli* or a baculovirus respectively, which has been modified to accommodate the introduced material so that it can be reproduced and the protein(s) that it encodes can be produced.

This biotechnological method leads to the production of large quantities of protein which can be custom designed according to the scientific requirements. As an example, herpes type 2 (HSV2) virus produces genital infections that are transmitted very efficiently between humans and there is currently no diagnostic kit available that utilises biotechnology to screen serum samples from potentially infected patients.

We have used PCR and recombinant baculovirus technology to develop a molecular probe that can be used in a diagnostic kit to identify HSV2 virus infections. A series of similar innovations has been applied to develop diagnostic probes for different infectious agents. Realising the potential of this technology, we are now developing the capacity to produce relatively large (Fig. 27) quantities of these custom-designed proteins and we have identified several small companies that can take these products to market. Under the name "PRIVEM" we are supplying a specialist market with custom-designed molecular biological products. Within one year of its inception PRIVEM had licensed two products and is currently developing a wider range of reagents for the user community. Profits generated by PRIVEM are ploughed back into IVEM's Core Strategic science.

Centre for Ecology & Hydrology

APPENDIX 1

IVEM is a component Institute of the NERC Centre for Ecology and Hydrology.

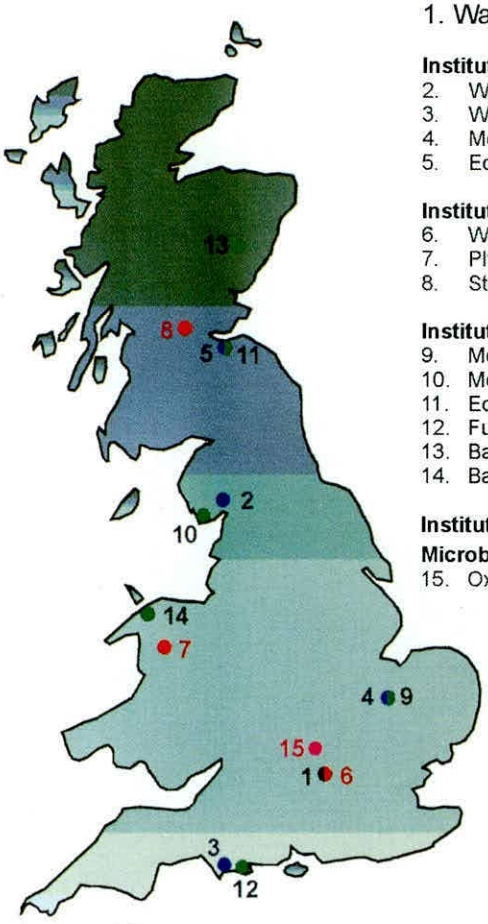
Centre for Ecology and Hydrology
1. Wallingford

Institute of Freshwater Ecology
2. Windermere
3. Wareham
4. Monks Wood
5. Edinburgh

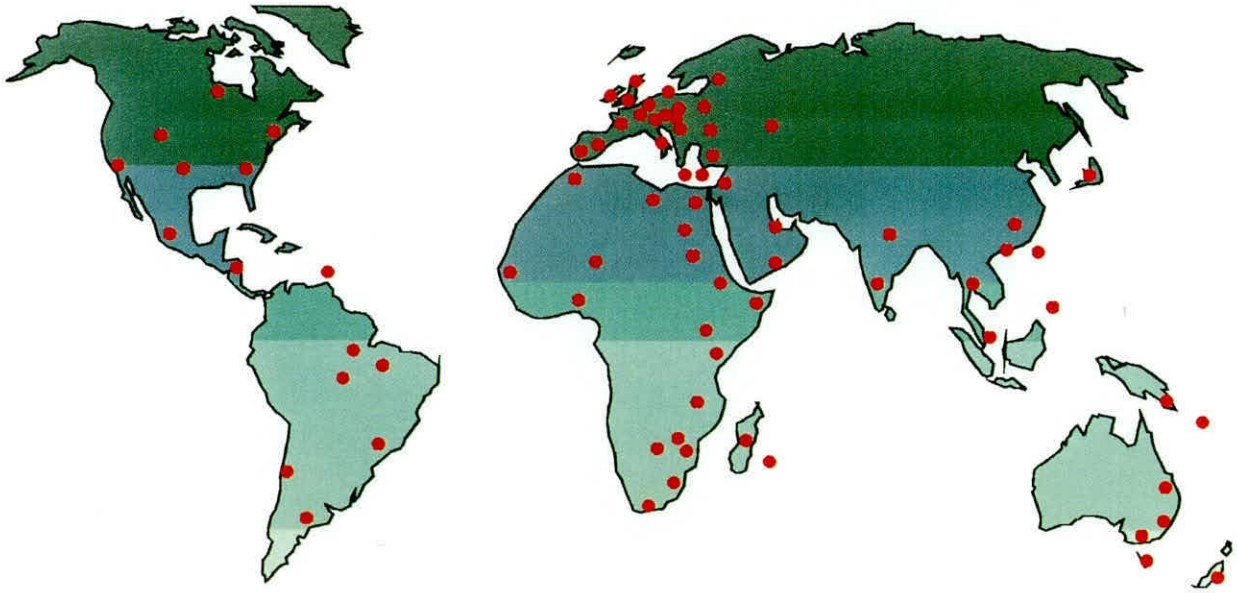
Institute of Hydrology
6. Wallingford
7. Plynlimon
8. Stirling

Institute of Terrestrial Ecology
9. Monks Wood
10. Merlewood
11. Edinburgh
12. Furzebrook
13. Banchory
14. Bangor

Institute of Virology and Environmental Microbiology
15. Oxford



Location of CEH Sites



Location of recent research contracts undertaken by CEH Institutes

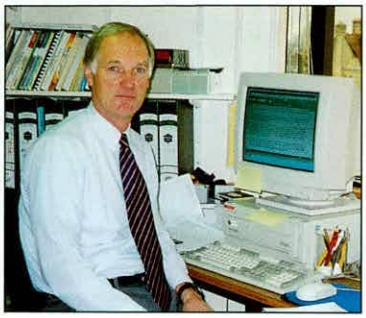
APPENDIX 2

CEH Integrating fund projects

Projects commencing 1995-96	IFE	IH	ITE	IVEM
The microbial basis of methane oxidation in soils	•		•	•
Interactions of viruses, aphids and wild *Brassica*			•	•
Modelling the chemical availability of radionuclides in upland organic soils	•		•	
Combined growth and water use modelling of mixed vegetation		•	•	
Upland forest canopy closure - its significance for chemistry, ecology and hydrology	•	•	•	
Molecular genetics and process level events in the biodegradation of xenobiotics in rhizosphere soils			•	•
Microbial diversity and ecosystem function - Phase I	•	•	•	
Projects commencing 1996-97				
The role of seabirds in the epizootiology of Lyme disease			•	•
Combined hydro-ecological and socio-economic models of land management and environmental degradation (CHASM)		•	•	
The environmental characteristics of urban environments	•	•	•	
The role of microbial diversity in regulating ecosystem function - Phase II	•		•	•
50 m solar grids for the UK		•	•	
Modelling the fate of viruses in the aquatic environment		•		•

APPENDIX 3

IVEM Organisation



Dr E.A. Gould
Assistant Director

Dr M.J. Bailey
Group Leader

Andy Reeson
Student

Staff (December 1995 - December 1996)

Director
Patricia A Nuttall MA PhD

Assistant Director
Ernest A Gould, PhD

Molecular Microbial Ecology
Mark J Bailey, *Group Leader*
Microbial Diversity
Ian Thompson, *Project Leader*
Kirsten Lawlor
molecular signalling
Andrew Lilley
gene mobilisation and plasmid transfer
Tracey Timms-Wilson
biocontrol agents
Han Zhang
environmental plasmids
Siân Evans
plasmids and survival factors

Virus Ultrastructures
Tim F Booth, *Project Leader*
Emma Nason
structural analysis
Claire Hill
structural analysis

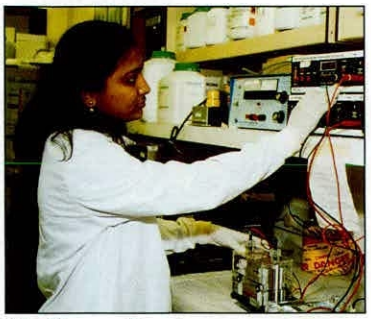
Plant Virology
Ian J Cooper, *Project Leader*
Mary-Lou Edwards
plant viruses
Delia McCall
plant propagation
Shi Jiao

Ecology and Biocontrol
Jenny S Cory, *Project Leader*
Rosie Hails
ecology and risk assessment
Bernadette Green
risk assessment
Steven Sait
ecology and risk assessment
Pedro Hernandez-Crespo
risk assessment
Robin Paul
virus biodiversity
Simao Vasconcelos
insect pathogen transmission
Kate Wilson
baculovirus control agents
Enda Clarke
virus-host interactions
Andy Reeson

Flaviviruses, Water-borne Viruses, Biotechnology
Ernest A Gould,
Project Leader
Steve Moss
biotechnology
Linda Jones
cell mediated immunity
Tamara Gritsun
tick-borne encephalitis virus
Sarah Butcher
water-borne viruses
Hui Wang
hepatitis c virus
Steve Howard
biotechnology
Kirsty McGuire
louping ill virus
Michael Gaunt
louping ill virus
Amadou Sall
African wildlife viruses

Virus Protein Functions
Ian M Jones, *Project Leader*
Uma Bhattacharyya
protein-protein interactions
Wenrong Jiang
hepatitis c expression
Rustem Krykbaev
CD4 mutagenesis
Carl Doyle
HIV expression
Wei Hong Zhang
virus assembly
Claire Perrin
glycoprotein mutagenesis

Mr S. Sangamnadech
Student



Ms Rama Devi
Student

Carole Thomas
Student

Louise Critchley
hepatitis c virus expression

Tick-borne Pathogens
Patricia A Nuttall,
Project Leader
Guido Paesen
tick biotechnology
Dorothy Carey
Lyme disease
Hans Dessens
tick-borne orthomyxoviruses
Klaus Kurtenbach
Lyme disease
Michael Leahy
tick-borne orthomyxoviruses
Miles Nunn
tick-borne orbiviruses
Nick Ogden
Lyme disease
Somchai Sangamnadech
tick biotechnology
Charles Lawrie
Lyme disease
David Strange
Lyme disease

Baculovirus Molecular Biology
Robert D Possee
Project Leader
Caroline Griffiths
minireplicon vectors
Claire Merrington
RNA polymerases
Melanie Bridges
baculovirus gene function
Carole Thomas
baculovirus pathogenesis
Anna Barnett
baculovirus host range
Susan Chapple
programmed cell death
Baresh Chauhan
yeast vectors
David Phillips
baculovirus gene promoters

Orbiviruses
Polly Roy, *Project Leader*
Catie Williams
orbivirus research
David Wright
orbivirus research
Nigel Horscroft
orbivirus research
Katia Monastyrskaya
orbivirus research
Geoff Sutton
orbivirus research
Paul Reay
orbivirus research
Norbert Staueber
orbivirus research
C K Yi
orbivirus research
Michel Mikhailov
orbivirus research
Piao Wang
orbivirus research
Adele Peek
orbivirus research
Javier Rodriquez
orbivirus research
Nobu Tetsu
orbivirus research
Sharifah Hassan
orbivirus research
Andrew Beaton
orbivirus research
N Rama Devi
orbivirus research
Naresh K. Kakker
orbivirus research

Administrative, Support Staff and Engineers
Dick Bamford
Stores Manager
Rex Bateman
Support services
Gavin Bird
Head of Administration
Carol Broadbent
Biomedical services
Ray Broadbent
Engineer
Marcelle Burden
Media prep
Tim Carty
Insectary Manager
Colin Cox
Computing consultant
Chris Hatton
Photographer
Pauline Henbest
Biomedical services
Lisa Heredge
Director's Secretary
Jennifer Jeacock
Finance Officer
Bridget Lewis
Computing consultant
R MacKenzie
Engineer
Sheila Morton

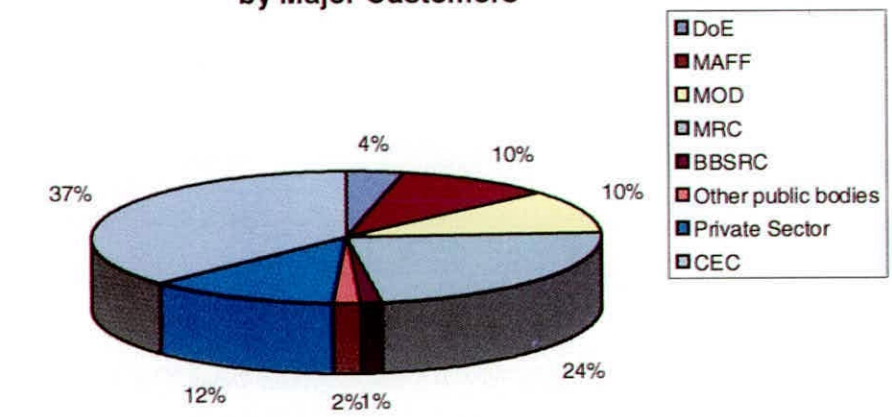
Mr T Primarolo
Mr R MacKenzie

Support services
Pat Newton
Receptionist
Gill Pinniger
Support services
Richard Pinniger
Support services
Stephanie Price
Secretary
Tony Primarolo
Engineer
Peter Selwood
Administration Officer
Joanna Sloley
Marketing and Research
Ann Sloper
Receptionist
Chris Wilson
Librarian

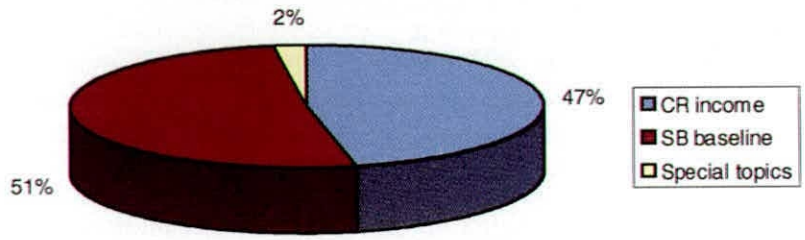
APPENDIX 4

IVEM Finance 1995/96

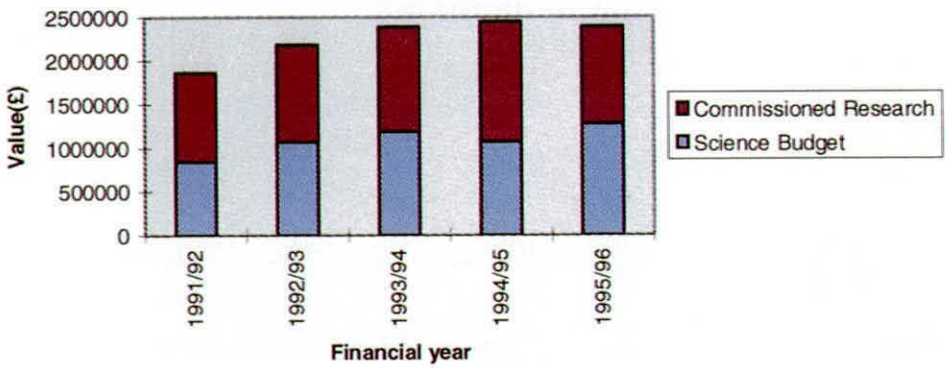
Division of 1995/96 Commissioned Research Receipts by Major Customers



Sources of funding for IVEM science in 1995/96



Total IVEM income 1991/92 onwards



Appendix 5

IVEM Publications 1995

(P = Peer-reviewed publications)

Dr Tamara Gritsun

Professor Bob Possee
Project Leader

Susan Chapple
Student

Bailey, M.J. (1995). Extraction of DNA from the phyllosphere. In: *Nucleic acid in the environment: Methods and applications* (Eds., Trevors, J.T. and Van Elsas, J.D.). Springer-Verlag, Berlin, pp. 89-110.

Bailey, M.J., Lilley, A.K., Ellis, R.J., Bramwell, P.A. and Thompson, I.P. (1995). Dispersal and persistence of GMMs and their introduced sequences. In: Unanswered safety questions when employing GMOs. Symposia proceedings, CCRO, The Netherlands. pp. 103-109.

(P)Bailey, M.J., Lilley, A.K., Thompson, I.P., Rainey, P.B. and Ellis, R.J. (1995). Site directed chromosomal marking of a fluorescent pseudomonad isolated from the phytosphere of sugar beet; stability and potential for marker gene transfer. *Molecular Ecology* **4**, 755-764.

(P)Belyaev, A.S., Hails, R.S. and Roy, P. (1995). High level expression of five foreign proteins by a single recombinant baculovirus. *Gene* **156,** 299-233.

Bishop, D.H.L., Hirst, M.L., Possee, R.D. and Cory, J.S. (1995). Genetic engineering of microbes: virus insecticides - a case study. In: *Fifty Years of Microbials* (Eds., Darby, G.K., Hunter, P.A. and Russell, A.D.): SGM Symposium Proceedings, Bath 1995, pp. 249-277.

(P)(Bonning, B.C.), (Hoover, K.), Booth, T.F., (Duffey, S.) and (Hammock, B.D.) (1995). Development of a recombinant baculovirus expressing a modified juvenile hormone esterase with potential for insect control. *Archives of Insect Biochemistry and Physiology* **30**, 177-194.

(P)Boublik, Y., Di Bonito, P. and Jones, I.M. (1995). Eukaryotic virus display: engineering the major surface glycoprotein of baculoviruses, gp64 for the display of foreign proteins on the virus surface. *Nature Biotechnology* **13**, 1079-1084.

Bramwell, P.A., (Barallon, R.V.), (Rogers, H.J.) and Bailey, M.J. (1995). Extraction and PCR amplification of DNA from the rhizoplane. In: *Molecular Microbial Ecology Manual* (Eds., Akkermans, A.D.L., Van Elsas, J.D. and DeBruijin, F.J.). Kluwer Academic Publishers, pp. 36-55.

Bramwell, P.A., (Barallon, R.V.), (Rogers, H.J.) and Bailey, M.J. (1995). Extraction of DNA from the phylloplane. In: *Molecular microbial ecology manual* (Eds. Akkermans, A.D.L., van Elsas, J.D. and de Bruijn, F.J.). Kluwer Academic Press, pp. 56-77.

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(Chuma, T.), (Le Blois, H.), (Sanchez-Vizcaino, J.M.), (Diaz-Laviada, M.) and Roy, P. (1995). Expression of the major core antigen VP7 of African Horsesickness virus by a recombinant baculovirus and its use as a group-specific diagnosis reagent. In: *Workshop for the development of diagnostic and preventative methods by genetic engineering for African horsesickness and related orbiviruses.* Tokyo. pp. 95-108.

Cooper, J.I. (1995). Use of genetically modified organisms in forestry. In: Quality for forest reproductive material in the field of the application of European Community rules (ed., Terrasson, D.), pp. 151-160.

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Cory, J.S. and Bishop, D.H.L. (1995). Biopesticides. In: *Issues in agricultural bioethics.* pp. 135-149.

Cory, J.S. and Bishop, D.H.L. (1995). Use of baculovirus as biological insecticides. In: *Methods in molecular biology - baculovirus expression vectors* (Ed. Richardson, C.). pp227-294.

(P)Cory, J.S. and Hails, R.S. (1995). Genetically-modified insecticides. *Commonwealth Forestry Review* **74(3)**, pp. 188-189.

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(P)Craine, N., (Randolph, S.E.) and Nuttall, P.A. (1995). Seasonal variation in the role of grey squirrels as hosts of *Ixodes ricinus,* the tick vector of the Lyme disease spirochaete, in a British woodland. *Folia Parasitologia* **42**, 73-80.

(P) Doyle, C.B., Bhattacharyya, U., (Kent, K.A.), (Stott), J. and Jones, I.M. (1995) Regions required for CD4 binding in the external surface glycoprotein gp120 of simian immunodeficiency virus - *Journal of Virology* **69,** 1256-1260.

(P) (Dizij, A.) and Kurtenbach, K. (1995). *Clethrionomys glareolus*, but not *Apodemus flavicollis*, acquires resistance to *Ixodes ricinus* (Acari: Ixodidae), the main European vector of *Borrelia burgdorferi. Parasite Immunology* **17**, 177-183.

(P) Ellis, R.J., Thompson, I.P. and Bailey, M.J. (1995). Metabolic profiling as a means of characterising plant-associated microbial communities. *FEMS Microbiology Ecology* **16**, 9-18.

(P) (Fenouillet, F.) and Jones, I.M. (1995) The glycosylation of human immunodeficiency virus type-1 transmembrane glycoprotein (gp41) is essential for intracellular transport of the envelope precursor gp160 *Journal of General Virology* **76**, 1509-1514.

(P) (Fuchsberger, N.), (Kita, M.), (Hajnicka, V.), (Imanishi, J.), (Labuda, M.) and Nuttall, P.A. (1995). Ixodid tick salivary gland extracts inhibit production of lipopolysaccharide-induced mRNA of several different human cytokines. *Experimental and Applied Acarology* **19**, 671-676.

(P) Gibbs, M.J. and Cooper, J.I. (1995). A recombinational event in the history of luteoviruses probably induced by base-pairing between the genomes of two distinct viruses. *Virology* **206**, 1129-1132.

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