# Scottish Crop Research Institute

## Annual Report 2000/2001

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## Annual Report 2000/2001

# Governing Body



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The Scottish Crop Research Institute (SCRI) is a major international centre for research on agricultural, horticultural and industrial crops, and on the underlying processes common to all plants. It aims to increase knowledge of the basic biological sciences; to improve crop quality and utilisation by the application of conventional and molecular genetical techniques and novel agronomic practices; and to develop environmentally benign methods of protecting crops from depredations by pests, pathogens and weeds. A broad multidisciplinary approach to research is a special strength of the Institute, and the range of skills available from fundamental studies on genetics and physiology, through agronomy and pathology to glasshouse and field trials is unique within the UK research service.

Das SCRI ist ein führendes internationales Forschungszentrum für Nutzpflanzen im Acker- und Gartenbau sowie in der Industrie und auf dem Gebiet der allen Pflanzen zugrundeliegenden Prozesse. Es hat sich zum Ziel gesetzt, die Grundkenntnisse in den Biowissenschaften zu vertiefen; die Qualität und Nutzung der Kulturpflanzen durch die Anwendung konventioneller und molekular-genetischer Techniken und neuer agrarwissenschaftlicher Praktiken zu verbessern; sowie umweltfreundliche Methoden zum Schutz der Pflanzen gegen Verlust durch Schädlinge, Pathogene und Unkräuter zu entwickeln. Ein breiter multidisziplinärer Forschungsansatz ist eine besondere Stärke des Instituts; und das zur Verfügung stehende Spektrum an fachlichen Ausrichtungen, das von genetischer und physiologischer Grundlagenforschung über Agrarwissenschaften und Pathologie bis zu Gewächshaus- und Feldversuchen reicht, stellt ein einmaliges Forschungsangebot auf den Britischen Inseln dar.

Le SCRI est un centre international majeur de recherche sur les cultures agricoles, horticoles et industrielles et les processus fondamentaux communs à toutes les plantes. Son but est d'accrôitre les connaissances des sciences biologiques fondamentales; d'améliorer la qualité et l'utilisation des cultures par l'utilisation de techniques conventionelles et de génétique moléculaire et par l'application de procédés agronomiques nouveaux; de développer des méthodes de protection moins dommageables pour l'environnement contre les préjudices causés par les ravageurs, les pathogènes et les adventices. L'une des forces majeures de l'institut est une large approche multidisciplinaire de la recherche. L'éventail des techniques disponibles allant des études fondamentales en génétique et physiologie en passant par l'agronomie et la phytopathologie jusqu'aux essais en serres et aux champs est unique au sein du service de recherche du Royaume Uni.

Lo SCRI e' uno dei maggiori centri internazionali nel campo della ricerca sulle colture agricole, orticole e industriali e sui meccanismi fondamentali comuni a tutte le piante. L'Istituto ha come obiettivo principale l'accrescimento del livello di conoscenza delle scienze biologiche fondamentali, il miglioramento della qualità e del potenziale di utilizzo delle colture tramite l'applicazione di tecniche convenzionali o di genetica molecolare e di nuove pratiche agronomiche, lo sviluppo di metodi ecologici di protezione delle colture da agenti patogeni o malerbe. Uno dei punti di forza dell'Istituto e' l'adozione di un approccio largamente multidisciplinare (probabilmente senza eguali nel servizio di ricerca britannico) fondato su una vasta gamma di capacità scientifiche derivanti da ricerche di fisiologia e genetica ma anche di agronomica e fitopatologia supportate da prove di campo o in ambiente controllato.

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# SCRI Health & Safety Policy

SCRI recognises and accepts its responsibilities for health, safety and welfare under the Health & Safety at Work Act 1974 and related legislation. The Institute has a senior member of staff responsible for health, safety and welfare management who reports to the Director. The health and safety team comprises a safety co-ordinator, first-aiders, fire officers, biological safety officers, hazardous waste managers, radiation protection officers and an occupational health adviser.

Training is made available for all staff and targeted groups of staff in order to maintain a high level of health and safety awareness. Regular inspections of the site and individual work areas are carried out by internal health and safety personnel and a 2-yearly external audit is carried out by a team of inspectors drawn from the other SABRI institutes and BBSRC.

# Foreword to Annual Report 2000/2001

James E. Godfrey, Chairman of the Governing Body

Last year, I commented on the reasons for research. This year, I want to consider the measurement of research output whether funded by government, research foundations or the private sector. There is no clear-cut single measure; a scientist may perceive and quantify success differently from government and commercial companies. Research should deliver end results, which are measurable and satisfy the needs and requirements of society as a whole, and of the different parts of society whether they be consumers, taxpayers, government, company shareholders or scientists, all of whom may have different views and expectations of research deliverables.

For this analysis, research needs to be dis-aggregated into various parts. In the government sector, policy, public good, food safety (balance of risk assessment), health, wealth creation and poverty reduction and environmental issues, which include amenity pollution and natural resource management, are high priorities. In the private sector, food and industrial output, the financial value of contracts, the profit to a production chain within a marketing cycle, and the efficient use of resources are important factors. For the research centre and scientist, specific measurements of number of research papers published (weighted to journal ratings) per research scientist and total staff, similarly cost of research per scientist and per total staff at a centre, need to be considered. There are, however, implicit conflicts for scientists who have been successful in gaining private sector research contracts, for these may require confidentiality clauses to allow lead time for commercial gain before publication and wider uptake of results. These issues need to be considered and balanced against the recognition, esteem and wealth-creating potential of innovative, commercially sponsored research. A dynamic research organisation such as SCRI must continue to diversify its portfolio of externally funded research, mindful of the differing pressures that this places on its staff.

For society as a whole to attain ever-higher standards of living, we need to create wealth in a sustainable way both in this and future generations. In its simplest form, in a free market economy, price will determine the goods and services which are most highly valued. This will be subject to large distortions when markets are exposed to external interference such as taxation, legislation and trade tariffs. However, when environmental aspects are considered, traditional economic analysis is often inadequate since it often fails to take into account the importance of public good and other externalities in calculations of wealth creation.

When research output is measured, then this poses the question of how research strategy and the appropriate criteria for the commissioning of research are set. In developing criteria for the commissioning of research, careful consideration must be given to ensure that adequate controls are in place. However, these procedures should not stifle innovative research and should be streamlined to minimise bureaucracy. It is often easy for government to justify policy research without overt measurable outputs; in such cases, to ensure high quality work and value for money, this should be accompanied by some form of independent evaluation such as international peer group review. If a research centre is too heavily dependent and focused on policy research, then it may lead to a lack of innovative wealth-creating research and drive to gain commercial contracts. In a period of economic restraint in the public sector, there must be a drive to create intellectual property and offer tangible returns to the sponsor, public or private. Another conflict arises between funding and measurement of outputs arising from research giving rapid but sometimes difficult to quantify benefits, perceived or otherwise, and those types of research with more distant though possibly greater long-term benefits.

We at SCRI can hold our heads up high and be proud of our record of achievements over many years. Our success, when measured against criteria such as commercial, financial and output per scientist, is outstanding. This has been achieved by the hard work of all at SCRI. Our collaborations locally, nationally and worldwide continue to flourish. The commercial environment in which we operate is changing with

company takeovers and amalgamations affecting our customer base. In this past year, I am pleased to report that our commercial activities through Mylnefield Research Services continue to be successful at a time of cutbacks in research programmes in the private sector associated with agriculture and food. I thank our commercial director, Nigel Kerby, and all his staff for their commitment and dedication in achieving this result.

Six new members were appointed to the Governing Body in April 2000; they have all contributed to and complimented the skill base and experience of the Governing Body. It has been gratifying to see the increasingly beneficial interaction between the Governing Body and senior staff over the last few years and I am sure that this aids the efficient and successful running of the Institute. Our Director, Professor John Hillman has been as dynamic as ever and I pay tribute to his vision and energy which drives SCRI forward.

# Introduction by the Director

John R. Hillman

The Scottish Crop Research Institute (SCRI) is a non-profit-making limited company established under the Companies Act, has charitable status, and is currently classified as a Non-Departmental Public Body because over 50% of the total funding is received as grant-in-aid from the Scottish Executive Environment and Rural Affairs Department (SEERAD). The Members of the Company are also members of the Governing Body and are appointed by the First Minister. Staff are not formally civil servants, but are members of the Scottish Executive Environment and Rural Affairs Department Superannuation Scheme, 1999. SEERAD also funds any redundancies, the site, and much of its fabric and capital equipment. There is also a Management Statement and Financial Memorandum embodying the formal relationship with SEERAD. The Pay and Grading System, and Staff and Management Codes under which the Institute operates are administered by the Biotechnology and Biological Sciences Research Council (BBSRC).

The Institute is committed to the implementation of Corporate Governance, which requires the highest standards in the three key areas of openness, integrity, and accountability. The Governing Body has therefore drawn up a Code of Practice to guide the conduct of its members and has established the appropriate procedures and remits to ensure adherence to these standards.

On 31 March 2000, six new members were appointed to the Governing Body and three current members were re-appointed. All the appointments were for a four-year period. The new members are Mr E Angus

### The Mission of SCRI is:

"To sustain excellence and our international reputation for strategic research into plant, crop, and environmentally related sciences of particular importance to Scotland and of wider international relevance, bringing added value and new opportunities to agriculture, forestry, horticulture, and the bioindustries in an environmentally sustainable manner".

### To achieve this Mission, SCRI aims

- to enhance its status as an international centre of excellence in plant and environmental science and to contribute fundamental knowledge of plant/crop systems to the UK and international science base;
- to provide a scientific and administrative infrastructure that supports high-quality, innovative, basic, and strategic research;
- to enhance the utility and value of plant and crop species, in particular, our mandate crops – barley, potato and soft fruit, to improve agricultural efficiencies and offer options to achieve profitability;
- to establish a critical mass in key strategic areas that is connected to a long-term vision;
- to develop more sustainable, environmentally sensitive and systems-based approaches to minimise inputs and maximise product quality and safety;
- to create value-added opportunities in our plant, crop, and environment-related industries by developing the technologies and knowledge required to sustain and to improve international competitiveness;
- to promote public awareness and understanding of relevant bioscience and environmental issues to assist informed public debate;
- to recognise excellence and encourage training and rewards to staff to ensure the success of the science programme;
- to provide a stimulating environment for research endeavours and student training;
- to sustain a dedicated knowledge- and technology-transfer operation that promotes and facilitates collaboration with global industry;
- to meet end-user needs.

MBE, Dr K Dawson, Dr M Eddie, Professor M Emes, Mrs W Goldstraw, and Mr I McLaren. The three re-appointed members are Professor R J Cogdell, Mr K Hopkins, and Mr P Whitworth. Three members have retired from the Governing Body, namely Mr J M Drysdale, Dr J M Sime, and Professor P C Young.

SCRI was established in 1981 by an amalgamation of the Scottish Horticultural Research Institute (SHRI, founded at Invergowrie, Dundee in 1951) and the Scottish Plant Breeding Station (SPBS, founded at East Craigs, Edinburgh in 1921). In 1987, the Institute assumed managerial responsibility for Biomathematics & Statistics Scotland (BioSS), formerly the Scottish Agricultural Statistics Service.

SCRI is a major international centre for basic, strategic, and applied research on agricultural, horticultural and industrial crops and on the underlying biological processes common to all plants. It is the only such institute in Scotland and Northern Britain, and the range of complementary skills assembled at the Institute, from fundamental molecular genetics to glasshouse- and field-trials, with exploitation of the SCRI-based international genetic resources in a region of high phytosanitary conditions, is unique within the UK.

The science is optimised by a matrix management system comprising three themes and nine inter-related research programmes. Management structures are regularly reviewed to ensure maximum effectiveness of the research at SCRI.

During the past year, the Institute has completed a major Science Strategy Review. The review team:

- assessed the quality, novelty, focus, and timeliness of current and projected research objectives and their relevance to SCRI's position within Scottish, UK, and International plant science communities;
- examined the management of science within the Institute, including the effectiveness of communication and synergies within and between the various research groupings;
- made recommendations on the future research direction and integration of research activities. Wealth-creating potential and industrial relevance were considered high-priority targets.

The review took into account views of staff, senior management, the Governing Body, and SEERAD.

The SCRI research programmes are peer-reviewed at many levels. Each year, the 'core' programme of research comprising a number of projects is assessed by the Agricultural and Biological Research Group of SEERAD. New projects are appraised by advisers prior to commissioning, progress is monitored annually and, ultimately, a final report is produced for evaluation.

Every four years, SEERAD commissions BBSRC to appoint a Visiting Group to review the work of the Institute. In 1998, a Visiting Group carried out a scientific audit of the quality and conduct of SCRI's core research programme and related work. It assessed SCRI's effectiveness in managing its resources to meet the needs of users and beneficiaries of research, and the Institute's strategic plans for the future development of its scientific programme.

The Mid-Term Implementation Report (November 2000) showed that all 25 recommendations of the VG Report had been implemented. Nineteen of the 25 recommendations related to the need to formulate a more focused and integrated science strategy based on reviews involving a corporate approach. In the past year, the management of the Institute has been substantially restructured, two new key members of staff appointed (Professor W Powell, Deputy Director and D Watt, Institute Secretary), a Corporate Plan acceptable to SEERAD was developed, new initiatives launched, and the composition and operation of the Governing Body amended to ensure best practice.

A broad multidisciplinary approach to fundamental and strategic research, and technology transfer, are special strengths of SCRI. Our programmes span the disciplines of genetics and breeding, molecular and cellular biology, biotechnology, plant pathology (bacteriology, entomology, mycology, nematology, and virology), plant physiology and cell biology, environmental science, plant chemistry and biochemistry, agronomy, molecular ecology, vegetation dynamics, bioremediation, serology, physics, mathematics, bioinformatics, and statistics. Genetics and enhanced breeding of selected crops, and biotechnology, lie at the core of all our substantial research, development, and training programmes.

The breadth and depth of knowledge, technical expertise, and infrastructural resources available at SCRI attract extensive contracts and consultancies from, and foster

collaborations with, numerous academic and corporate organisations around the world. Synergistic liaisons with other institutes, universities and colleges in the UK and overseas are also integral to the scientific growth, development, and validation of the Institute's research activities. New links are being forged continuously, as well as existing contacts being developed and strengthened.

SCRI and Mylnefield Research Services (MRS) Ltd, the commercial arm of the Institute, are successful in gaining competitive research contracts from government departments and agencies, Levy Boards, grower organisations, international agencies, the European Union, commercial companies, local government, and some Charities, Research Councils, and Trust funds, although we are largely excluded from submitting applications to the latter three sources.

In February 2000, the Mylnefield Trust was registered. The objectives of the Trust are:

- to promote research and scientific work in the life, environmental and related sciences, in particular production of agricultural, horticultural, and forestry crops, methods of limiting or eradicating pests and diseases, wood sciences and biomathematics, methods of increasing production or growth, improving cultivation, and research into improved cultivars;
- to promote the dissemination of such research.

The Trust will support scientific research at SCRI by making gifts, grants, loans, or payments to the Institute subject to the above objectives being met.

Also in February 2000, Mylnefield Holdings Ltd was established. Mylnefield Holdings Ltd is legally separate from SCRI and MRS Ltd but will obtain licences to SCRI technology and other necessary third-party technology that will enable it to establish spin-out and start-up companies. The new company will transfer money to SCRI and MRS Ltd through royalty and/or milestone payments.

SCRI provides the base and secretariat for The Scottish Society for Crop Research (SSCR), a registered Friendly Society formed in 1981 by the amalgamation of The Scottish Society for Research in Plant Breeding and The Scottish Horticultural Research Association.

The SSCR provides an important link between SCRI research scientists and farmers, growers, processors and other interested companies in the private sector.

The Society:

- organises interactive field walks and end-user/researcher discussion sessions;
- finances science-based advisory publications for the benefit of its members;
- stimulates crop-based sub-committees to support targeted research projects ;
- reinforces SCRI representation with trade associations, Levy Boards, and other user-groups;
- administers the biennial Peter Massalski Prize to the most promising young scientist at SCRI.

SCRI is one of five Scottish Agricultural and Biological Research Institutes (SABRIs: Scottish Crop Research Institute; Hannah Research Institute; Macaulay Land Use Research Institute; Moredun Research Institute; Rowett Research Institute); and together with the Royal Botanic Garden, Edinburgh; the Scottish Agricultural College (SAC); the Scottish Agricultural Science Agency (SASA); the Fisheries Research Services; and Forestry Commission Research Agency, comprise the Committee of Heads of Agricultural and Biological Organisations in Scotland (CHABOS).

BioSS was established to cover the biomathematical and statistical needs of the five SABRIs and SAC. High-level consultancy, training and research inputs from BioSS give a major advantage to the SABRI and SAC research programmes, as well as to the work of SASA and several other bodies for whom it carries out contracts.

This Report details a small selection of the recent research achievements of SCRI, BioSS, and MRS Ltd, briefly describes the commercial rôles and successes of MRS Ltd, and summarises the important linking rôle of SSCR. Significant advances continue to be made in both fundamental and strategic science, with contributions to the protection and understanding of the environment. SCRI contributes to the debate on genetically modified crops, providing independent and unbiased information on this important subject. Discoveries are reported of direct and indirect benefit to agriculture, horticulture, forestry, land management, and biotechnology. Dedicated and talented scientific and support staff in the Institute, BioSS, and MRS Ltd, account for our stature, successes, and delivery of achievements.

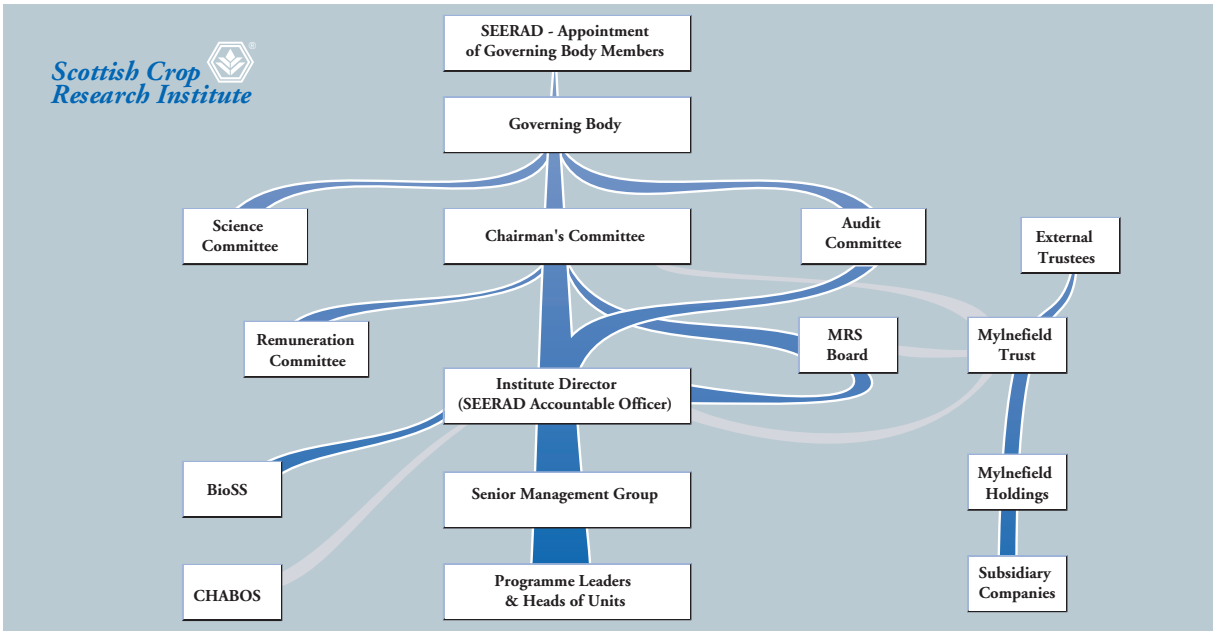


**Figure 1** SCRI company and committee structure.

Details of the annual accounts, Corporate Plan, health and safety provisions, and the SCRI/MRS quality-assurance arrangements are available on request. Figures 1 and 2 illustrate the SCRI company and committee structure, and the management structure.

On behalf of the staff and Governing Body, it is a pleasure once again for me to acknowledge with gratitude the staff of SEERAD for their continuing support of, and demonstrable commitment to, our research programme and to our development. Grants, contracts, donations, advice, and joint participation in our activities from the SSCR, other government departments and their agencies, non-governmental agencies, our sister CHABOS institutions, and BBSRC institutes with whom we coordinate our research, grower levy boards, local and regional authorities, commercial companies, farmers, and other individuals, and learned societies, are also warmly appreciated.

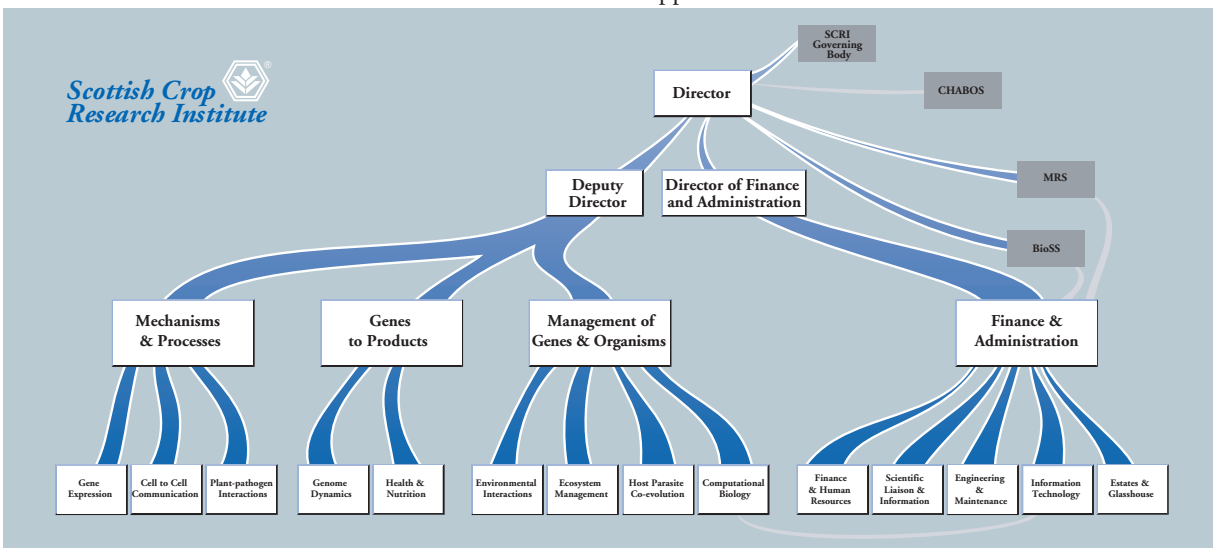


**Figure 2** Institute management structure.

# Report of the Director

John R. Hillman

## Global perspectives of factors influencing agricultural, biological, and environmental sciences, and their associated industries : 2000-2001*

### Preamble

For many, the third millennium did not begin until 1 January 2001, given that the widely adopted Gregorian calendar ordained by Pope Gregory XIII in 1582 did not include the year 0 in the transition from Before Christ (BC or *Ante Christum*) to *Anno Domini* (AD - in the Year of Our Lord). A miscalculation – a shortcoming - by Dionysius Exiguus (Dennis the Short), a 6$^{th}$-century monk, was apparently integrated into the Christian calendar, according to M. Bolt, an historian of matters astronomical, masking the widely held view by scholars that Christ was born around 4 or 6 B.C. If true, it would seem that the third millennium is not as new as it is widely assumed to be, festivals and celebrations notwithstanding. Fortunately, no apocalyptic or cataclysmic predictions came true in 2000 or in the transition from 2000 to 2001. There were no major wars, and the 'Y2K' computer problem was barely detectable. A new President of the USA, G.W. Bush, was elected by a small margin. Globalisation in its various guises; the onward march of science, engineering, and technology; and social protection and public health programmes were occasional headline issues alongside the plethora of entertainment and sporting frivolities. Politics at all levels became more complex and demanding, and on a global level, taxation levels increased. Protesters of diverse types from liberal democracies coalesced to direct their ire at multinational institutions and companies. There was disturbing evidence of destabilisation of Central Asian states fomented in Afghanistan, disruption founded as with many conflicts elsewhere and in times past, on dogmatic and intolerant religious interpretations of lifestyle. Centrifugal nationalist and regional forces counteracted globalisation and integration trends. Agriculture was not regarded in the more-developed countries (MDCs) as a growth industry and investments declined, as did the numbers of scientists engaged in agricultural research and development (R&D). In contrast, the array of modern bioindustries linked by common-denominator molecular-genetics technologies, advanced natural product chemistry, and computational developments thrived intellectually, particularly in the private sector, although the downturn in stock markets during the year curtailed the number of products entering the market place. There were further signs of economic weakness towards the end of the year as expenditure in advertising and several areas of R&D declined, the usual early indicators of impending economic stress, and company contractions, mergers, and acquisitions became more frequent.

* This review updates and enlarges on themes developed in my previous accounts in the *SCRI Annual Report* series.

Greatest amongst the sustained achievements of the human race is modern agriculture. Food is no longer the preoccupation of most human beings, a feature distinguishing them from other life forms, and one permitting them to engage in all the facets of civilisation, including the quest for knowledge and understanding.

The most prominent scientific achievement in 2000 was the announcement in June of the completion of a rough draft of the sequence of the human nuclear genome (see www.sanger.ac.uk/HGP/policy-forum.shtml#ref1). Founded on the legacy of Gregor Mendel's laws developed in the mid-1800s, the analysis of nuclei by F. Miescher, the discovery by O.T. Avery that DNA carried genetic material, W. Flemming and E. Strasburger's work on chromatin and mitosis, the proposed double-helix structure of deoxyribonucleic acid (DNA) by Watson and Crick in 1953, and various technological and conceptual advances in the separation, identification and mode of action of nucleic acids, the public-sector-funded Human Genome Project and a private-sector company, Celera Genomics, finally came together to speed up completion of the rough draft sequence. With such profound positive implications for medical and veterinary science in new forms of diagnostics and treatments, it is important to stress that other benefits have come from related technological, engineering, and software developments, and have strongly influenced, and been influenced by parallel studies in other animals, microbes, and plants. Most striking was the commonality of genes between different groups of organisms. Gene functionality, mode of action, location, and control need to be unravelled, and represent the real target of human endeavour – understanding the processes underpinning the creation, functioning and interactions of phenotypes. The genomes of humans are more than 99% identical but unique to an individual (see www.ncbi.nlm.nih.gov/genome/guide). It is still too early to determine what can be considered as being in the 'normal' range of sequences.

Professor John Hillman, and Mrs Irene Geoghegan (left), present prizes to the recipients of the special needs section of the painting competition sponsored by SCRI and Ladybird Books.

Annotating the entire genome of all organisms, *i.e.* characterisation of all the genes and working out their functions, will firstly require identification of all the protein-coding regions which will indicate the total number of functional genes. Thereafter, gene structures, identification of regulatory elements and then assignation of functions will ensue. Complicating matters are questions about the accuracy of the many public databases that receive deposited sequences and annotations. Intellectual property (IP) considerations will lead to a reappraisal of the so-called Bermuda Rules. These were derived from an agreement reached at the International Strategy Meeting on Human Genome Sequencing held in Bermuda in February 1996, which stated that "All human genomic sequence information, generated by centres funded for large-scale human sequencing, should be freely available and in the public domain in order to encourage research and development and to maximise its benefit to society". Equivalent rules do not apply to plants and microorganisms, but there are strong international collaborations.

Although the future of medicine was advanced by the draft sequencing of the entire human genome, the year was marked by outbreaks of infectious diseases, principally cholera, new variant Creutzfeld-Jakob disease, dengue fever, Ebola haemorrhagic fever, hantavirus, Legionnaire disease, leptospirosis, malaria, measles, polio, tuberculosis, *West Nile virus*, and yellow fever. About 130 million new cases of malaria occurred in 2000, over 90% of which were in Africa, fully justifying calls for increasing the relatively modest investments by the World Bank and MDCs in the Roll Back Malaria programme. Recombinant vaccines, DNA vaccines and prime-boost approaches, transgenic vaccines, and genomic and proteomic approaches represented promising routes to control the protozoan parasites that cause malaria. Many believe that DDT (dichlorodiphenyltrichloroethane) remains the most cost-effective tool to control the vectoring mosquitoes.

During the year, the World Health Organisation (WHO) documented the extent to which certain infectious diseases, particularly AIDS, diarrheal diseases, malaria, pneumonia, and tuberculosis, were developing antimicrobial drug resistance, a phenomenon which is also shared by many food-poisoning organisms. The misuse of antibiotics and other antimicrobial agents was regarded as the primary cause of the spread of resistance.

Agrarian economies are affected by the health of the population. The UN calculated that by the end of 2000, 21.8 million people had died of AIDS, and that an additional 36.1 million were infected with the HIV virus, 24.5 million of whom lived in southern Africa; most were desperately poor. Around 70% of the AIDS cases were in sub-Saharan Africa, with projections that life expectancy in that region would drop to around 30 years by 2010 in the absence of effective control measures. As a prelude to the 13th International AIDS Conference in Durban, South Africa, 5 228 physicians and scientists from 80 countries felt obliged to sign the Durban Declaration which stated that the evidence that AIDS is caused by the human immunodeficiency virus, HIV-1 or HIV-2, is clear-cut, exhaustive and unambiguous. AIDS was recorded as spreading rapidly in the Caribbean, China, Eastern Europe, India and the Far East. Vaccines are now regarded as the single intervention most likely to control the AIDS pandemic; during the year more than 70 different vaccines were being trialled.

New medical treatments introduced included constraint-induced-movement therapy and robot-aided exercising for stroke-disabled victims, islet-cell transplant techniques to eliminate need for insulin injections with poorly controlled type 1 (insulin-dependent) diabetes, and minimum surgery treatments to treat gastroesophogeal reflux disease. Two promising treatments that are likely to be introduced as a result of trials were the use of interferon-la (Avonex) to delay the development of established, clinically definite multiple sclerosis, and novel vaccine-like approaches to treat kidney cancer. Embryonic stem cell research in humans attracted widespread attention from medical scientists, the healthcare industry and pressure groups opposed to research on human embryos. As in plants, adult stem cells do not have the same level of totipotency as embryonic cells. Controlling the differentiation of stem cells into cells, tissues and organs offers new vistas for repairing damaged or defective tissues and organs. As usual, there was confusion in public and political debates over the distinction between research and commercial application.

Plant scientists were intrigued by the current emphasis in the marketplace on herbal products, plant-derived dietary supplements, nutraceuticals and functional foods. In most instances, but not all, the efficacy, and sometimes the safety of the products were not known but assumed with little evidence (see www.consumerlab.com) and much wishful thinking. One feature of many products was the variation in the amounts of so-called active ingredients both between apparently similar products and batches of the same product. Some dietary products were also noted to contain dangerous contaminants. Other products, however, were efficacious. More science and regulation will have to be directed to this neglected facet of oral intakes (see also *SCRI Annual Report for 1999-2000,* pp 83-94).

The 'Snowball Earth' concept was reinforced by its originator J. L. Kirschviak and colleagues who presented evidence for two periods when the earth was more or less completely covered with ice, at around 2.4bn and 600-800 million years ago. These periods coincided with mass extinctions, and the ensuing global warming created favourable conditions for the surviving prokaryotic Cyanobacteria (blue-green algae, formerly Cyanophyta) leading to oxidising conditions for large-scale geological banded iron formations and postglacial cap carbonates. It was suggested that the enzyme superoxide dismutase (see *SCRI Annual Report for 1999-2000*, p93) may have had a key rôle in the adaptation of organisms to the changed environment. Fascinating hypotheses were presented by I.W.D. Dalziel (University of Texas) and colleagues, C.R. Scotese (University of Texas at Arlington) and others about the formation of the supercontinents Rodinia, Pangaea, and Pangaea Ultima (see www.scotese.com). Together with reports using geochemical analyses, greater understanding of plate tectonics revealed the nature and extent of continental drift. Continuous geophysical monitoring using the satellite-based Global Positioning System gave unprecedented insight into dynamic phenomena such as earthquakes, tectonic plate motion, and volcanoes, and aided more sophisticated predictive models to be generated.

In mathematics, prizes were offered for the solution of eight famous problems: (i) a proof for Goldbach's conjecture that every even integer greater than 2 is the sum of two prime numbers; (ii) P *versus* NP (are there

more efficient algorithms for computations?); (iii) the Poincaré Conjecture that if every loop on a compact three-dimensional manifold can be shrunk to a point, is the manifold topologically equivalent to a sphere? (iv) the Riemann hypothesis that all zeros of the Riemann zeta function lie on a specific line; (v) the existence of solutions for the Navier-Stokes equations, describing the motions of fluids; (vi) the Hodge conjecture on algebraic geometry; (vii) the existence of Young-Mills fields in quantum field theory and particle physics; and (viii) the Birch and Swinnerton-Dyer conjecture on elliptic curves. There were no equivalent prizes in the life sciences.

In organic chemistry, scientists at the University of Chicago reported that difficult-to-synthesise octanitrocubane may be the most powerful non-nuclear explosive, reflecting its highly strained 90° bonds and eight oxygen-rich nitro groups. Cubane derivatives may interact therapeutically with enzymes involved in Parkinson's disease. Relevant to many branches of life sciences was an automated oligosaccharide synthesiser reported by P.H. Seeberger and colleagues at the Massachusetts Institute of Technology. In nuclear chemistry, A. Türler of the Paul Scherrer Institute, Switzerland, and colleagues, reported that contrary to the belief of certain theoreticians that the periodic table of elements was unable to assist in the prediction of properties of extremely heavy elements beyond uranium in the table, the strangeness or relativistic effects associated with the interpretation of Einstein's theory of relativity, when applied to superheavy elements do not alter the predicted properties of element 107, bohrium. In applied chemistry, R.H. Grubbs and colleagues at the California Institute of Technology reported the development of a new family of nickel-based catalysts that could simplify the production of polyolefins, including polyethylene and polypropylene, and designer plastics with desired electrical, mechanical and optical properties. K.E. Sickafus and colleagues at the Los Alamos National Laboratory announced a new family of ceramic materials virtually impervious to the damaging effects of ionising radiation. By developing a fluorite-structured oxide of erbium, zirconium, and oxygen, the group may have generated an important class of compounds that could be used for the safe long-term encapsulation of nuclear waste, suitable for storage in geologically stable mines. The stability of plutonium oxide, used in commercial nuclear reactor fuels and as the storage form for plutonium from dismantled nuclear weapons, was called into question by the work of J.M. Haschke and associates at the same Los Alamos National Laboratory in New Mexico. They demonstrated that water can slowly oxidise solid crystalline plutonium oxide to a new less-stable phase with greater than 25% of the plutonium atoms in a higher oxidation state, releasing hydrogen gas and becoming relatively water-soluble. This phenomenon may explain the relatively rapid spread of plutonium from the nuclear tests in Nevada into groundwaters. The search for non-toxic, environmentally friendly 'green' solvents to replace organic solvents has highlighted the potential of supercritical carbon dioxide, which has fluid-like properties. Its utility, however, depends on high pressures and the presence of solubility-enhancing compounds termed $CO_2$-philes which are typically fluorocarbons. E.J. Beckman and colleagues at the University of Pittsburgh reported the synthesis of an environmentally effective series of $CO_2$-philes, co-polymer compounds termed poly (ether-carbonate(s)).

An international team of physicists at the DONUT (Direct Observation of the Nu Tau) experiment at the Fermi National Accelerator Laboratory provided in 2000 the first direct evidence for the existence of the tau neutrino, the only one of the 12 kinds of matter particles (fermions) yet to be confirmed in the current standard model. There are three kinds of neutrino; the electron, muon and tau; and there may possibly be a fourth type of neutrino – the sterile neutrino. Six of the fermions are termed quarks, two of which, the up quark and down quark, comprise the protons and neutrons, or nucleons, that constitute nuclei of matter. Quarks were confirmed within the nucleons, bound together by the exchange of particles called gluons. At the time of the formation of the universe, it was postulated that quarks and gluons existed freely in the form of a quark-gluon plasma. At the European Laboratory for Particle Physics (CERN), physicists reported evidence for a new state of matter akin to a quark-gluon plasma.

Advances in solid-state physics came from continuing development of semiconductor quantum dots – isolated groups of atoms with the crystalline lattice of a semiconductor coupled quantum mechanically such that electrons in the dot can only exist in a limited number of energy states. Accordingly, the dot has light-absorbing and emission properties that could be used in astronomical spectroscopy, optical communication, and quantum computing. Laser technology was advanced by the development at

Lucent Technologies's Bell Laboratories of the first electrically powered semiconductor laser based on an organic material. Here, a crystal of tetracene was placed between two different kinds of field-effect transistors which triggered a yellow-green light pulse when applied with a voltage. The speed of light as a fundamental speed limit, essential to the theory of relativity, was a topic for deep philosophical debate following the experiments of A. Ranfagno and colleagues of the Electromagnetic Wave Research Institute, Florence, who modulated a microwave pulse to send microwave-frequency radiation through air faster than that of light, and those of L. Wang at the NEC Research Institute, Princeton, who propagated a pulse of visible light through a chamber of optically excited cesium gas such that it exceeded the speed of light in a vacuum. The fundamental principle of causality would be undermined should usable information travel faster than the speed of light, thereby allowing a preview of the future that could be used to alter the present! Politicians could be interested in further developments of this work.

The year 2000 was also noted for evidence from detailed studies by J.M. Dohm and R.C. Anderson on the high-resolution images of Mars, taken by NASA's Mars Global Surveyor spacecraft in 1997, that liquid water may have flowed just beneath the Martian surface, raising interest in the possible existence of life on that planet. The largest of the near-Earth asteroids, 433 Eros, an odd-shaped s-type asteroid, was surveyed in close detail by the Near Earth Asteroid Rendezvous Shoemaker spacecraft. In orbit since 1995, the Galileo spacecraft continued to operate in its official extended mission to study Jupiter's large ice-covered moon, Europa: initial data may support the notion that the ice sheets cover liquid water, a prerequisite for life. New solar system objects were discovered. Nine new extrasolar planets were described, including one revolving around the star Epsilon Eridani, 10.5 light-years distant, and one of two planets orbiting HD 83443 likely to have a mass about 50 times the mass of Earth. It may be possible for the Hubble Space Telescope to make direct recordings. A planet about 30% larger than Jupiter was thought to orbit HD209458, a star similar to the Sun lying at a distance of 174 light years in the constellation of Pegasus. According to theory, elements heavier than iron can be formed in the centres of massive stars and spread into space in Supernova explosions. The Chandra X-ray Observatory, operating in Earth orbit since July 1999, provided evidence in 2000 for the detection of newly formed iron in the Supernova remnant Cassiopeia A., formed from a star that exploded in 1680, but the iron was ejected beyond the region where lighter elements such as silica were located. The Chandra Observatory also helped resolve the conundrum over the uniform glow of S-rays, radiation with energies 1 000 to 1 000 000 times that of visible light, apparently coming from all directions. About 80% of the radiation was produced by about 70 million discrete sources uniformly spread over the sky, a third of which appeared to be distant galaxies with black holes at their centres.

Based on the motions of stars in the neighbourhood of the Sun, W. Dehnen of the Max Planck Institute for Astronomy provided evidence in support of the view that the Milky Way Galaxy, which contains the Sun and all the stars visible with the naked eye, is a barred spiral in which the spherical central hub is replaced by a barred structure obscured by interstellar dust.

Manned space exploration was boosted by the ongoing assembly in orbit of the International Space Station and the start of permanent human occupancy. Associated with this was the flight testing of the Crew Return Vehicle, its lifeboat. Long-term sustainability of humans in space will require investments in R&D on recycling waste solids, liquids and gases; combating the effects of microgravity; and the supply of liquids and foodstuffs: plants and microorganisms will be crucial to this work. My own experience during the late 1970s and early 1980s, dealing with the European Space Agency and others, is that this type of research has very low priority in the UK but not elsewhere. As an aside, graviperception is a special feature of differentiated organisms, and although there is a useful pioneering literature on the topic, the advent of space travel means that greater attention will be required to elucidate the biophysical and biochemical factors underpinning growth and other physiological and biophysical responses to changes in gravity.

A computer model possibly explaining the formation of the Sahara Desert was generated by a team led by M. Claussen at the Potsdam Institute for Climate Change. The change from a fertile and inhabited region to a barren desert might be explained in part by the as yet unexplained change in the tilt of the Earth's spin axis about 9 000 years ago from 24.14º to 23.45º over a period of 3 000 years, to 23.40º currently. In addition, there was a movement in the perihelion (the nearest point of the Earth to the Sun), such that it

occurred in July, 9 000 years ago, and is now in January. These two changes were sufficient to modify the weather systems in the northern hemisphere, so that the African monsoons and the vegetation cover declined, altering the hydrology of the area. In turn, less rain fell, the rivers dried up and desertification ensued. The transition from fertility to aridity took place in just a few hundred years. Meanwhile, the changes in the tilt of the Earth's spin axis and timing of the perihelion continue. Computer modelling studies highlighted the close interrelationships between vegetation cover and composition, atmosphere, and ocean currents, with small changes leading to vast effects. This work is germane to the international Convention to Combat Desertification, and to predictions of the effects of climate change.

Experiments using liquid sodium in Riga, Latvia, and Dresden, Germany, confirmed the dynamo theory which proposes that the Earth's magnetic field is produced in the centre of the Earth by the movement of the fluid liquid outer core (consisting of molten iron and nickel) surrounding a solid inner core. According to the theory, a moving conductive fluid can generate and sustain its own magnetic field, but needs to be initiated by a disturbance such as a small electric field. This field can be stretched and distorted by the moving fluid giving rise to new currents and magnetic fields, and eventually a large stable magnetic field.

In previous editions of this *Annual Report*, I have described the essential characteristics of science and the societal need for research; both are fundamental to our appreciation of truth and progress. Reports of misconduct over the past two decades led to several investigations at institutional level and to the intervention of national government, and even to a definition of misconduct generated by the US Office of Science and Technology Policy (see also *Responsible Science,* US National Academy of Science, 1992, and *What Price Progress?* N. Steneck, *Times Higher Education Supplement*, March 16, 2001) Misconduct and behaviour that undermines confidence in science and scientists, and therefore compromise integrity, include falsification of results, wanton or reckless misinterpretation of results, plagiarism, bias in peer-review exercises, clogging the literature with insignificant work, failure to cite correct authorship attributions, misuse of statistics, failure to acknowledge the efforts of colleagues, lack of attention to detail, and inadequate supervision and mentoring. The setting of high standards should not be compromised whatsoever by the source of funding – public or private. Litigation will flush out misconduct or misleading work as it enters the marketplace, or becomes incorporated into public policy, so it is not in the interest of any organisation or company to tolerate bad practice.

A growing divide was noted in higher education between the well-endowed centres of excellence, almost exclusively in the USA, and the rest. A dependence on accessing public funding, which brings in its wake bureaucratic processes and liabilities to ensure public and political accountability as well as significant extra costs and enforced uniformity, caused problems in the UK and many other countries. The scale of the problems faced by the universities related directly to their financial reserves, the quality of their management, the economic well-being of the host country, its attitude to higher education, and fostering high-profile R&D. Measures to increase efficiency included increasing class sizes, creating popular low-cost courses, better utilisation of space, tight financial management, a focus on diversifying funding sources, a switch to profitable R&D, better publicity, and policies to recruit and retain key personnel. Traditional areas of scholarship, particularly in some of the expensive underpinning sciences such as chemistry, physics, and many areas of the life sciences including botany, were under threat. In contrast, distance and e-learning were regarded positively, as they offered the promise of greatly improved efficiency and quality. Tentative steps were made towards institutional mergers in some countries. Underlying the higher education sector was a growing awareness by administrations of students and prospective students as discriminating and vocal customers. The sector also was much more international in outlook, with almost universal interconnection to the Internet *via* institutional intranets.

Public education at the tertiary level (higher education provided mainly in universities and colleges of higher education) should not be assumed to be exempt from the negotiations surrounding the General Agreement on Trade in Services, part of the World Trade Organisation (WTO) agenda. Unless properly protected by robust legislation, this could result in the loss of domestic government control over Research Council funding, degree awards, and the restriction of education funding to public-sector providers. In the UK, the universities are legally regarded as essentially part of the private sector, albeit with heavy

dependence on public funding and strong central controls – only the University of Buckingham is truly independent. Advice to government on universities is provided by the Higher Education Funding Councils (HEFCs) for England, Wales, and Scotland, and by the Higher Education Council in Northern Ireland. The Councils receive block grants from central government for allocation to the institutions.

Libraries as repositories of knowledge as well as culture have been transformed by the Internet. Print materials were increasingly being scanned into digital form. Nonetheless, the costs were rising of maintenance of collections, new acquisitions, security, computer software and hardware, archiving, classification, and improving access to information. Scientific organisations, particularly in the public sector, found the costs of sustaining subscriptions to scientific journals burdensome, let alone subscribing to the stream of new journals. The numbers of on-line scientific journals increased. (See also *Publishers agree on deal to link journals on the web. Nature*, 402, 226, 2000).

Study of the major trends in the contemporary world, *i.e.* cultural anthropology, brings together science, public policy, cultural perceptions, attitudes to change and risk, non-empiricism, ethnography, immigration and identity, codes of ethics, perceptions of the built and natural environment, human rights, kinship and gender, and postmodernist writing. The reissue in 2000 of the 1998 *Handbook of Methods in Cultural Anthropology*, edited by H.R. Bernard, described quantitative and qualitative approaches and methods in important areas of human activity, many of which represent the interface between science, technology and society. More emphasis will have to be placed on this neglected interface, introducing much greater intellectual rigour.

Freedom of the press was actively curtailed in Angola, China, Iran, Malaysia, Swaziland, Zambia, and Zimbabwe. Elsewhere, newspapers and magazines moved to establish on-line readerships, and convergence of print, Internet and television journalism was starting to take place, most notably in Canada. The deaths of the influential cartoonists Jeff MacNelly and Charles M. Schultz depleted the newspapers they served so admirably and journalism generally. Magazines were reported as taking 12.9% of the world-wide expenditure for advertising in 1999. Similar to certain newspapers, and scientific magazines and scientific journals, book publishing began to explore 'e-publishing', the publishing of books in electronic format. There were no universal hardware and software standards for downloading and reading e-texts. Difficulties in safeguarding intellectual property (IP) rights in the new e-format, and taxation and royalty strategies, were not resolved.

### Economics and Politics

In sharp contrast to 2001, the year 2000 witnessed the world economy growing at the fastest rate since 1988, largely as a result of consumer demand in the USA - the global economic dynamo, although a downturn was detected in the second half of the year. According to provisional data from the International Monetary Fund (IMF), real output in 2000 rose by 4.7%, compared with a revised 3.4% in 1999. Relative price stability was maintained for consumer products as a result of tight monetary policies world-wide, although there was volatility in the oil and stock markets, and inflationary hotspots in some countries. For countries undergoing transition from centrally controlled to market economies, consumer prices rose by 18.3%, unacceptably high but markedly less than the 43.8% level of 1999. In the economically advanced countries, consumer prices rose by 2.3%, up from the 1.4% level in 1999 which had then raised the spectre of deflation. Inflation in the less-developed countries (LDCs) fluctuated around an average of 6.2%. In African LDCs, the percentage change from 1999 was 12.7%, and 17.4% for LDCs in the Middle East and eastern Europe.

Growth, as measured by the annual change in real gross domestic product (GDP), was faster in LDCs (5.6%) than in the more-developed, advanced countries (MDCs, 4.2%). Provisional data from the Organisation for Economic Co-operation and Development (OECD, see *Economic Outlook*, November 2000) and the IMF (see *World Economic Outlook*, October 2000) indicated that growth in the USA (5.2%) and Canada (4.8%) significantly exceeded that in other major OECD economies (France 3.3%, Germany 3.0%, Italy 2.8%, Japan 1.9%, and UK 2.9%). By the end of the year, the current-account deficit of the USA was around $450bn. Foreign direct investment (FDI), one of the important features of globalisation, was aided by favourable changes in the regulatory environments of more than 50 countries. FDI outflows exceeded $1 trillion worldwide in 2000, some 20% more than in 1999. The number of companies classified as transnational increased to 63 000, with 690 000 affiliates and sales of $14bn. Transnational mergers

and acquisitions, leading to market consolidations, tended to be 'horizontal' rather than 'vertical', involving closely similar industries and commercial activities, and were valued at around $720bn. The principal mergers and acquisitions involved the automobile, beverage, chemical, food, pharmaceutical, and tobacco industries, and France, Germany, UK, and the USA were the chief beneficiaries.

In the UK, real GDP was estimated by the Bank of England to be 2.9%, compared with 2.1% in 1999. The RPIX changed by 2.1%, earnings growth was 4.6%, and the unemployment rate 3.6%, the lowest rate among the MDCs. After the withdrawal in 1992 of sterling from its ill-fated involvement in the European exchange-rate mechanism, the UK experienced its longest period of sustained growth since 1945. As in nearly all the strong economies, growth was led by domestic consumer demand at a rate (3.6%) that exceeded that of household disposable income (2.5%). An enhanced level of net lending to individuals meant that the household savings rate fell from 5.1% in 1999 to just 3.6% in 2000. The UK was expected to be the fastest-growing nation in the G-7 MDCs, with a relatively resilient, domestic-demand-oriented economy deriving benefit from an easing of monetary policy.

In Scotland, the growth in GDP in 2000 was 1.5% compared with 2.9% in the UK, a lesser performance attributed to its greater dependence on exports, principally to the EU. Even so, GDP growth was driven for the most part by an expansion in the service sector, with a strong reliance on public services. Consumer spending lagged behind that of the rest of the UK (see *Scottish Economic Report*, Scottish Executive, June 2001). Manufacturing exports declined, most notably in the electronics sector. Overall, there was a falling unemployment rate, although it still remained above the UK average.

In the euro zone (or euro area), growth increased from 2.4% in 1999 to 3.5%, aided by a weak euro which made exports more competitive. In the second half of the year, rising oil prices generated rises in consumer prices and falls in real income, aided by an almost doubling of interest rates imposed by the European Central Bank (ECB). As in the previous year, growth was most pronounced (8.7%) in the Irish Republic, compared with 3.5% in France, 3.1% in Italy, and 2.9% in Germany, the three larger euro economies. Elsewhere in the euro zone, growth ranged from 5.1% in Luxembourg, 5% in Finland, 4.1% in Spain, 3.9% in The Netherlands and Belgium, and 3.5% in Austria, Greece, and Portugal. Variations were also noted in inflation rates, reflecting constraints of a single euro-zone interest rate in diverse economies, and ranged from about 4.8% in Ireland to between 2% and 3% in the other countries. The average unemployment rate declined from 9% in 1999 to about 8.3%, but in Belgium, Germany, Greece, and Italy between 8% to 15% of the workforce were unemployed.

According to the IMF (*World Economic Output*, October 2000), the rate of growth in the LDCs was expected to increase from 3.8% in 1999 to 5.6% in 2000, and there were no longer marked differences on a regional basis. Individual countries, however, varied from the 6%-7% range in China and India, to 4%-4.5% in Latin American countries, 2.6% in South Africa or even lower in those African countries disrupted by conflict or political unrest. Zimbabwe was unique in suffering a 6% decline in output coupled to burgeoning inflation, as racially motivated interference by the Marxist Zimbabwe African National Union-Popular Front (Zanu-PF) government in the white-owned commercial farming sector – the mainstay of the economy - undermined confidence.

Another measure of economic activity is the measured increase in the volume of world trade in goods and services, estimated to have more or less doubled from 5.3% in 1999 to 10% in 2000. Thus, the difference in the rate of growth of production (4.7%) and trade was wider than hitherto. Compared with 1999, the dollar rise in global exports was estimated to have been $7 497bn, reflecting trading buoyancy in all regions, and the economic absorptive capacity of the USA. Sales of fuel, manufactured goods and primary products rose in volume terms in both MDCs and LDCs. Import volumes rose by 13% in Canada and the USA, 8.9% in the euro-zone, 8.2% into the UK, 6.8% in Japan, and 14.1% in the newly industrialised countries (NIC)s.

When considered in terms of value, the rise in the rate of exports from the LDCs exceeded 20% and their imports rose to 15% in 2000. From a figure of 17% in 1990, the LDCs attained a level of 27.5% in the share of world exports in 2000, as new manufacturing facilities were opened, and metal prices recovered somewhat. Contrasting with the globalisation trend as expressed in the internationalisation of economies, industries, trade, government policies, legal systems,

political movements, the World Trade Organisation (WTO), pressure groups, science, diets, publishing and broadcasting, terrorism and the Internet – regional trading arrangements were much in vogue in 2000. Although regional trading bloc arrangements shared with the WTO the same vision and processes of integrating trade and services, incompatibilities were seen in several crucial matters. Around 170 regional agreements operated in 2000, with another 70 under consideration. One potentially influential agreement enacted in 2000 was the two-decade partnership, the Cotonon Agreement, between the EU and the African, Caribbean and Pacific (ACP) group, replacing the Lomé Convention.

Globalisation created tensions in those anxious to sustain national sovereignty, preferences and prejudices, culture and independence. Institutions such as the IMF, World Bank, and WTO were subject to protests, sometimes violent in nature by those resentful of their influence, and who claimed that such institutions were unresponsive to domestic civil society and tended to be secretive. Protestors demonstrated at the WTO meeting in Seattle in November 1999, the IMF and World Bank meeting in Washington in April 2000, at the World Economic Forum in Melbourne on 11 September 2000, and at the Prague Summit Meeting of the World Bank and IMF on 26 September 2000.

Globalisation to some is an undesirable manifestation of the spread of international capitalism from the MDCs, but mainly from the G-7 nations, reinforced by the IMF, the World Bank, and the WTO. Free choice, the uptake and exploitation of technologies that improve efficiency and reliability, the free flow of information, the operation of free markets and individual choice which can develop the common good, and lightweight governance, will inexorably favour globalisation. Competition and the deployment of profit for social progress drive improvements, as do enlightened democratic processes that quell corruption and support strong legal systems. Opponents of globalisation, many of whom wished to target inequality, often presented feeble and dubious arguments which were couched in anti-business, anti-mixed-economy, anti-American, anti-technology, anti-capitalism terms and were based on the application of excessive market-distorting regulations, trade barriers, and taxation. International economic integration has become an anathema to others who wish to resist the loss of cultural diversity. Yet foreign direct investments (FDIs) mentioned above affect both the providing (outward) and recipient (inward) economies, sometimes seemingly adversely in the short term, but almost without exception there are net benefits for consumers, government taxation returns, and the higher-paid workers appointed to the new enterprises. Technologies are taken up quickly. According to the *Economist* (29 September – 5 October 2001), in these early stages of globalisation most outward FDIs tend to create exports and represent net complements because affiliates of multinational companies trade with each other. W. Dobson of the University of Toronto and G. Hufbauer of the Institute for International Economics, USA, noted that longer-term capital contributes to economic growth in emerging markets. Whereas there is little evidence that bank loans and trade credits contribute to higher GDP, for every 10 percentage point rise in the ratio of FDI stock to the economy, GDP rises by 4%. Clearly, and perhaps controversially, free trade should properly go hand-in-hand with mobility of appropriate labour.

Complexed with globalisation is the rôle of democratic governments, and the need for checks and balances that prevent democracies operating insensitively as monolithic tyrannies over minorities. General taxation levels have risen in the MDCs, largely to fund social protection, defence, infrastructural, and public-good programmes. Globalisation presents, however, a challenge for governments in the raised expectations of the populace (good public services with low taxes), diminished control over the flow of capital across borders, ownership transcending national borders, international comparators that raise malcontentment, and rapid surveys of public opinions (usually influenced by the media) capable of changing the direction of policy. Biologists at least realise that all forms of diversity affecting human behaviour – cultural, ethnic, linguistic, political, and economic, have their roots in differential gene expression. Governments are beginning to realise that a better-informed population tends to generate contradictory demands and expectations that in turn create political difficulties.

Much criticism during the year was directed at the 'Washington consensus'. This term, introduced in 1989 by the economist J. Williamson, encapsulates the promotion of trade and FDI, fiscal discipline, reduced subsidies, simplified and reformed taxation systems, liberalised financial systems, competitive exchange rates, privatisation, deregulation, and strengthened property rights. Concerns were

specifically expressed at the imposition of these policies on LDCs, leading to unsustainable debts, vulnerability to the excesses of economic cycles, and political instability. Regulatory failures, corruption, and unreasonable expectations undoubtedly caused problems in the inward economies. Although the IMF and World Bank have been justifiably criticised for their prescriptive and inflexible impositions and policy blueprints, and unwillingness to permit longer periods of economic transition, they are usually only called in as a matter of last resort (as did the UK, the last MDC to do so, in the 1970s). At least, it is the case that all countries now have access to the global capital markets, and it is the politics of a country that determine its fate.

Included in the criticism of the IMF and World Bank is the WTO, an organisation specifically mandated to promote international trade. Some believed erroneously that it overrode democracies through newly applied international quasi-judicial routes and was both unaccountable and unrepresentative. Yet the WTO is wholly intergovernmental, operating solely by consensus. All 142 members, soon to be joined by China after its 15-year quest, have a veto. The dispute-resolution rules were agreed unanimously, and require objective and open analyses, rather than subjective imposition. Small nations can participate actively in the struggles between the main trading blocs. With time, it is expected that the secrecy attached to trade negotiations will have to go, even though governments currently insist on the control of information. Eventually, politically tender areas of trade *viz.*: agriculture, textiles, exploitation of natural resources such as minerals and forestry, and corporate interests, look likely to be assimilated without special protection measures in all the remaining areas of trading interchange.

After six years of surplus, the overall current account of the balance of payments in the advanced economies moved into deficit in 1999, rising to a projected $176bn in 2000 and was mainly attributable to the deficit of $420bn in the USA. Of the Group of Seven (G-7) MDCs, only the USA and UK ($20.9bn) had significant deficits. The euro zone remained in surplus. Across Europe, significant surpluses were noted in France ($35.7bn), Switzerland ($24.2bn), Belgium/Luxembourg ($22.9bn) and Norway ($22.6bn). Deficits were posted in Spain ($12.6bn), Portugal ($11bn), Greece ($5.7bn), and Germany ($3.7bn). Outwith Europe, surpluses are projected to have been recorded in Japan ($110bn), Singapore ($22.1bn), South Korea (*circa* $12bn), Hong Kong ($11.2bn), and Taiwan ($6.6bn). Australia and New Zealand reduced their deficits to $18.6bn and $3.2bn, respectively.

An overall current account surplus of $21.1bn was expected for the LDCs, largely as a result of higher oil prices. At a regional level, the Middle East had a surplus of $43.9bn and Asia £39.4bn, but deficits were provisionally recorded for Africa ($3.6bn, a large improvement over $16.8bn in 1999), and Latin America ($58.7bn). Indebtedness of the LDCs rose slightly to $2 068bn, of which $270bn was short-term debt. The most heavily indebted region was Latin America ($775bn).

Investor nervousness and volatility characterised the world's stock markets in 2000. Although 'Y2K', the 'millennium bug', did not materialise to jeopardise trading, a raft of factors contributed to a generally bearish sentiment later in the year. Chief amongst these were the economic outlook of the USA and the bifurcation between 'old economy' and the fragile 'new economy' stocks, especially businesses in the information technology (IT) sector which had been encouraged to develop disproportionately high price-earnings ratios compared with all other sectors, such that the increased weighting of IT stocks in national indices in the early part of the year led to vulnerability as investor and analyst sentiment changed later on. Other factors included rising oil prices, the threat of conflict in various parts of the Middle East, and a weak euro. As several 'dot.com' or Internet-based companies collapsed - the 'Internet bubble' - throughout the year, the technology, media, and telecommunications (TMT) sector suffered, leading to a sharp decline in the National Association of Securities Dealers automated quotations (Nasdaq) composite index and related technology indices in other countries. One of the features of information technology and its close association with aspects of globalisation was the internationalisation of capital markets, cross-border correlation of stock prices and international trading. A debate yet to be resolved concerns the true weighting of stocks in the various key national indices to reflect accurately and openly those that could be bought and sold, as opposed to those constrained by corporate cross-holdings, government holdings or control, or other restrictive devices. Several blue-chip companies would be adversely affected by re-weighting. Attribution of company pension liabilities was also a lively topic of discussion related to true company valuations.

The collapse in technology stock and share prices in April 2000 cut a swathe through public stock offerings for Internet start-up companies. Valuations of existing dot-com and e-commerce companies began to relate more sensibly to their earnings and profitability in the short-to-medium term rather than an evanescent potential for the long term. Similar declines in investment and valuations were noted in media and biotechnology companies. Meanwhile, technological developments in computing and information systems included web-enabled digital wireless telephones, personal digital assistants (PDAs), and other hand-held computers, but limited screen sizes and modem speeds severely restricted their utility. Recordable and rewritable compact-disc drives were beginning to be built into personal computers (PCs) during a year when growth in sales started to tail off, prices declined but computer performance increased. Initial estimates by the Semiconductor Industry Association put global sales of semiconductors at $205bn in 2000, a 37% increase over sales in 1999. Demand for semiconductors came from the PC, data networking, wireless, broadband and optoelectronics (especially laser devices and image sensors) sectors. Rapid growth was noted in programmable logic devices, digital signal processors, microcontrollers, flash memory, and dynamic random access memory.

At a time when e-commerce companies were forced to retrench, raising prices and focusing on profits, the so-called 'bricks-and-mortar' conventional companies began to use the Internet for marketing. In the USA, legal status given to electronic signatures began to permit on-line electronic contracts. Business-to-business web sites also began to take shape, but globally the complex issue of Internet taxes and bartering challenged national and more locally based taxation centres.

Loss of privacy and confidentiality in cyberspace by the actions of commercial profilers of personal on-line activities, and other surveillants, became major concerns. The breaching of commercial confidentiality of financial information and intellectual property was a closely related issue. Internationally, there was no robust cross-border legislation to deal with Internet crime, and hackers and destructive software 'viruses' caused destruction of computer files, immobilisation of corporate computer servers, and extortion. One growing problem was the use of the Internet by people, sometimes anonymously, to criticise, vilify or even libel individuals and corporations, currently without proper redress.

According to the *Financial Times*, only the Toronto Composite (Canada), ISEQ overall (Republic of Ireland), Milan Banca Comm.Ital. (Italy), and SBC General (Switzerland) stock markets recorded increases above the levels at the end of 1999, whereas declines were noted in the remaining major global stock markets. The Morgan Stanley Capital International World Index declined by 15%. Cross-border mergers of European exchanges were entertained, and, by the end of the year, the outlook for equities was depressed.

A hotly debated topic was the rôle of hedge funds which, in the view of some, weaken stock markets by short-selling, break down exchange-rate pacts, and target shares leading to a collapse in share prices and thereby to bear markets. Hedge-fund managers, however, pointed out their rôle in the creation of balanced markets, as well as their investment potential in recent years as an alternative to traditional equity investments.

A dramatic rise in oil prices from relatively low prices to a high in September 2000, caused popular unrest in Europe and malcontentment in the UK of high fuel-tax impositions. The partial cartel operated by the Organisation of Petroleum Exporting Countries (OPEC) was mildly effective at stabilising relatively high prices, but a shortage of refinery capacity; low strategic reserves in importing, fuel-dependent economies; the threat of conflict; the increasing influence of non-OPEC exporting countries such that they more than matched OPEC output; and a downturn in manufacturing, all contributed to a non-stable situation. Low prices still afflicted agricultural commodities, and consequently the economies of those countries with large agricultural sectors. As input costs rose, and quality-control measures were becoming the norm, competitive pressures suppressed agricultural prices. In many MDCs, a combination of statutory controls relating to health and the environment, the influence of supermarkets, new technologies, pressure groups and marketing strategies led to bleak predictions for the future of their indigenous agricultural and horticultural industries.

Rural tourism interdigitates with agriculture and horticulture. It is reliant on the visual amenity and other free benefits, as well as paid benefits, provided by

these underpinning primary industries. Only when large-scale deleterious changes in the landscape take place, or when travel restrictions are introduced following pest and disease outbreaks - amplified by ghastly media reports as in the UK's 2001 foot and mouth disease outbreak, is this dependence revealed. The UN International Labour Organisation estimated that in 2000 the tourism industry as a whole employed 207 million people, equivalent to 8% of global employment. According to the World Tourism Organisation, international tourism expenditure in 2000 was *circa* $65bn in the USA, $46bn in Germany, $35bn in the UK, $32bn in Japan, $16bn in France, $156bn in Italy, and $11bn each in Canada and the Netherlands. International tourist arrivals were 75 million in France, 50 million in the USA, 48 million in Spain, 41 million in Italy, 30 million in China, 25 million in the UK, and around 20 million each in Russia, Mexico, and Canada.

In the forest products industry during 2000, pulp prices increased whereas lumber prices declined. The textile industries in the MDCs suffered from low prices and competition from the LDCs and NICs. Despite political attacks on the US pharmaceutical industry during the presidential campaign, and the threats posed by generic pharmaceuticals, the major pharmaceutical companies prospered. Even so, many politicians and pressure groups were beginning to regard the industry world-wide as a taxation milch-cow at best, or target for protest at worst, despite its massive investments in biomedical R&D. The tobacco industry also prospered irrespective of actual and threatened legal actions, anti-smoking initiatives in most MDCs, and the imposition of high taxes by certain countries.

Consolidation in the banking and securities industries reshaped the global banking and financial services sectors. Procedures were put in place to eliminate local currencies in the euro zone to replace them with euro banknotes and coins by 1 January 2002. Regulatory and supervisory relationships in the banking and securities industries exercised governments of most countries, not least in the light of the formation of highly complex international groupings, and the need to reduce unnecessarily convoluted restrictive processes without undermining confidence. Loan loss provisions, classification of assets, pension provisions, risk-management processes, financial-reporting practices, and the elimination of money laundering were the main common themes. In June 2000, the report of the international Financial Action Task Force on Money Laundering identified 15 jurisdictions operating with inadequate measures, including The Bahamas, Cayman Islands, Dominica, Israel, Liechtenstein, the Philippines, and Russia.

Comparative performance indicators of countries were (widely) used by analysts to evaluate their contributions to global and regional trade, their gross national product, labour force, the effectiveness of their governments and body politic, their educational and health and other social indicators, transport and communications, demographic indicators, taxation levels, military expenditure, and standard of living. Other indicators included the seaward claims for territory and fishing and economic rights, and land claims. These wide-ranging indicators were used extensively in 2000 for international negotiations in both the public and private sectors; they were important for inward-investment decisions. The principal indicators (i) were rarely up-to-date; (ii) were subject to methodological problems whereby data may be compiled on different bases; (iii) suffered from the fact that the sources of the data varied to a considerable extent; (iv) were difficult to compare because some data were classified as preliminary, final, revised or adjusted; (v) varied because the accounting interval may vary from Gregorian calendar year, a fiscal year, an Islamic or other national or religious year, a cropping year, a multiyear period or average, especially when a single year would be unrepresentative; and (vi) may use data sets that are discontinuous. Interpretation of these data to gauge national effectiveness, or even to present the data in a straightforward manner, are fraught with difficulty, and it is rare to access crisply presented authoritative global data comparable to the *Britannica World Data* published by *Encyclopaedia Britannica.* Other valuable sources of reference include the *World Bank Atlas*, and the associated *Global Development Finance*, and the *World Development Report*; the IMF's *Direction of Trade Statistics Yearbook, Government Finance Statistics Yearbook* and the monthly *International Financial Statistics*; *Keesing's Record of World Events*; the OECD's *Economic Surveys*, and *Financing and External Debt of Developing Countries*; Pennwell Publishing Company's *International Petroleum Encyclopaedia*; the UN's *Demographic Yearbook, International Trade Statistics Yearbook, Population Studies, National Accounts Statistics,* the quarterly *Population and Vital Statistics Report, Statistical Yearbook,* and the biennial *World Population Prospects*; UNESCO's *Statistical Yearbook*; UNIDO's

*Global Report*; the US Central Intelligence Agency's annual T*he World Factbook;* and the WHO's *World Health Statistics Annual*.

Increasing attention was given to membership and adherence to the precepts of the relevant premier international organisations, *viz*. the main UN organs and affiliated intergovernmental organisations (United Nations Conference on Trade and Development, UNCTAD; United Nations Children's Fund, UNICEF; International Court of Justice, ICJ; Food and Agriculture Organisation, FAO; International Atomic Energy Agency, IAEA; International Bank for Reconstruction and Development, IBRD; International Civil Aviation Organisation, ICAO; International Development Association, IDA; International Finance Corporation, IFC; International Labour Organisation, ILO; International Monetary Fund, IMF; International Maritime Organisation, IMO; International Telecommunications Union, ITU; United Nations Educational, Scientific and Cultural Organisation, UNESCO; United Nations Industrial Development Organisation, UNIDO; Universal Postal Union, UPU; World Health Organisation, WHO; World Intellectual Property Organisation, WIPO; World Metereological Organisation, WMO; and the World Trade Organisation, WTO); the Commonwealth of Nations; six regional multiple-purpose bodies (the European Union, EU; Gulf Cooporation Council, GCC; League of Arab States, LAS; Organisation of American States, OAS; Organisation of African Unit, OAU; and the South Pacific Commission, SPC), and finally nine economic organisations (African Caribbean and Pacific [Lome IV] Convention, ACP; Asian Development Bank, ADB; Asia-Pacific Economic Cooperation Council, APEC; Caribbean Community and Common Market, CARICOM; Economic Community of West African States, ECOWAS; The Franc Zone, FZ; Inter-American Development Bank, I-ADB; Islamic Development Bank, IDB; and Organisation of Petroleum Exporting Countries, OPEC).

Afghanistan was reported by the UN Office for Drug Control and Crime Prevention to be the largest opium producer in the world and becoming a major heroin manufacturer. Lack of funding and local political involvement in the trade closed the drug-control programme in that country.

Political turbulence affected several member countries of the Commonwealth of Nations at a time when it appointed a new Secretary-General (D. McKinnon). There was the military takeover in Fiji; the enforced removal of the Prime Minister of the Solomon Islands; the land-reform/land-seizure crisis in Zimbabwe mentioned above; a delay in the return to civilian rule in Pakistan, a developing country hosting large numbers of Afghani refugees and in dispute with India over Kashmir; and the civil war in Sierra Leone. Political advancement took place, however, with progress in adopting tighter rules of membership and stopping the attendance of military leaders at its meetings. In addition, pressure was put on the World Bank and the OECD to deal sensitively with small nation states.

Expansion of the EU into former communist countries in Eastern Europe was retarded by the slowness in reforming the Common Agricultural Policy (CAP) and the efforts primarily of France and Germany to engage in deeper political and economic integration. Disapproval of the election in Austria of a government coalition involving the far-right Freedom Party led to the unprecedented act by the other 14 EU partners of freezing bilateral contacts. By September, a review team concluded that the new coalition had not strayed from 'European common values', in effect rescuing the EU from deadlock in its reform programme which requires unanimity. Further internal political crises in the EU were spawned by the proposal floated by the German Foreign Minister, K. Fischer, of a fully fledged European government with a written constitution, elected President, diminished national parliaments, and therefore even weaker regional assemblies. The proposal was seized on by those opposed to seemingly inexorable further political integration or who wished to unwind the current level of central control. Similarly, there was debate as to whether the EU should run its own foreign policy and defence, thereby creating disharmony with the North Atlantic Treaty Organisation (NATO) and some of the more prominent member states. Plans were drawn up in the face of opposition by the staff unions to modernise the operation of the European Commission itself, tightening up staff training, discipline, and processes to permit 'whistle-blowing'. The disgraceful treatment in 1999 of P. van Buitenen, the auditor, led to the resignation *en masse* of the Commission on 16 March 1999 on the grounds that lax management had allowed fraud and nepotism in the Commission's services.

Throughout 2000, the euro was generally weak against the dollar and yen, leading to the stimulation of inflation in the euro-zone. A stated reluctance of

the European Central Bank (ECB) to intervene in the market place was thought to have undermined confidence.

Against this background of a slump in the value of the euro, and the rejection in a referendum in Denmark of adopting the single currency, reinforcing the views held by many in Sweden and the UK that adoption of the euro was not inevitable, European integration and enlargement were fraught issues. Preliminary and technical analysis continued nonetheless to prepare for the five economic tests to determine whether the UK Government should recommend entry to the euro (see www.hm-treasury.gov.uk/pdf/2001/p11901.pdf; for business implications of the introduction of the euro see www.euro.gov.uk; and www.europa.eu.int/euro;). Concerns were expressed about the disconnection between the public and politicians in the speed and direction of change. Plans were nonetheless advanced to admit 13 new countries to the EU: ten former communist countries, Bulgaria, the Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Romania, Slovakia, and Slovenia; plus Cyprus, Malta, and Turkey. The Commission president, R. Prodi, announced that negotiations on the terms of entry of the "more prepared" nations (Cyprus, the Czech Republic, Estonia, Hungary, Malta, and Poland) should be concluded by the end of 2002, with full membership by 2004-2005. At the Nice Summit in December, national interests soon prevailed over European integrative moves. Vetoes were retained, *e.g.* over taxation policy, and there were disputes over voting weights.

Partnership and co-operation agreements based on regulating and improving political and economic relations, and mutual trade concessions, but which exclude any possibility of membership, have already been signed with Moldova, Russia, and Ukraine (1994); Belarus, Kazakhstan, and Kyrgyzstan (1995); and Armenia, Azerbaijan, Georgia, and Uzbekistan (1996). The procedure for accession to the EU is laid down in the Treaty of Rome. Thus, nation states must be stable European democracies, governed by the rule of law and with free-market economies. In essence, the procedure involves a study of the membership application by the Commission which leads to an Opinion which, if positive, leads to negotiations followed by an Accession Treaty. Approval of the Treaty is required from all member state governments and parliaments, the European Parliament, and the government and parliament of the applicant state.

The European Parliament expanded its influence through the Single European Act of 1986, the Maastricht Treaty (1991) and the Amsterdam Treaty (1997). It is deliberately biased in favour of multi-national political groupings, and has incrementally increased its degree of democratic control. The legislative process is formally described as a dialogue between the European Commission, the Council of Ministers and the European Parliament, but the European Commission and the European Central Bank (ECB) have limited legislative powers within their fields of competence. Funding of the European Community budget is derived from four sources from each member state : levies charged on agricultural imports into the EU from non-member states; custom duties on imports from non-member states; contributions based on the share of a notional Community harmonised Value-Added Tax base; and contributions based on the share of community gross national product (GNP). The latter is a so-called budget-balancing item and covers the difference between total expenditure and the revenue from the other three sources. According to the *General Budget of the European Union for the Financial Year 2000*, agriculture accounted for 44% of the total spend of 92.7 billion ECU, regional and social aid 35.1%, external action 5.1%, pre-accession aid 3.4%, research and technology 3.9%, other internal policies 2.6%, administration 5%, and reserves 1%.

Manifold difficulties were faced in 2000 by members of the Association of South-East Asian Nations (ASEAN, Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar (Burma), the Philippines, Singapore, Thailand, and Vietnam). Northeast Asia was clearly advancing economically faster than Southeast Asia; Indonesia faced political, religious, and separatist strife; concerns were expressed about the differences between North and South Korea, and between China and Japan, and China and Taiwan; the UN had reported that the region had 1.3 million new cases of HIV infection in 1999. The member states voiced their commitment to developing a free-trade area despite the desire of some members to protect sensitive industries. The free-trade theme was also shared with the Asia-Pacific Economic Co-operation (APEC; Australia, Brunei, Canada, Chile, China (Hong Kong), Indonesia, Japan, Republic of Korea, Malaysia, Mexico, New Zealand, Papua New Guinea, Peru, the Philippines, Russia, Singapore, Taiwan, Thailand, USA, and Vietnam). In

November, members agreed to begin negotiations to eliminate trade barriers, but did not address the fears of some LDCs that the EU and the USA would impose labour and environmental standards through the WTO and bilateral arrangements that would reduce the competitiveness of poorer nations.

The creation of any single market area requires a common patent system. At the European Council of Lisbon, a decision was taken to introduce a 'community patent' from the end of 2001. Three profound problems will need to be addressed, namely: (i) the choice of language; although English is widely accepted as a suitable technical language, cultural sensitivities mean that the ongoing enormous costs of translation are likely to continue; (ii) an intellectual property (IP) tribunal will have to be set up at the European Court of Justice in Luxembourg, and on grounds of cost it may have to set up decentralised channels, but the vested interests of many national patent lawyers will strongly oppose this aspect; and (iii) the bureaucratic processes to bring the common patent system into being are not fully integrated, and are unlikely to be changed in the short term, given the fact that the various national patent offices will have to concede power, and the essential services they provide users may suffer. Presumably, the European Patent Office in Munich will have to examine and grant new single patents.

Patenting costs, including associated litigation and licensing arrangements, mean that most public sector R&D bodies and all but the most affluent companies have to review constantly their patenting strategies and tactics. Failure to patent could mean that one or more competitors could gain the patent and protect it by closing off a market or demand licensing fees. On the one hand, failure to patent properly often means that incremental improvements and derivatives have to be patented too, or the original patent becomes surrounded or 'picket-fenced' by competitors. On the other hand, existing and potential competitors can be disqualified from patenting by 'defensive' publishing of information about the invention so as to create 'prior art'. In order to facilitate this defensive strategy, specialist journals such as *Research Disclosure*, websites such as *IP.com,* and its associated *Priorart.org*, and others act as public disclosure routes, with ready access to patent offices. Defensive publishing of incremental improvements could stop picket-fencing, but may have several drawbacks. Thus, it could be used to undermine or even dismantle the patenting system, adversely affecting investments in research-intensive organisations and technology-transfer companies, and quelling enquiry and investments more generally in innovation.

Agricultural biotechnology (agbiotech) experienced difficulties in IP matters internationally, mainly in restricted access to IP (or lack of freedom to operate), variable regulatory regimes, overlapping patents and patent claims, inadequate technology-transfer systems, and the burden of transaction costs associated with convoluted IP rights. Almost akin to orphan medicines, minor crops (especially horticultural crops) and many crops which are essential for life in LDCs are for the most part ignored in agbiotech R&D programmes in the MDCs. Helpful proposals to improve the situation were made in *IP Strategy Today No. 3-2001* (see www.bioDevelopments.org), including IP 'clearinghouses' to reduce the complexity and transaction costs of the 'patent thicket' and IP 'aggregators' for bringing together smaller interested parties, as well as for mutually interdependent patents over a common technology system scattered across multiple parties. As pointed out in *Slow Magic. Agricultural R&D a Century after Mendel* by P.G. Pardey and N.M. Beintema, International Food Policy Research Institute, 2001, there are 114 members of the Patent Co-operation Treaty administered by the UN World Intellectual Property Organisation. All 141 members of the WTO must comply with the terms of the Trade-Related Aspects of Intellectual Property by 2005. Moreover, 49 countries, including 28 LDCs, are signatories to the UPOV Convention (the International Union for the Protection of New Plant Varieties) that provides certain rights to plant breeders.

A measure of IP awareness in the UK was detailed in the *Annual Report and Accounts of the UK Patent Office (2000/2001)* (see www.patent.gov.uk/about/reports/index.htm). Patent applications (more than 31 000), registrations for trade marks (76 290), and design registrations (9 380) were substantially greater than in 1999. Most patents were granted in the telecommunication sector (1 110), civil engineering (535) and data handling (526) sectors. There were 6 856 applications published for scientific trade marks.

At a time when governments and individuals have become conscious of health costs and the efficiency of public and private health systems, the report from the World Health Organisation *The World Health Report 2000 – Health Systems: Improving Performance* was

timely. In an analysis of standards, effectiveness and responsiveness in 191 countries, it was recognised that it would be difficult to adopt a single healthcare model. Medical advances, ageing populations, rising expectations, and rapidly accelerating costs caused political and social problems in many countries, not least in publicly funded health systems such as that of the UK National Health Service (NHS). A 'socialised' medical system which purported to offer free access to high-quality healthcare, the NHS was under great pressure, rather like the public transport network, and despite increased funding and dedicated staff, was unable to meet expectations, denying expensive treatments to those that needed them and delaying treatments generally. Dissatisfaction was also voiced about public health systems in Canada, The Netherlands, Norway, and Spain. *Per capita* health expenditure in 1997 was greatest in the USA, $3 724, 41% of which was public expenditure, but in overall terms of efficiency was only ranked 37th as it did not cover uninsured people adequately. France was considered to have the best healthcare system, although its *per capita* expenditure, $2 125, 77% of which was public expenditure, was less than that of the USA, Switzerland ($2 644), and Germany ($2 365). The UK had a relatively lowly ranking at $1 193, 97% of which was publicly funded. Little R&D was supported by the NHS.

Many MDCs were concerned about the long-term funding and viability of their social security (protection) programmes. The rôles of private-sector providers, new technologies to enhance delivery, and policies to improve fairness but discourage dependency, were under continuing scrutiny. Combinations of insurance schemes, means-testing, payroll taxation, tax credits, involvement of charities, employer-based schemes, and a policy focus on the most needy were operated with varying levels of success. To this was factored in many MDCs a rapidly expanding proportion of older people in the population, early retirement schemes, healthcare and pension expectations, and pressure exerted by the broadcast and publishing media. In the newly emerging countries and LDCs, reform efforts were made against a backdrop of poverty and ill-health. There was disagreement in 2000 on the five main ways to alleviate poverty in the LDCs: loans, grants, debt-forgiveness, economic and market restructuring to improve productivity and wealth creation, and increasing the proportion of MDCs GDP to donate to LDCs to reach the UN target of 0.7%. Insufficient attention was given to confronting corruption and terrorism. International or globalised social security agreements operated only to a limited extent.

Globalisation strongly influenced the development of international law in 2000, and numerous international agreements were introduced. The establishment of a Caribbean Court of Justice was agreed in principle, to join three existing permanent international courts: the International Court of Justice, the European Court of Justice, and the European Court of Human Rights. Non-permanent bodies, such as the International Criminal Tribunal for the former Yugoslavia, the International Criminal Tribunal for Rwanda, and the Marshall Islands Nuclear Claims Tribunal, met during the year. By the end of 2000, 139 nations, including the USA and Israel, had signed, and 27 nations had ratified the treaty – the Rome Statute – to establish the permanent International Criminal Court, under the auspices of the UN, and first voted for in 1998. It would bring to international justice those individuals accused of the most serious violations of international humanitarian law or values. Harmonisation of national and international laws was particularly challenging in the functioning of the WTO, and in the imposition mentioned above of EU sanctions against Austria, a member state. During the year, there was settlement of the first cases under the new Uniform Domain Name Dispute Resolution Policy of the Internet Corporation for Assigned Names and Numbers. In May, the G-7 countries and Russia convened the first international gathering of law-enforcement agencies, civil servants and prominent private-sector individuals to combat computer crime. In January 2000, more than 130 countries signed the Cartegena Protocol on Biosafety, which requires notification if any transported material has been genetically modified.

International and local terrorism was evident, but at a relatively low level in 2000. Drug trafficking, however, was a significant factor in international conflicts, murder, terrorism, and other violence. Transparency International, a non-governmental organisation (NGO) based in Berlin and established to expose and prevent corruption, reported in September that Nigeria was the most corrupt country. The eight cleanest national administrative environments were Canada, Denmark, Finland, the Republic of Ireland, New Zealand, Norway, Singapore, and Sweden.

### Conflicts and Populations

Population growth and changes in the age composition of the population can be predicted on the basis of (i) fertility and whether the number of births per woman exceed, match or fall below the level required for each generation to replace itself; (ii) mortality and life expectancy data; (iii) effects of migration on particular regions and countries; and (iv) age distribution, particularly the ratio between young and old. Global population projections by the UN and World Bank since the 1950s have been remarkably accurate, within 4% of the measured totals. Difficulties were noted for country projections, most notably for LDCs, not only in respect of acquiring reliable primary data, but there were inherent errors, arising from trying to quantify the uncertainty of trends. In recent times there has been a continuing decline in fertility in MDCs, a rise in life expectancy world-wide, and a persistence of migration to a number of major receiving countries. According to *Beyond Six Billion: Forecasting the World's Population* by the Panel on Population Projections, edited by J. Bongaarts and R.A. Bulatao, current world population projections to 2050 are based on reasonable assumptions but several improvements could be made to the forecasting methodology, principally by (i) reducing the assumed rate of fertility decline as fertility approaches the replacement level in countries now in transition; (ii) removing an assumed ceiling on life expectancy; (iii) maintaining net migration around current levels for several decades for receiving countries; (iv) using more reliable baseline data, requiring investments in censuses, surveys and vital registration; and (v) timely updating of projections. Beyond 2020 and in the absence of catastrophic happenings, uncertainty accumulates rapidly and nonlinearly. It is reasonable to estimate that the global population will grow from the current 6bn level to between 8 and 11bn in 2050. Nearly all of this growth will take place in the LDCs, and there will probably be population declines in several MDCs. All of the major agencies (the UN, the World Bank, the US Census Bureau, and International Institute for Applied Systems Analysis, and related institutions) accept that world population growth will continue at least to 2050. By this date, it is expected that the most populous countries will be India (1 529 million), China (1 478 million, USA (349 million), Pakistan (345 million), Indonesia (312 million), Nigeria (244 million), Brazil (244 million), Bangladesh (212 million), Ethiopia (169 million), and the Democratic Republic of the Congo (160 million). Thus, the USA will be the only MDC in the listing. Periurban and urban agriculture will become pressing topics for planners, scientists, and politicians. The European population is projected to decline markedly and the proportion of the elderly in the population will rise rapidly. According to *Encyclopaedia Britannica*, at midyear 2000 there were an estimated 6 080 141 683 people on Earth, some 77 632 256 more than in the year before.

The UN was involved in 15 peacekeeping operations, deploying more than 37 000 military personnel and civilian police from 88 countries. Africa was the most troubled continent, with wars affecting Burundi, the Democratic Republic of the Congo, and other countries in Central Africa as an aftermath of the 1994 genocide in Rwanda. A virulent, nine-year civil war in Sierra Leone was quelled by British special forces. Rebel forces disrupted the border areas of Guinea and Liberia. In December, Eritrea and Ethiopia signed a peace agreement after a prolonged series of offensive actions. Civil war persisted in parts of Asia. Afghanistan was racked by the efforts of the Taliban Islamic militia to seize overall control from an assortment of warlords. The separatist Liberation Tigers of Tamil Eelam fought with the government troops of Sri Lanka. Muslim separatist guerrillas fought the Philippine armed forces. China, an undemocratic country, blatantly threatened to use force to retake Taiwan, a wholly democratic country. The Middle East remained politically fragile, with little sign of peace between the Israelis and Palestinians. Israel terminated its occupation of southern Lebanon in May but the West Bank and Gaza witnessed scenes of violence. A suicide attack was mounted against the USS *Cole* in Aden. Enforcement of the no-fly zones in northern and southern Iraq was sustained by the UK and USA, and Turkish forces made an incursion into northern Iraq to combat Kurdish rebels.

Russia was engaged in attempting to take full control of Chechnya, virtually destroying its capital, Grozny, but failing to pacify the mountainous southern zone. Related in part to the activities of other militant Islamic fighters in the inhospitable mountainous region where Kyrgyzstan, Tajikistan, and Uzbekistan share common borders, the six member states (Armenia, Belarus, Kazakhstan, Kyghyzstan, Russia, and Tajikistan) of the 1992 Commonwealth of Independent States Collective Security Treaty agreed to create a joint rapid-reaction force to deal with any external aggression or terrorism.

Under the protection afforded by the Kosovo Force (KFOR) of over 40 000 troops in the Serbian province of Kosovo, and the 32 000 troops of the Stabilisation Force (SFOR) in neighbouring Bosnia and Hercegovina, the Balkans settled down to an uneasy peace. The profundity of the animosity between the communities would indicate that peacekeeping would be needed for several years.

In April 2000, the Russian government finally ratified both the 1993 Strategic Arms Reduction Talks (START-III) treaty and the Comprehensive Test Ban Treaty. START-III talks to make further cuts in the nuclear arsenals floundered on the claim by Russia that the development of a national missile defence system by the USA would undermine the Anti-Ballistic Missile Treaty, signed with the former Soviet Union. Even so, at the Review Conference of the Nuclear Non-Proliferation Treaty (NPT), the avowed nuclear weapon states (China, France, Russia, UK, and USA) unequivocally undertook to reduce and eventually eliminate their nuclear weapons; no timetable was set. India, Israel, and Pakistan were called on to become signatories of the NPT. Bioterrorism and biological warfare were beginning to be regarded as worrying as nuclear warfare.

There was an increase in the number of people classified as refugees and persons of concern to the Office of the United Nations High Commissioner for Refugees (UNHCR), from 21.5 million in 1998 to 22.3 million in 1999. The scale of the humanitarian crisis was that the latest figure represented more than 1 in 300 of the global population, and that more than 50% of the number were women, and 41% were children under 18 years old. Yet even the numbers cited did not record adequately those affected by conflict.

Africa was the continent of most concern. Almost half of the population of Sierra Leone, *i.e.* 2.5 million people, were not able to access relief aid because of the fraught situation and despite a cease-fire agreement. About 11 000 of the population had been displaced. Liberia was also unsettled although 38 000 Liberians were repatriated in 1999. The largest refugee population in West Africa, more than 500 000 people, was in long-suffering Guinea, largely unrecognised by MDC donors for its hospitality. Refugees also flooded into the Central African Republic from the adjacent Democratic Republic of the Congo. Instability persisted in the Great Lakes region irrespective of the 1999 Lusaka cease-fire agreement. Since 1998, over 90 000 Congolese refugees resided in Tanzania, and 25 000 in Zambia. Over 38 000 Rwandan refugees were repatriated, but by the beginning of 2000, Tanzania faced the burden of nearly half a million refugees from unstable Burundi, the Democratic Republic of the Congo, and Rwanda. UNHCR was involved in the repatriation of 100 000 Eritrean refugees in the Sudan, and large numbers of Somali refugees from Djibouti, Ethiopia, and Kenya. Angola was enmeshed in its 26-year civil war, leading to sub-populations of more than 2.6 million internally displaced persons, and tens of thousands of refugees in Namibia, Zambia, and the Democratic Republic of the Congo.

In Asia, the principle areas of concern were Afghanistan, Bhutan, Myanmar (Burma), parts of the former Soviet Union (Chechnya, Ingushetia, Georgia, and Kazakhstan), Indonesia, Kashmir, East Timor, and the Middle East. Millions of refugees from conflict and drought in Afghanistan sought refuge in Iran and Pakistan, and were the largest refugee group in the world. As of March 2000, UNHCR provided relief assistance to 180 000 displaced Chechens in Ingushetia. It was estimated that the violence in East Timor led to the displacement of 75% of its population, and the conflict in Sri Lanka caused the displacement of 700 000 people with an additional 70 000 refugees in India.

During the past two years, the UNHCR estimated that 600 000 people had been displaced within Colombia, joining 500 000 who had been displaced by earlier troubles. It was the area of most concern in South America.

Disruption continued in the Balkans with the return of displaced ethnic Albanians to Kosovo in July 1999. By mid-July 2000, 210 000 Serbs, Roma (Gypsies), and other ethnic minorities were forced to flee Kosovo. In contrast, large numbers of refugees and internally displaced persons returned to Bosnia and Hercegovina, and Croatia.

For most countries, repatriation was regarded as the preferred solution to the situation of refugees, rather than offer resettlement in their new host country. In 1999, 45 000 refugees were resettled in receiving countries; far fewer countries offered local full integration, an option dependent on the willingness of refugees to adopt the local culture and language. For many refugees, particularly Afghanis and Palestinians, their refugee status has persisted over decades. The

difficulties faced by returning displaced persons and refugees should not be underestimated. In many instances, their homes and livelihoods have been destroyed or taken by others, the infrastructure of the country or region has been damaged, their families broken up, and they are subject to a life of poverty, often in politically unstable areas. Feeding them is a challenge to the MDCs.

Failure to prevent the 1994 genocide of 800 000 people in Rwanda led to a panel from the Organisation of African Unity (OAU) to propose that nations and institutions such as Belgium, France, the UN, USA, and the Roman Catholic and Anglican churches were culpable and should pay reparations. The Panel asked the UN Secretary-General, K. Annan, to establish a commission to deal with the issue, and also requested that creditors cancel the international debts of Rwanda.

In *Deliver Us from Evil: Warlords & Peacekeepers in a World of Endless Conflict,* William Shawcross questioned the ability of the UN to solve the problems of local wars, refugees and genocide without the member nations fully implementing its Charter.

According to The World Bank, in *World Development Indicators 2000*, 2.8bn people, nearly half the global population, survive on an income of less than $2 per day, while 1.2bn live on less than $1 a day, 291 million of whom live in sub-Sahara Africa, and 522 million in South Asia. In the UNDP *Human Development Report 1999*, it was estimated that about 1.1bn people are rated as malnourished, 1.2bn do not have access to clean water, and for the poor, contaminated water, and diseases such as cholera, tuberculosis, and AIDS have become acute.

### Agriculture and Food

According to the UN Food and Agriculture Organisation (FAO), total agricultural production increased by around 1%, in both MDCs and LDCs. Total food production increased likewise, but *per capita* food production fell in the LDCs because the increase in food output did not adequately match the increase in population, contrasting with an increase in *per capita* food production in the MDCs.

Particular problems in production were noted in the former Soviet Union and Eastern European countries undergoing transition from centrally planned to market economies, such that agricultural outputs remained depressed to levels some 30% lower than a decade earlier. Despite potentially highly productive land resources, a combination of poor infrastructural development, corruption, old-fashioned agronomic practices and cultivars, failure to take on board entrepreneurial approaches, and constraints on land ownership, impeded development, a situation made worse by the downward pressure on international commodity prices. Sub-Saharan Africa, including Central Africa, also suffered production difficulties, not aided by expanding populations leading to declining *per capita* food production. Many countries experienced drought. Mozambique had catastrophic flooding; other countries such as Ethiopia, Eritrea and Angola were embroiled in conflict.

Food aid, as measured by shipment in cereals, declined to low-income, food-deficit countries (LIFDCs) from 7 908 million metric tonnes (mmt) in the period July 1998-June 1999 to 6 779 mmt in the period July 1999-June 2000, but increased to other countries, such as Russia, from 3 126 mmt to 3 449 mmt. The primary source of food aid was the USA (6 693 mmt), followed by the EU (1 324 mmt), Canada (349 mmt), Japan (303 mmt), Australia (264 mmt), Norway (48 mmt), and Switzerland (40 mmt). Russia was the single largest recipient of food aid from the USA.

Trade liberalisation fostered by the WTO did not extend to matters agricultural, following the unproductive Seattle event in late 1999. Trade-distorting subsidies were criticised by the Cairns Group (Argentine, Australia, Brazil, Canada, Chile, Colombia, Fiji, Indonesia, Malaysia, New Zealand, Paraguay, Philippines, South Africa, Thailand, and Uruguay), highlighting the extraordinary protectionist measures adopted by the European Union, Japan, and South Korea, mainly on the basis that agriculture provides indirect, difficult-to-quantify benefits such as visual amenity, food security, and rural cultures. A raft of production-related subsidies, export credit and credit-guarantee programmes, peripheral subsidies, and import barriers seen in the protectionist countries is associated with complex bureaucratic processes and regressive attitudes to agriculture. Potential enlargement of the EU to include agriculturally dependent countries in Central and Eastern Europe would create enormous pressures on the EU total budget unless substantial reforms including subsidy downsizing were to take place.

In the EU, agriculture continued to enjoy relatively high levels of protection from the vagaries of the marketplace. Despite a series of reforms since its inception in 1957, including the reforms of 1984,

1988 and the MacSharry Reforms of 1992 and the modifications brought about by the Uruguay Round Agreement on Agriculture (1997-1999), the Common Agricultural Policy (CAP) absorbed a disproportionately large component of the EU budget. Born of a drive to increase agricultural production, provide a fair standard of living for farmers, and ensure the availability of food at reasonable prices, as well as protect small-scale, sometimes peasant-level agriculture and rural culture, the bureaucratically complex CAP distorted international trade and weakened the international competitiveness of EU agriculture. By maintaining a combination of (i) common pricing systems with associated market intervention; (ii) sustaining a dual-pricing system such that internal EU prices were kept above those in the rest of the world through direct intervention, import duties, and export refunds; and (iii) direct payments to farmers for production and output; the CAP was responsible for unfair trading, creating inducements for excess production, stultifying technological advancement, and generating anomalous actions such as 'set-aside', quotas, corruption, and overburdening paperwork. Transformation of the CAP requires unanimity of agreement between the Member States, so given the level of vested interests, it is unlikely that substantial changes will be brought about unless the deficiencies are likely to imperil the existence of the EU as a whole during enlargement or economic stress.

In 2000, France received the largest allocation (£5.7bn) of EU funds for agriculture, compared with Germany (£3.6bn), Italy (£3.2bn), UK (£2.6bn) and the Republic of Ireland (£1.1bn). The major net contributors to the EU budget were the UK, Austria, Germany, Luxembourg, The Netherlands, and Sweden, with Belgium and France as minor net contributors. Major net financial transfers were made to Greece, Portugal, Spain, and the Republic of Ireland - the so-called cohesion countries. Given the disparities in *per capita* standards of living, contributions, and receipts, the current distributions are not tenable in the medium term.

No progress was made in the undertaking (Article 10(2)) to develop "suitable" rules at international level on subsidised export credits specified in the Uruguay Round Agreement on Agriculture (URAA). According to the OECD, the total support for agriculture in the MDCs was $327bn in 2000, or 34% of gross farm incomes, a figure slightly below the proportion in the mid-1980s. At a special session of the WTO Committee on Agriculture in July 2001, the EU proposed (i) a single reduction mechanism to reduced expenditure on export credits whilst recognising the special needs of LDCs, and (ii) clarification of the EU's application of the precautionary principle (see COM(2000) 1 final of 2 February 2000). On the latter, reference was made to preventing the placing on the market of products that cause 'legitimate concern' and the obligation on signatories of the URAA to implement the Agreement on the Application of Sanitary and Phytosanitary Measures – the so-called SPS Agreement, permitting countries to set higher levels of health protection than the accepted international norms (Article 14 of the URAA, and Article 5(7) of the SPS Agreement). Debate will ensue on how these contributions from the EU will accord with the guidelines laid down by the WTO Appellate Body.

Enlargement of the EU will mean reconsideration of the CAP, and during 1999-2000 progress was made in four respects. First, screening of all legislation was undertaken as a prelude to transposing Community law into the national law of acceding countries. Thereafter, nine of the candidate countries submitted their negotiation positions (Cyprus, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Slovakia, and Slovenia). Following submission of their negotiating positions, the Commission will adopt a common position for the negotiation which is expected to be adopted unanimously by Member States prior to negotiating sessions, the preparation of a draft accession treaty to be approved by Council and given assent by the European Parliament. Ratification of the treaty by the Member States and candidate country leads to incorporation of the candidate country into the EU. Bilateral agreements between the EU and each of the candidate countries were signed in 2000, addressing agricultural trade liberalisation. Associated with these agreements was approval of Sapard programmes in ten central and eastern European candidate countries (notably Bulgaria, Romania, Slovakia, and Slovenia). About 530 million euros were allocated to prepare the agricultural sector and rural areas in those countries for EU membership. Other assistance programmes implemented included Phare (investments related to institution building and economic and social development) and ISPA (pre-accession support for transport and environment infrastructure projects).

In terms of international agricultural trade, the EU was the world's leading importer of agricultural

products, and the second leading exporter after the USA. Under the auspices of the WTO, the EU's position on agricultural trade liberalisation mentioned above was agreed by the General Affairs Council in December 2000. A largely defensive posture was taken. Thus, there was a commitment to an overall average reduction of bound tariffs, and a minimum reduction *per* tariff line, but EU advocated retaining the special safeguard clause to ease tariff reductions. The right to continue to use geographical indications (*i.e.* the current Protected Designation of Origin register, of special interest in Scotland), and a guarantee of consumer protection through the regulation of labelling (*e.g.* Traditional Speciality Guaranteed labelling) were proposed for world-wide adoption. The EU stated its willingness to negotiate further reductions in export refunds in tandem with firmer controls over other instruments used to boost exports. With regard to the domestic support of agriculture, the need for continuing reform was recognised, using the blue and green box framework of measures agreed in the Uruguay Round. More controversially, the EU wished to recognise the specific rôle of agriculture as a provider of public goods – which it is, incorporating the multifunctional rôle of agriculture in sustainable development, the protection of the environment, the sustained vitality of rural areas, and poverty alleviation. These less tangible aspects are seen externally as routes of special protection. Similarly, the EU proposed to use specific measures, including the precautionary principle, to address concerns which arose over food safety and animal welfare, again areas that might be used blatantly to distort trade, or pander to prejudice. Finally, the EU proposed measures to open up duty-free access to products from LDCs by provision of trade preferences and other forms of assistance.

Through the year, the EU interacted with Mercosur (a trade bloc established in 1995 comprising Argentina, Brazil, Paraguay, and Uruguay) and Chile, established an EU-Mexico free-trade agreement in July 2000, as well as the Agreement on Trade, Development, and Cooperation with South Africa, and was engaged in related talks with Israel, Morocco, and Tunisia.

Agri-environmental measures became obligatory in the programmes of the Member States in 2000, with the aim of compensating farmers for losses of income they will incur by using more environmentally friendly practices that are more labour-intensive and which may lead to lower yields than by other methods. The measures must be in addition to good farming practice and not circumvent or be used to comply with mandatory legislation such as the Directives on nitrates and habitats. Amongst the measures are water-resources management, protecting specific production techniques such as terracing, production techniques that encourage richer biodiversity and wetlands management, landscape conservation, less-intensive livestock farming, and developing the 'organic' sector.

Organic farming, as defined by the Codex Alimentarius Commission, involves holistic crop and livestock production management systems that emphasise the use of management practices in preference to the use of off-farm inputs. (see also www.ifoam.org, the website of the International Federation of Organic Agriculture Movements, and www.soilassociation.org, the website of the UK Soil Association). Thus, it is expected that cultural, biological, and mechanical methods are used in preference to synthetic materials. The stated aims of organic farming include the application of farming methods that do not "damage the environment", that encourage a "more respectful use of the countryside", promote "concern for animal welfare", and achieve "high-quality" products (see the *SCRI Annual Report 1998-1999).* By 1999, over 50% of the 121 055 holdings in the EU certified as organic or in conversion were in Italy (40.5%) and Austria (16.3%). None of the other countries had more than 10% of their holdings organic; the UK total was 1.9%. Claimed benefits of strict organic systems were under review, particularly animal welfare and commodity quality criteria, and there were trends in Austria and Denmark of lowered price premia as a result of supply exceeding demand.

More than 7 million hectares were committed to 'organic' farming world wide, a tenfold expansion over a decade, and dominated by EU countries. The global organic food market was estimated to be $22bn annually, a tiny percentage of the total food market (see *Organic Agriculture World-Wide* by H. Willer and M. Yussefi, 2000; and *Organic Food and Beverages*: *World Supply and Major European Market,* UN Conference on Trade and Development, 1999). Differences of some magnitude exist between practitioners of organic farming and conventional farming in the criteria distinguishing 'organic' from 'conventional', the relative rôles of profitability and lifestyle, the rôle of new technology, and vision for the future of organic production systems.

According to Eurostat *Economic Accounts for Agriculture*, the shares of the EU member states in final agricultural production were France (23.1%), Germany (15.4%), Italy (15.4%), Spain (12.1%), UK (8.7%), the Netherlands (6.8%), Greece (4%), Denmark (2.9%), Belgium (2.6%), Portugal (2.3%), Republic of Ireland (2%), Austria (1.8%), Sweden (1.6%), Finland (1.3%), and Luxembourg (0.1%).

Against a background of concerns about the spread of bovine spongiform encephalopathy (BSE) from the UK to five countries in mainland Europe (France, Germany, Portugal, Spain, and Switzerland), and its possible linkage to a new variant form of Creutzfeldt-Jakob disease (CJD), dioxin contamination of poultry in Belgium, continuing problems of *Escherischia coli* O157 and other food-poisoning microorganisms, a rapidly escalating foot and mouth disease outbreak, and a mistrust of scientists and regulators, genetically modified (GM) crops received bad – often hostile – publicity in Europe. Regardless of the proven safety of current GM crops in agriculture, their widespread cultivation outwith Europe, their potential contribution to wealth-creation and the quality of life - including their potential benefits to the environment, various non-governmental organisations (NGOs) such as Friends of the Earth and Greenpeace and other groups strongly opposed GM crop cultivation and utilisation in Europe. Demonstrations, sometimes violent, against GM foods took place, and meetings on the topic were frequently discomfited by implacably polarised views, prejudice, misinformation, inability to understand risks, and outright intolerance. Religious views on the inviolate separation of species (taxonomic taboos), attitudes on 'ownership' of genes by species, ingrained suspicions about multinational corporations based in the USA, opposition to market-based capitalist economies (anti-globalisation), anti-Americanism, anti-science tendencies, unwillingness to accept expert views or the opinions and actions of regulators, and urban-based perceptions on the rôle and functioning of agriculture as a business, all acted against a change in direction to adopt GM technology even on a gene-by-gene, crop-by-crop, place-by-place basis. Indeed, the actions and publicity engendered by the anti-GM groups created concerns about GM organisms (GMOs) in countries throughout the world.

Pressure was brought to bear on governments in Europe to retard the development of GM crops, a position aided by the results of opinion polls. Retailers and food producers in Europe and especially in the UK responded by withdrawing GM products, and insisting that such products were withdrawn by suppliers from the food chain. This tactic operated in tandem with the marketing impetus given to 'organic' produce and products, but will undoubtedly create problems for those organisations when a change takes place on the grounds of quality, cost-benefit studies, and supply regularity. Herbicide-tolerant GM oilseed rape, one of the first GM crops of general release, proved to be a public-relations problem in the UK in respect of its outbreeding, gene flow induced by widespread pollen movement, and the inadvertent appearance of GM types in a purportedly conventional cultivar (see section on Plant Biotechnology). Another highly publicised crop was StarLink maize, an animal-feed type with the potential to cause allergies in humans. Analysis of foodstuffs, principally taco shells, revealed StarLink contamination, some caused by cross-fertilisation with human-feed cultivars and also by inadequate segregation of grain stocks. There were estimates of the StarLink genes being detected in nearly 50% of the maize crop harvested in Iowa.

The debates in 2000 about gene flow from genetically modified (GM) plants spread beyond the EU, extending to discussions on the merits and disadvantages of gene-use restriction (terminator) technology sanctioned in August by the US Department of Agriculture (USDA), and the nutritional, environmental, and medicinal benefits of GM crops. Meanwhile, S. Padulosi of the Rome-based International Plant Genetic Resources Institute stated that of 5 300 species of food plants collected world-wide, more than 50% had but a single sample left in a seed bank, even though each species may have hundreds or even thousands of cultivars. The preservation, viability, and characterisation of many collections are neglected in most countries. It is ironic, therefore, that biotechnology will be central to the rescue of these genetic resources.

Massive investments in GM technology by companies such as Monsanto and DuPont, and by many governments in MDCs, and the utility of the technology to a wide spectrum of new industries in healthcare, nutrition, industrial feedstocks, and the environment, provided market optimism in North America, and to the mainstream international scientific community. Food safety standards in the highly litigious society of the USA, and the openness and independence in the USA of their Department of

Agriculture, the Environmental Protection Agency, and the Food and Drug Administration, coupled to the credibility of their investigative media, clearly reinforced confidence in GM crops specifically, and biotechnology in general. European opposition to the technology and importation of its products represented a trade barrier that was difficult to justify scientifically and in trade terms. Perhaps the resistance to GM crops was able to blossom in an environment of poor marketing strategies, incomplete data sets for the impacts of both conventional and GM-based agriculture, ignorance of gene flow in agriculture, horticulture and forestry, and the paucity of scientists able to present in debate their knowledge and opinions without complex jargon and concepts. By appreciating the slow incremental improvement of knowledge, recognising risks in all human activities, welcoming the heterogeneity of scientific opinion, having a high regard for peer evaluation, knowing the difficulty of generating 'soundbites' without leading to over-simplification, and having the good manners to avoid personal vilification led to a marked reduction in participation in public debates by scientists. Most biotechnologically based scientists were described by pressure groups as 'GM advocates', as opposed to the more correct description of scientists able to understand both 'sides' of GM debates but aware of the huge potential of their studies. Understanding and appreciation of the behaviour of the public represented a challenge to scientists, and were areas of study rapidly taken up by social scientists and a growing population of ethicists. 'Shaping' of views, 'avoidance of unwanted outcomes', political 'preferences', and dubious sampling and questionnaire processes were noted in some studies. The high-quality social science publications supported the view that the general public operated rationally and justifiably according to the information that was made available. With regard to GM crops, the introduction of tighter EU regulatory controls, overarching committees that are socially inclusive, wider consultation, and close monitoring of crops and their products post-release should give greater public reassurance, as the policy of crop-by-crop, gene-by-gene, place-by-place analysis continues, and the newer metabolic profiling, gene-flow measurement, and environmental impact technologies are introduced (see section on Plant Biotechnology). Even so, neither multinational corporations nor scientists *en bloc* seemed able to deal with well-orchestrated attacks on them.

Strident anti-science views, even in pluralistic, multicultural societies, had the capacity to destabilise the numerically expanding political classes who were noted for the scarcity of scientists in their ranks. There was widespread confusion over such straightforward distinctions between research for curiosity, serendipitous discovery, invention, development, and commercial application. Rarely has it been possible to suppress inquiry throughout the world; the major challenge is to foster its socially acceptable application. Ridicule of scientific aspirations and vision (*e.g.* feeding the world, curing disease *etc.*) - easy because for obvious reasons there are not many irrefutable examples in younger areas of science, and hostile questioning of well-intentioned motives, also undermined scientific endeavours. In the UK, both the Office of Science and Technology in the Department of Trade and Industry, and the Scottish Executive produced strategies that were aimed at strengthening the science base.

Repeated health scares also undermined the confidence of consumers in food-safety regulations and the integrity of conventional farming. The linking by the EU of agriculture and the environment in world trade discussions was essentially aimed at legitimising barriers to farm trade, based theoretically on one of many definitions of the precautionary principle, and was an approach largely refuted by virtually all the other WTO members.

Domestic gardening in many MDCs was a major leisure activity both participatory and as broadcast entertainment. Modern trends were evident of containerised growing, use of planting modules as opposed to rearing from seed, elaborate garden designs, a preponderance of floral rather than vegetable growing, and a willingness to seek new species and cultivar introductions. For most countries, the horticultural introductions were species alien to the area. As the horticultural industry adjusted to these trends, the challenge of horticultural plant breeding gained wider respect. The sheer diversity of species and types, growing systems, pests and diseases, and markets presents geneticists and breeders with great opportunity offset only by a paucity of medium- to long-term funding. It is likely that horticultural benefits in the form of new vegetable types will come indirectly as collateral benefits from the agricultural crop breeding and selection programmes.

## The Environment

One of the most interesting sources of information and analysis on environmental matters is the annual *State of the World* report on progress towards a sustainable society. Such accounts give an overview of the scale of the challenge facing the human race.

Pollution of groundwaters has become a serious issue as 97% of the Earth's liquid fresh water is stored in aquifers, some of which are not recharged by incoming water, or which are recharged slowly over hundreds of years (see UNEP *Groundwater : A Threatened Resource*, 1996 and P. Sampat *Uncovering Groundwater Pollution* in *State of the World 2001*). Groundwater is the primary source of drinking water for up to 2bn people. Agriculture accounts for around 65% of the fresh water drawn from rivers and wells each year, and manufacturing industry consumes 22%.

Removal of water from an aquifer can magnify the concentration of pollutants, enhance the inflow of saline water, cause a collapse in the aquifer structure, and affect the hydrology of the area. The major threats are pesticides, petrochemicals, chlorinated solvents, arsenic, other heavy metals, radioactive materials, nitrates, fluoride, and other salts. In the UK, low-level but bioactive pharmaceutical pollution has become an issue. Strategies to overcome these problems include lessening the impact of agriculture so that it requires less water and agrochemicals, restricting the use and disposal of pollutants above aquifers, use of closed-loop systems, changed manufacturing systems, restricting wasteful use of water, use of end-of-pipe filters, attempts to clean or treat aquifers, use of other sources of water *e.g.* desalination of sea water, lakes, dams, *etc.*, and a search for new technological solutions.

Lester R. Brown, in one of his characteristically hard-hitting articles, *Eradicating Hunger: A Growing Challenge* in *State of the World 2001*, made the point that 1.1bn of the world's population are undernourished, and this goes in hand with poverty. With the exception of Africa, though, the proportion of the population that is hungry is diminishing in all regions. In recognising that malnutrition is for the most part a manifestation of rural poverty, the World Bank started to replace conventional agricultural development strategies largely centred on subsistence agriculture with rural development strategies. Population growth is a pivotal factor to consider in the ability of a country to meet its needs. Most of the world's poor live in countries with rapidly growing populations. Calculations based on farm size, area of cultivated or grainland, or fresh water *per capita* are sobering for most LDCs; millions are effectively landless and the statistics are worsening. Thanks to the efficiency of modern agriculture, global food production (see section on Agriculture and Food) has more or less kept pace with population growth, although there are signs of a growing mismatch. From a figure of 247Kg *per capita* in 1950, grain (mainly wheat, rice, and maize) production reached a peak of 342Kg *per capita* in 1984, declining to 308Kg in 2000. Intensive livestock protein production (principally beef, sheepmeat, and chicken) and fisheries are unlikely to contribute a great deal to the world's future food supply, although the demands for these products is increasing. Thus, to avoid damaging or eliminating large tracts of forests and other natural and semi-natural habitats, it will be necessary to raise productivity. This can be addressed by raising yields with improved cultivars and agronomic practices, multiple cropping, use of residues and eliminating waste, controlling pests and diseases, restoring damaged agricultural habitats, operating with better water-use efficiency, and planning for long-term sustainability. Competition for non-farm uses of water will shackle agriculture and horticulture in the future. Few individuals and organisations world-wide pay the true price for water. Reduction of animal protein production will be enforced under highly competitive conditions for access to foodstuffs. Livestock are poor converters of energy from fodder, and can degrade the environment, even in low-density, extensive systems. Competition for agricultural products could be accelerated in the short-to-medium term if there are major weather perturbations, rising sea levels that flood low-lying coastal lands and river basins which are major agricultural production areas, outbreaks of pests and diseases, and clearly, rapid population growth. (See also International Food Policy Research Institute *Fourth Report on the World Nutrition Situation*, 1999; the UN Food and Agriculture Organisation *The State of Food Insecurity in the World 1999* and *2000*; the UN *World Population Prospects: The 1998 Revision.*

S. Dunn in *Decarbonising the Energy Economy* in *State of the World 2001,* highlighted the fact that the release of carbon atoms has been the byproduct of the human harnessing of energy, from the combustion of wood, coal, lignite, oil and through to natural gas. He noted that carbon output can be decoupled from economic growth. From the reports of the Intergovernmental

Panel on Climate Change and others, it is estimated that 42 trillion tonnes of carbon are fixed in or circulate between the three main reservoirs: the atmosphere, oceans and biosphere (*e.g.* vegetation, detritus, marine and freshwater biota, terrestrial fauna, soil and other microorganisms *etc.*). Since the start of the Industrial Revolution, more than 271bn tonnes of carbon have been added to the atmosphere by the oxidation of fossil fuels (coal, oil, natural gas); present-day emission rates are *circa* 6.3 billion tonnes. The capacity of the main reservoirs is not known, as is a precise understanding of the social, economic and environmental impacts of the anthropogenically added greenhouse gases (carbon dioxide, methane, nitrous oxide, and halocarbons) able to trap infra-red radiation reflected from Earth. Renewable energy sources have the potential to substitute for fossil sources, and hydrogen as a fuel of choice is a promising line of enquiry. Rarely, though, is nuclear energy regarded by reviewers as an appropriate major source for generating electricity until the issue of nuclear waste is put to rest.

Carbon sequestration and storage could be achieved by technological routes such as its incorporation into inert and long-lived composite products, injecting it into oilfields which are thought by the International Energy Agency to be capable of storing 126bn tonnes of carbon dioxide, or locking up carbon dioxide by forming stable hydrates, or reacting it with naturally occurring mineral oxides to form carbonates. The most effective approach would seem to be a reliance on photosynthesis, whereby the carbon is bound into terrestrial and aquatic plants, but there are uncertainties as to the rates at which the carbon will be released by degradation processes, unless there are large-scale increases in the biomass of forestry plantations, the planting of perennial amenity and horticultural species, and iron-fertilisation of oceans to stimulate phytoplankton growth. Of course, the oceans with their ability to store carbon dioxide in the form of carbonates represents a major buffering reservoir, and perhaps the deep oceans could also store injected carbon dioxide, but again there are uncertainties as to the possibility of large-scale, uncontrolled releases. Many environmental activists are opposed to technological fixes, and advocate the termination of burning fossil fuels.

Renewable energy from biomass, wind, solar and geothermal energy, and hydroelectric schemes, the so-called 'green' energy, does have the capability to be

| Title | Date | Number of Parties |
|---|---|---|
| Ramsar Convention on Wetlands of International Importance, Especially as Waterfowl Habitat | 1971 | 123 |
| Convention Concerning the Protection of the World Cultural and Natural Heritage | 1972 | 161 |
| London Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter | 1972 | 78 |
| Washington Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) | 1973 | 152 |
| International Convention for the Prevention of Pollution from Ships (MARPOL) | 1973/78 | 112 |
| Bonn Convention on the Conservation of Migratory Species of Wild Animals (CMS) | 1979 | 70 |
| UN Convention on the Law of the Sea (UNCLOS) | 1982 | 135 |
| Montreal Protocol on Substances that Deplete the Ozone Layer | 1987 | 175 |
| Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and Their Disposal | 1989 | 141 |
| Convention on Biological Diversity (CBD) | 1992 | 178 |
| UN Agreement Relating to the Conservation and Management of Straddling Fish Stocks and Highly Migratory Fish Stocks | 1995 | 28 (not yet in force) |
| Kyoto Protocol to the 1992 UN Framework Convention on Climate Change | 1997 | 30 (not yet in force) |
| Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade (PIC convention) | 1998 | 11 (not yet in force) |

**Table 1** A selection of international environmental accords.

operated on a smaller scale than conventional coal- and nuclear- and natural-gas powered stations. Around 70% of the world's carbon emissions in 1999 were produced by the USA (25.5%), EU (14.5%), China (13.5%), Japan (6%), Russia (4.6%), and India (4.5%).

Hilary French and Lisa Mastny in *Controlling International Environmental Crime*, in *State of the World 2001*, pointed out the existence of nearly 240 international environmental accords (see UN Environment Programme *Register of International Treaties and Other Agreements in the Field of the Environment*, 1999). The principal agreements (Table 1) may be regarded as expressions of good intentions, at least, but resources to police the agreements are limited.

There were countless incidents of illegal dumping and pollution world-wide. Phasing out production of some of the 95 ozone-depleting chemicals (CFCs, halons, HCFCs, methyl bromide *etc.*) was confounded by a sizeable illegal trade and fraud. Some environmental NGOs were constructively active in policing compliance to the international codes, others merely sought publicity for enrolment purposes. The book by the Danish statistician, B. Lomborg, *The Skeptical Environmentalist*, *Measuring the Real State of the World*, challenged the mantras and assumptions of the more aggressive members and organisations in the environmental movement, and pointed out the selective use of scientific evidence and exaggeration of environmental damage. His views were contested by the World Resources Institute (see www.wri.org).

Environmental issues were politically prominent in 2000. Settlement of the debate between the EU countries on one hand, and Australia, Canada, Japan, and the USA on the other, over the Kyoto Protocol was not attained. The reluctance of LDCs and rapidly industrialising countries to restrict their greenhouse-gas emissions and jeopardise their economic growth meant that targets were sidelined. Decarbonising the economy; supporting the Clean Development Mechanism in LDCs; supporting agriculture, forestry and sustaining or improving natural habitats; emissions trading; and enforcing low-emission domestic and industrial activities, were all reviewed, but the challenge remained of reaching an agreed, fair system. Closure of the Shenyang Smeltery in north-east China, which was responsible for Shenyang being classified as one of the 10 most polluted cities in the world, was a refreshing approach to tackling environmental degradation in China. The Environmental Protection Agency of the USA reclassified dioxins from 'probable' to 'known' carcinogens, and stated that the compounds were 10 times more likely to cause cancer than thought hitherto; but this opinion was contested.

In Germany, Russia, and Sweden, the nuclear industry came under political pressure on safety grounds. Mainly as a result of the efforts of 'green' political activists, the nuclear industry in Germany was given operating limits, and safety regulations were tightened on the transport of spent nuclear fuel. In Sweden, the closure of the Barsebäck 2 nuclear reactor was postponed as a result of the demand for electricity and the inability of the renewable energy sector to make good any shortfall. As a result of the attention given to climate change issues, there was evidence of a rethink on the future of the power-generating industries. Although the transport and custodianship of spent nuclear fuel and waste were of concern, as were catastrophic radiation leaks and terrorism, the huge potential of nuclear industry to generate carbon-dioxide-free power was becoming widely recognised. Gas- and coal-powered plants were seen to create difficulties for countries trying to meet their Kyoto obligations. Alternative energy strategies were seen to be attractive yet remained under-resourced in respect of R&D investment into the associated areas of science, engineering and technology. Complex energy taxation arrangements were, in contrast, starting to be introduced.

Early release of the draft of the third assessment report by the Intergovernmental Panel on Climate Change – a group acting under the auspices of the World Metereological Organisation and other sections of the UN – showed that three of the past five years had been the warmest in recent history, and dendrochronological records from the past millennium demonstrated that the abrupt twentieth century warming is unique. Human-induced (anthropogenic) warming was identified as the factor responsible for a climate warming of 0.6ºC over the past century. Predictions of future 'greenhouse' warming and their consequences were thought to have made little progress given the levels of uncertainty over climate models, cloud behaviour, use of fossil fuels, impacts of changing land use, solar activity, *etc.* (see Fig. 1).

In the USA, a panel of the National Research Council, an offshoot of the US National Academy of Sciences, noted in January 2000 that there was little or no warming detected in the upper atmosphere, but

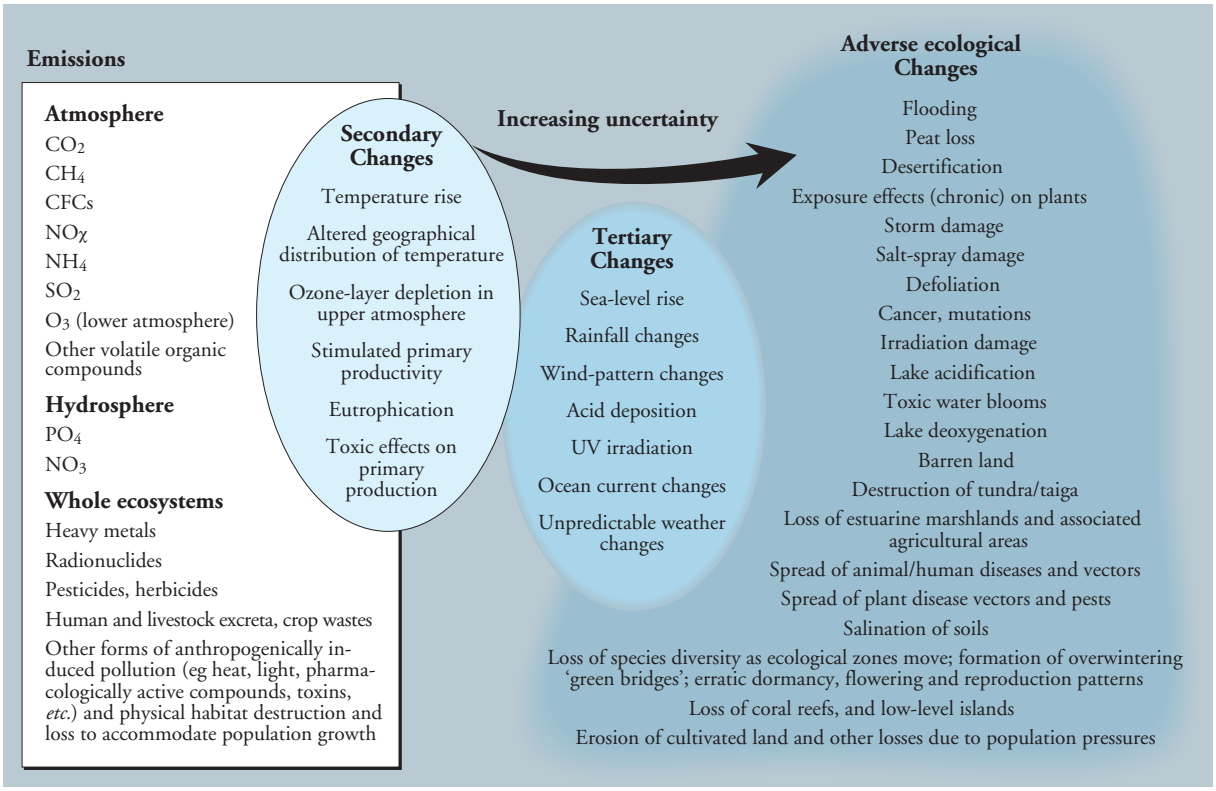


**Figure 1** Principal global changes and their likely consequences.

confirmed warming at lower levels. The panel did not provide evidence to support the largely accepted view that the warming was a result of the accumulation of greenhouse gases, or would even continue.

Studies in the melting of the Greenland ice sheet, the retreat of glaciers, world-wide, melting of the Antarctic ice sheet, and the major depletion in the average thickness of summer polar ice in recent years, were all indicative of global warming. Any threat to the oceanic conveyor belt – specifically the Gulf Stream that accounts for the temperate condition of the UK, to the global flora and fauna, and to current weather patterns justifiably caused concern and raised questions as to whether global climate change can be favourably modified or, delayed anthropogenically, or is inevitable.

A combination of four governments (Finland, The Netherlands, Norway, and Sweden) and several companies funded a World-Bank global-market-based project to reduce greenhouse-gas emissions and to promote appropriate technologies in LDCs. It was envisaged that the World Bank would act as a broker, aiming at a price of *circa* $15 *per* tonne of carbon.

Notable pollution events in 2000 included (i) the failure in January of a containment dam holding water contaminated with cyanide and heavy metals at the Aural gold-tailings retreatment mine near Baia Mare, Romania; (ii) a series of fires during March in the forests of Sumatra, Indonesia; (iii) a damaging leakage from an oil pipeline into the Guanabara Bay in January; and (iv) in August, employees of the Shengli Chemical Company in Taiwan were charged with dumping dimethyl benzene into the Kqop'ing River, the principal source of drinking water in southern Taiwan.

In March, the European Commission and Norway persuaded the Marine Environment Protection Committee of the International Maritime Organisation, an independent specialised agency of the UN, to designate the North Sea as a low-sulfur-fuel zone *i.e.* to restrict fuels to no more than 1.5% sulfur as opposed to the 4.5% permitted globally. Implementation of designation, however, would require ratification by at least half of the world's fleet of an annex to the Marpol convention.

Effects of the lingering La Niña over the Pacific Ocean accounted for much of the unusual weather effects in 2000. The below-normal sea-surface

temperatures in the eastern and central equatorial Pacific, and above-normal temperatures in the western Pacific led to increased rainfall in the western Pacific, western USA and Indian Ocean basins, tropical storms in Australia, southeastern Africa, and the southern Indian Ocean.

Green architecture aimed at designing and constructing buildings that leave a small environmental footprint, consuming few resources and emitting few greenhouse gases, began to influence architecture and civil engineering in 2000. When the extent of ongoing construction world-wide of domestic housing, factories, airports, aquaducts, bridges, commercial centres, hotels, towns and villages, dams, roadways, railways and subways, tunnels, and harbours are taken into account, plus the costs of maintenance, it is clear that the pressures on the national environment and cultivated land are inexorable, and 'green' construction will need to become widespread.

## UK Perspectives

Devolution developed during the course of 2000. The Westminster-based UK Parliament remained the supreme law-making authority, legislating for the UK as a whole, or for any parts of it. Sovereignty resides with the Queen in Parliament. The main functions of Parliament are to pass laws, to provide the means of carrying on the work of government by imposing taxes, and to scrutinise government policy and administration. All international treaties and agreements are presented to Parliament before ratification. Contrasting with most countries, the UK constitution is not contained in a single document but has slowly evolved, and been shaped by statute, common law, and by convention. Membership of the European Union poses constraints and overrides, however. Following its election on 6 May 1999, the Scottish Parliament exerted legislative power over all devolved powers, *i.e.* matters not reserved to Westminster; primary legislation can be introduced, and the Scottish Parliament has the power to raise or lower the basic rate of income tax by three pence in the pound. The areas of responsibility cover agriculture (including agricultural R&D), economic development, education, environment, financial assistance to industry, fire services, food standards, forestry, heritage and the arts, health, housing, law, planning, police, and some aspects of transport. A busy year was experienced by the Scottish Parliament (see *Scottish Parliament Annual Report 2000 to 2001*). Thirteen bills were passed, 14 bills received Royal Assent, 71 members' business debates were held, large numbers of questions were lodged, and answered either in Chamber or in writing. The Committees of the Scottish Parliament gained a higher profile. Of relevance to this report was the work of the Rural Development (formerly Rural Affairs) Committee which devoted considerable time to the controversial Protection of Wild Mammals (Scotland) Bill, but became increasingly involved in the alarming outbreak of foot and mouth disease. Other topics covered by this committee included an inquiry into the effect of changing employment patterns in rural areas. Following a petition, GM crops were considered in a report by the Transport and the Environment Committee; this Committee also considered a petition on the environmental effects of an oilseed-crushing plant in Arbroath. Petitions from the public were firstly reviewed by the innovative Public Petitions Committee, a noteworthy democratic initiative. Cross-party groups were also active. The National Assembly for Wales was also elected in 1999 and has the power to make secondary legislation in the areas where it has been granted executive functions. The New Northern Ireland Assembly elected in 1998 was due to be formally established by legislation in 1999. It was suspended in February 2000 and resumed sitting in June 2000, and had legislative authority in the fields administered by the Northern Ireland departments.

Alongside the changes taking place with devolution, the relationship with the EU came into sharper profile during the year. It is through the European Communities Act, 1992, incorporating the Treaty of Accession, that sovereignty is shared with the European Union, and it is assumed that the courts follow primarily the statute of Parliament. Potential expansion of the EU, sensitive issues such as taxation

and constitutional matters, Single Market laws and harmonisation, environment policy, health and safety, regional aid, transport policy, research and development, immigration and human rights, consumer protection, and overseas aid, generated both heat and light in the media. Eurostat, the official EU statistics agency, recorded a £20bn deficit on Britain's visible trade with the 11 original members of the eurozone last year, whereas Britain's Office for National Statistics estimated the deficit at just £1bn. This provided strong evidence of the over-valuation of the pound.

From an estimate of 59.8 million for the population of the UK in 2000, the Office for National Statistics in another of its functions forecast that the population will rise by 5 million in 2025, more than 3 million of whom will be immigrants arriving at a rate of 135 000 annually. Already immigration is responsible for more of the population increase than natural expansion by births in England, with slight increases in Northern Ireland and Wales, but the population of Scotland was projected to fall by about 200 000 to 4.9m. The average age of UK citizens will increase from 38.8 to 42.6 years by 2025, and more than 13 million people will be of pensionable age compared with less than 11 million in 2000. Life expectancy will rise from 76 to 79 years for men and 80 to 83 years for women of this time, raising questions on the quality of life in people in their final years and the need for companies to review marketing strategies. Under present arrangements, the tax take will decline, and the ratio of workers to pensioners will fall from 4:1 to 2.5:1 by 2025 unless attitudes to the elderly, retirement ages, and pension conditions change substantially in the interim.

Risk management is an intrinsic component of research organisations. It involves the removal of traditional barriers within the organisation, the application of vision and foresight, and the recognition of the rôle of risk tolerance – or risk appetite. The biggest risk to research organisations is risk avoidance. To capitalise on risk, and optimise opportunities, without endangering the future of the institution, there must be due levels of accountability, with measures to identify, quantify, and prioritise risk at the strategic and operational levels, bearing in mind reputation, regulation, and asset protection. Audit committees are a particular focus. Their oversight responsibilities and effectiveness were emphasised by the recommendations of the Blue Ribbon Committee and the O'Malley Panel Report. During the year, public-sector bodies were encouraged to emphasise the probity and process-checking rôles of audit committees. Corporate governance procedures were beginning to be widely applied in the public sector, codifying arrangements to deal with risk, responsibility, and communication. Decisions impacting on finances, effectiveness, and reputation were deemed to be best shared, rather than remain under the control of a single person. Disaster plans to enable rapid business recovery were widely discussed, with emphasis on protection of assets including IP, inter-institutional linkages, command chains, identification of choke points, and back-up processes. Institutional integrity, ethics and corporate values, and their relationship to stakeholders, were concepts beginning to be inculcated into the *modus operandi* of public- and private-sector bodies. Ethics represent a less-tangible concept in business terms, but relate to values, policies, and the behaviour of individuals. Honesty and integrity are needed at all levels, sustaining trust between and within businesses of all types; they inhibit illegality and failure to comply with regulations, and clearly diminish the risk of damaging litigation.

Investment in the future lies at the heart of the rationale for companies and countries to commit to a complex set of activities under the heading of R&D. Translation of the products, tangible or otherwise, into market or social advantage requires additional skills. In the UK, the Department of Trade and Industry has published for more than a decade, an annual *Research & Development Scoreboard* (see www.innovation.gov.uk/finance). The European Commission started to produce a related benchmark report – *The 2001 Innovation Scoreboard* (www.cordis.lu/scoreboard).

Rather like advertising, R&D spend is vulnerable during recessionary periods as short-term thinking pervades decision-making. Comparisons of sector mixes and intensity of spending on company R&D within and between countries, and between trading blocs give indications of future company, if not public sector, performance. Historical analyses show that R&D-heavy companies perform, on the whole, significantly much better than their competitors and are more likely to withstand successfully downturns in the economy. From the company reports and accounts of 597 companies received by 4 July 2000, as analysed by Company Reporting Ltd who prepared the 10$^{th}$ (2001) anniversary issue of the R&D Scoreboard in conjunction with the DTI Future and Innovation

Unit, it was possible to conclude that overall, the levels of business expenditure on R&D in the UK rose from 1.18% of GDP in 1997 to 1.25% in 1999. The overall total of R&D for the 597 companies was £15bn, excluding all the R&D carried out by UK companies overseas. The Scoreboard excluded R&D paid for by governments and other non-commercial sources. As always, there were uncertainties of definition and classification. The UK definition is contained in Statement of Standard Accounting Practice 13, and for international companies, the definition is that governed by International Accounting Standard 9. Both definitions are based on the OECD 'Frascati' manual, whose linear-thinking taxonomy of the spectrum of R&D has caused strong disagreement in the research practitioning communities concerned about subjective classification and complex inter-relationships between different components of R&D, disagreements not necessarily shared by those that administer R&D.

Some trends were obvious. R&D intensity (R&D/sales) in the USA rose from 1.9% to 3.5% during the 1980s, ostensibly at the expense of realisable profit because R&D intensity plus profitability remained approximately constant at around 7%. During the 1990s, however, R&D intensity increased further to over 5%, while R&D intensity plus profitability rose from 9-10% to 13-15%. At the start of the 1990s, UK profits exceeded the US level, but the R&D effort was much lower. By the end of the decade, UK company profits were less than 90% of US companies, and R&D expenditure was 60% less. The R&D intensive part of the economy, which includes 51% of the companies listed in the FTSE 100 and 39% of the FTSE 350 indices, was heavily biased towards what the Scoreboard described as chemistry-based industries, with over 39% of UK R&D in pharmaceuticals. In other words, in R&D terms, biotechnology is the UK's most important industry. Other advanced economies were, conversely, skewed towards physics-based industries, but the position is changing as the bioindustries grow in stature and integrate with the physics-based sector. That the overall R&D intensity in the UK was less than 50% of the US level, and that R&D intensity was higher in the UK than elsewhere for pharmaceuticals, aerospace and defence, means most other industries, the physics-based sector included, were underperforming. Almost 50% of the sales turnover in the UK Scoreboard was accounted for by sectors with R&D intensities of less than 1%, contrasting sharply with 22% of sales turnover attributable to low R&D-intensive companies for the US Scoreboard, and only 12% for the international Scoreboard. With the exception of the automotive industry, the UK subsidiaries of overseas companies invested at a lower R&D intensity than their UK equivalents, but some of the R&D supported was crucial to key sectors of the economy. In conclusion, overall UK profitability *per se* does not appear to be a constraint on R&D spend, if viewed in the medium term. Certainly, R&D intensity is positively linked to sales growth, and there is strong evidence to support the view that the amount of R&D per employee translates into sales per employee. Most UK companies in R&D intensive sectors are below international averages in these measures, a fact that indicates room for improvement in productivity and competitiveness. Concern was expressed in the Scoreboard that the proportion of medium-sized companies (over £60m annual sales) with R&D intensities of over 10% in the UK economy is about one sixth of the level in the USA. It is interesting that R&D is not greatly valued by the UK market, with the honourable exception of the pharmaceutical sector. Spend, of course, does not signal efficiency or effectiveness of the investment, or even how well the companies perform. Intellectual property owned or controlled in the form of patents or licences, and innovative power, measuring the R&D effectiveness of each employee – as is adjudged in visiting group and research assessment exercises in the public-sector – will surely start to be a part of the market analyst's armoury.

Distinct from the approaches adopted by the DTI R&D Scoreboard, the EC Innovation Scoreboard analysed relevant statistical data on 17 indicators in human resources; knowledge creation; transmission and application of new knowledge; and innovation finance, output, and markets. With the data available, there was evidence of declines in public-sector R&D, business R&D, and value added from high-technology manufacturing. The USA held a lead over the EU in tertiary education, business R&D, home internet access, and high-technology patenting. The EU led the USA in the supply of new science and engineering graduates, public-sector R&D, and information and communication technology (ICT). Compared with Japan, however, the EU led only in ICT expenditure. Of particular interest was a tentative national summary innovation index (S11) which scored performance within a range of whether all indicators were above average (+10) or all indicators were below average (-10).

The leading country was Sweden (+6.5), followed by the USA (+5.6), Finland (+4.7), UK (+4.4), Japan (+3.8), Denmark (+3.5), Netherlands (+3.0), the Republic of Ireland (+1.0), Germany, (+0.6), France (-0.6), Austria (-2.4), Belgium (-2.4), Luxembourg (-4.4), Spain (-5.9), Italy (-5.9), Greece (-7.9), and Portugal (-8.7). The UK gained from its educational system, the availability of high-technology venture capital, and Internet access. Two observations in the report were that the European innovation leaders managed to increase innovation performance and reduce poverty, as evidenced by the negative correlations between S11 and two indicators of social exclusion (percentage of the population living below the poverty line and inequality of income distribution), and that highly innovative countries tended to give high priority to sustainability.

Agriculture did not figure in the Scoreboard analyses. Its links with the international and top 300 UK listed companies would come in the beverages, chemicals, food processors, forestry and paper, general retailer, pharmaceuticals, and tobacco sectors. So, regardless of its underpinning rôle in the provision of foodstuffs and other aspects of civilisation, and the huge volumes and values of agricultural commodities in international, national, and regional trade, its once-high profile has been usurped by upstream and downstream industries.

### UK Agriculture

As a consequence of the high levels of support to the agricultural industry, UK agriculture was with few exceptions uncompetitive in world markets. When compounded with animal health problems reflected in bans on exports, as well as dramatic falls in incomes and profitability, the industry had a bleak short-term future. The annual monetary support transferred to producers from taxpayers and consumers more generally is usually described officially as the Producer Support Estimate or Producer Subsidiary Equivalent, comprising direct payments (Budgetary Payments), and the indirect or invisible support (Market Price Support) leading to the elevated prices paid by EU consumers.

Despite a stream of reforms and external pressures to reduce Market Price Support measures, agriculture in Scotland was heavily supported. Reference to the keynote publication *Economic Report on Scottish Agriculture 2001 Edition* from the Scottish Executive Environment and Rural Affairs Department (SEERAD) reveals that CAP Budgetary Payments in 1999/2000 were estimated to have been about £473m, and Market Price support in the region of £293m. The bulk of the Producer Support Estimate in 1999 (£766m) was directed towards five sectors: such as beef (£115m) and wheat (£32m). Extensive grant and subsidy schemes have operated in recent times (Table 2).

| |
|---|
| **Cattle** |
| Beef Special Premium Scheme, including Top Up, Extensification Premium, and Agrimonetary payments |
| Suckler Cow Premium Scheme, including Top Up Re-Distribution, Agrimonetary, and Extensification payments |
| Extensification Payment Scheme |
| Hill Livestock Compensatory Allowance |
| Calf Processing Scheme |
| Veal Calf Slaughter Premium Scheme |
| Over Thirty Months Scheme |
| **Sheep** |
| Sheep Annual Premium Scheme, including Least Favoured Areas Support and Agrimonetary payments |
| Hill Livestock Compensatory Allowances |
| **Milk** |
| Dairy Agrimonetary Compensation |
| **Arable Area Payments Scheme** |
| Cereals |
| Oilseeds |
| Protein Crops |
| Linseed |
| Grain Legume Scheme |
| **Other Subsidies** |
| Arable Area Payment Set Aside |
| Chernobyl Compensation Payments |
| Environmentally Sensitive Areas Payments |
| Countryside Premium Scheme, including aspects of the Habitats and Heather Moorland Schemes, as well as the organic aid scheme |
| Selective Cull Compensation |
| Annual Diseases Compensation |
| Compensation for Milk Quota Cuts and Quota |
| Objective 1 (H1AP) |
| Objective 5B |
| Agriculture Business Development Scheme |
| Farm Business Development Scheme |
| Farm and Conservation Grant Scheme (EC) |
| Agriculture Improvement Scheme (EC) |
| Crofting Buildings Grants and Loans Scheme |
| Crofting Counties Agricultural Grants Scheme |
| FEOGA Processing and Marketing Scheme |

**Table 2** Agricultural grants and subsidies 1998-2000

The gross output at basic prices in 2000 was estimated to have been £1869m, the gross input (total intermediate consumption) £1043m, the gross value added (gross product) £826m, and the net value added at basic price £545m. The measure Total Income From Farming (TIFF) which estimates business profits plus income to workers with an entrepreneurial interest, decreased from £261m in 1999, to £230m in 2000.

The numbers employed in Scottish agriculture (working occupiers including working spouses of occupier, full-time employees, part-time employees, and casual and seasonal employees) was 26 808. Analysis of the data reveals that out of a total land area of 7 708 010ha, agricultural land accounted for 6 074 789ha. The areas devoted to various crops and other uses, with few exceptions, did not change substantially from 1999. Sole-right and common rough grazing (3 974 420ha) and mowing and grazing grass, under or over five years (1 187 792ha) dominated land use. Winter and spring barley (316 823ha), woodland (200 227ha), wheat (108 859ha), set-aside (77 875ha), 'other' land (*i.e.* farm roads, yards, buildings, excluding glasshouses, ponds and derelict areas, 60 826ha), rape for oilseed (36 410ha), potatoes (29 791ha), crops for stockfeeding (turnips, swedes, kale, beet, *etc.*; 24 245ha), oats (22 562ha), fallow (11 220ha); vegetables (excluding potatoes; 10 776ha); linseed (2 543ha); fruit (2 021ha); peas for combining (1 640ha); triticale (1 544ha); beans for combining (1 138ha); bulbs, other flowers and nursery stock (864ha); and all other crops (3 215ha) collectively accounted for Scottish agricultural land use, as estimated for June 2000.

In order to analyse UK agriculture generally, and the comparative performance of agriculture in Scotland specifically, reference has to be made to several publications produced by the Department for Environment, Food and Rural Affairs (DEFRA, derived mainly from the Ministry of Agriculture, Fisheries and Food); the Scottish Executive Environment and Rural Affairs Department (SEERAD, formerly Scottish Executive Rural Affairs Department, Scottish Office Agriculture, Environment and Fisheries Department, Scottish Office Agriculture and Fisheries Department, Department of Agriculture and Fisheries for Scotland), the Department of Agriculture and Rural Development (Northern Ireland), (formerly the Department of Agriculture for Northern Ireland), and the National Assembly for Wales Agriculture Department (formerly Welsh Office Agriculture Department). The principle document is *Agriculture in the United Kingdom*. Other valuable documents include *Agricultural Census Statistics in the UK,* the *Agricultural Atlas,* the *June Census Analyses, Historical Agricultural Data, Farm Incomes in the United Kingdom 1999/00, Economic Report on Scottish Agriculture, Agriculture Facts and Figures Scotland 2001* (http://www.scotland.gov.uk), *Agriculture in Scotland, Farm Incomes in Scotland 1999/00, Agricultural Census, Agricultural Sample Census Sheets by Geographical Area*, and *Abstract of Agricultural Statistics* (see http://www.defra.gov.uk and http://www.scotland.gov.uk).

Provisional data in the calendar year 2000 edition, at a stage prior to the 2001 Foot and Mouth Disease (FMD) outbreak, indicated that the contribution of agriculture to the total UK economy gross value added (GVA, at basic prices less intermediate consumption of goods and services, *e.g.* feed, seeds, agrochemicals) declined further from 0.9% in 1999 to 0.8% in 2000, contrasting with a figure of 1.5% in 1996. At current prices, the GVA in 2000 equated to £6 646m. About 2% of the UK work force was employed in agriculture (557 000), illustrating the rapid decline of around 100 000 in rural employment over a decade, a decline that has not received media attention in the same way as redundancies in the manufacturing and mining industries. Nonetheless, these figures do not reveal that there is a large tranche of the UK workforce involved directly with agriculture, namely parts of the public sector such as government departments, their agencies and institutes, Research Councils and their institutes, higher-education and further-education bodies, and various groups associated with the EU; the food-processing, distribution and retail sectors; and the industrial feedstock industry. Importation of food, feed, and drink amounted to £17.004bn compared with the revised figure of £17.385bn in 1999, possibly reflecting the decline in commodity prices, and amounted to 7.6% of total UK imports. Imports of alcoholic drinks from the EU amounted to £1.619bn, and £0.848bn from the rest of the world. Exports of food, feed, and drink declined from a revised figure of £8.948bn in 1999 to £8.720bn in 2000, of which exports of alcoholic drinks to the EU contributed £1.262bn, and £1.707bn to the rest of the world. These agriculturally related exports represented 4.6% of total UK exports, down from 5.4% in 1999, 5.6% in 1998, and 6.3% in the period 1989-1991. The UK was 66.5% self-sufficient in all food types in 2000, contrasting with an average of 72.8% in the period 1989-1991. For indigenous-type food, the UK was 79% self-sufficient, declining slightly from the revised

figure of 80.5% in 1999. Household final consumption expenditure on household food and alcoholic drinks at current prices was once again up from a significantly upgraded revision of £87.245bn in 1999 to £88.6bn in 2000. Some £33.2bn (over 37%) of that figure was attributable to alcoholic drinks! Household food and alcoholic drinks accounted for only 15% of total household final consumption expenditure, continuing a declining trend over several years and reflecting the remarkable efficiency of agriculture. In the period 1989-1991, the figure was 18.8%. Domestic food expenditure alone was only 9.4% of total household expenditure compared with 5.6% for alcoholic drinks.

In June 2000, the total area of UK agricultural land plus common rough grazing was 18 306 000 hectares, of which 4 665 000 hectares were devoted to crops, and 37 000 hectares were left fallow. In the period 1989-1991, an average of 18 887 000 hectares was committed to agriculture, 5 037 000 hectares of which were harvested for crops. More detailed analysis of the cropping data reveals that the area devoted to cereals rose from 3 141 000 hectares in 1999 to 3 348 000 hectares in 2000. This was mainly attributable to an increase in the wheat area from 1 847 000 hectares in 1999 to 2 086 000 hectares in 2000. Slight increases were noted in the area devoted to oats (92 000 hectares to 109 000 hectares), and triticale (13 000 hectares to 16 000 hectares), but the barley area declined from 1 179 000 hectares to 1 128 000 hectares and the rye and mixed corn area remained at 10 000 hectares. The potato area declined to 166 000 hectares, similar to that of 1997. Other arable crops, excluding potatoes, took up less land than in 1999, declining from 1 211 000 hectares to 979 000 hectares. Pronounced declines were noted in the area of oilseed rape (417 000 to 332 000 hectares), linseed (209 000 to 71 000 hectares) and sugar beet not for stockfeeding (183 000 to 173 000). Peas for harvesting dry and field beans increased from 202 000 to 208 000 hectares, and the area for other crops reduced from 197 000 to 192 000 hectares. Reflecting tough market conditions, the area of horticultural land continued its long-term decline, to reach just 172 000 hectares. Of this area, the bulk (119 000 hectares) was given over to vegetables grown in the open; commercial and non-commercial orchards accounted for 28 000 hectares, soft fruit (including strawberries, raspberries, blackcurrants and wine grapes) 10 000 hectares, ornamentals (hardy nursery stock, bulbs and flowers) 14 000 hectares, and glasshouse crops 2 000 hectares.

Without taking account of direct subsidy payments, the average price of agricultural products fell by 2.4% between 1999 and 2000, and inputs rose by 3%. The starkness of the situation is illustrated by the fact that agricultural product prices fell by 26% over the last five years.

In terms of production, the area of land devoted to cereals increased by over 6%, and with a slight improvement in yield, the volume of harvested production reached 23.98 mmt in 2000, an 8.3% increase over the 1999 figure. The value of production, however, only increased marginally to £2.35bn. Provisional cereal yields in 2000 were 8.01 tonnes per hectare for wheat, 5.76 for barley, and 5.87 for oats. Wheat production increased sharply from a revised figure of 14.87 mmt in 1999 to 16.7 mmt in 2000, valued at £1.545bn. Barley, one of SCRI's mandate crops, recorded a small decline in production, down from a revised figure of 6.58 mmt in 1999 to 6.49 mmt in 2000 and the value declined from £735m to £693m. Oat production increased over the same period from a revised 540 mmt to 640 mmt, and the value increased from £58m to £65m.

Potato production in the UK in 2000 was affected both by planting decisions arising from oversupply and poor return in the previous year, and by bad weather in the autumn of 2000. The volume of harvested production of this key SCRI mandate crop in 2000 was 6.611 mmt, 290 000 tonnes of which were attributable to the early crop, and the remainder to maincrop. A value of production of £501m, represented a major fall from the previous year. Processed potato products in the UK were valued at around £2.16bn. Potato imports amounted to 1 035 000 tonnes, the bulk in the form of processed potato product from the EU (727 000 tonnes raw equivalent), and exports amounted to just 369 000 tonnes, the bulk of which (173 000 tonnes) was as raw material. Total domestic use of potatoes in the UK (7.447 mmt) was divided into 5.724 mmt for human consumption, 452 000 tonnes for 'seed' for home crops, including seed imports of around 162 000 tonnes, and retained stockfeed, 'chats', and waste of 1.272 mmt. Oilseed rape production suffered a steep decline from a revised 1.737 mmt in 1999 to 1.129 mmt in 2000, valued at just £249m, less than the UK crop average of £289m in the period 1989-1991. The 32% drop in value from 1999 reflected the 25% decline in planting area, and lowered yields. Subsidy payments for the crop declined by 36%, even though subsidy and compensation payments were made to cover an area of

the crop inadvertently planted with unauthorised GM oilseed rape. Sugar beet production in 2000 was provisionally assessed at 9.335 mmt, adjusted at standard 16% sugar content, and was valued at £253m. Refined sugar production for the UK was 1.37 mmt, an 11% reduction from 1999; imports amounted to 148 000 tonnes from the EU, and 1 097 000 tonnes from the rest of the world. Total exports were around 700 000 tonnes. A decline was noted in linseed production during a year of lowered yields from 302 000 tonnes in 1999 to 43 000 tonnes (40 000 tonnes on a standard 9% moisture content) in 2000. The value of production fell by 74% to just £34m, with subsidies amounting to £30m. In the previous year, linseed was seen as a subsidy-dependent, financially safer, planting option than crops such as oilseed rape.

Horticulture, an industry dominated by financially stretched, small-scale producers without ready access to subsidies and with little market muscle, overall saw a small increase in the value of production from £956m in 1999 to £974m in 2000, despite a decline in cultivation area from 149 100 hectares to 144 500 hectares. Horticultural crops grown in the open in 2000 on an estimated 143 300 hectares were valued at £650m, and £324m for protected crops grown on an estimated 1 200 hectares. The highest value horticultural crops were mushrooms (£168m), followed by lettuces (£103m), carrots (£86m), tomatoes (£75m), cabbages (£57m), peas (£55m), and cauliflowers (£43m). Orchard (top) fruit production on an area of 25 300 hectares was valued at £101m, and soft fruit at £139m on an area of 8 900 hectares, mainly attributable to two crops of importance to SCRI and MRS Ltd, strawberries (£81m) and raspberries (£42m). Ornamental production on an area of 20 000 hectares was valued at £714m, a little down from the revalorised figure of £719m for 1999, representing £398m for hardy ornamental stock, £274m for ornamental protected crops, and £42m for flowers and bulbs, including forced flower bulbs, in the open.

Purchased livestock feedingstuffs, valued at £2.167bn, declined from 20.446 mmt in 1999 to 19.827 mmt in 2000, affected by reductions in milk and pigmeat production. Purchased seeds totalled 956 400 tonnes: potatoes (471 200 tonnes including farm-saved seed), certified cereal seed (413 400 tonnes), root and fodder crops (45 000 tonnes), vegetables, bulbs, seeds for hardy nursery stock, flowers, sugar beet and oilseed rape (15 600 tonnes), and grass and clover (11 300 tonnes). The total cost of all purchased seeds at £296m was an 11% decline from the position in both 1998 and 1999. A healthy seed industry that can offer a flow of improved cultivars is of prime importance to the revitalisation of the agriculture, horticulture and forestry industries; the poor financial returns bode ill for commercial plant breeding in the UK. The diminution in public-sector plant-breeding research also bodes ill for the future provision of plant breeders, and advanced germplasm, and access to modern technology and IP.

Total Income From Farming (TIFF) is a valuable if somewhat complex measure. It refers to those with a direct entrepreneurial interest in the agricultural industry (*e.g.* farmers, growers, partners, directors, sponsors and most other family workers). Official reports stress that TIFF is acutely sensitive to relatively small changes in the values of outputs and inputs, as well as the changes in statistical methodology and sourcing of data. It is derived by deducting interest, rent and paid labour costs from Net Value Added at factor cost, the best measure of value added by the agricultural industry because it includes all subsidies. According to *Agriculture in the United Kingdom,* TIFF was forecast to fall by 25% compared with its 1999 level, to £1 882m. In real terms, the fall was 27%, equivalent to a £696m attrition, prior to any judgements yet to come for 2001 from the impacts of the FMD episode afflicting UK agriculture. Productivity measures, based on volume indices with 1995=100, show that final output (that output leaving the industry *i.e.* gross output less transactions within the agricultural industry) has remained relatively static from 1989 onwards, varying between 97.2 to 100.1, On the other hand, labour productivity as given by the index of Net Value Added per annual work unit of all labour, increased from 117.4m in 1999 to 123 in 2000, as annual work units (*i.e.* the number of average full-time persons in agriculture) fell. There was a 2.6% rise in total factor productivity as given by the final output per unit of all inputs, including fixed capital and labour.

Across the Member States of the EU, there was great variation in the income derived from agricultural activity. Changes in income as measured by Eurostat's Indicator A (see Eurostat Statistics*: Statistics in Focus,* December 2000), which is based on Net Value Added per whole-time person equivalent, showed, provisionally, rises in Denmark (24.1%), Finland (22%), Belgium (12.2%), Germany (6.9%), the Republic of Ireland (6.5%), Sweden (4.9%), Spain

(4.6%), The Netherlands (3.7%), France (1.3%), and Luxemburg (0.4%). No rise was indicated for Greece but declines were noted for Italy (-4.3%) Austria (-4.8%), Portugal (-7.5%) and alarmingly most of all, the UK (-10.8%). Reasons for the poor UK performance may include the strength of sterling, weak commodity prices, poor weather, regulatory and bureaucratic impositions, lower subsidy regimes, lack of innovation, lack of market muscle relative to the supermarkets and processors, and changed political and public attitudes to, and perceptions of, agriculture.

Within the UK, the weakening economic, political, and social rôle of agriculture varied between the constituent parts. Thus, the Gross Value Added at basic prices in 2000 was £5.038bn in England, £824m in Scotland, £320m in Wales, and £464m in Northern Ireland. The TIFF estimates reflected the impacts of the different components of farming: England £1.552bn, Scotland £228m, Wales a remarkable and worrying £-2m, and Northern Ireland £98m. Estimates of the share of agriculture of total regional Gross Value Added at basic prices, revealed marked declines over time and that it was lowest in England (0.7%), followed by Wales (1%), Scotland (1.2%), and then Northern Ireland (2.6%). A slightly different pattern existed for the share of total regional employment by agriculture: England (1.6%), Scotland (3%), Wales (4%), and Northern Ireland (7.9%). This measure may well indicate future economic vulnerability to any continuing downturn in agriculture

The total UK public expenditure in agriculture was forecast to rise by £21m from £3.161bn in 1999-2000 to £3.182bn in 2000-2001. Of this, spending under the CAP regime was forecast to increase from £2.816bn in 1999-2000 to £2.869bn in 2000-2001, of which 39% was apportioned to the Arable Area Payments Scheme, 4% to sugar and 1% to cereals. Beef, veal and sheepmeat accounted for 45% of the spend. Unlike these sectors, the pig industry was subject to heavy regulation with little support. In addition to classical swine fever, the industry suffered from outbreaks of two linked diseases: post-weaning multi-systemic wasting syndrome, and porcine dermatitis nephropathy syndrome, both accounting for losses of about £21m a year.

Environmental accounting as a measure of the environmental impact of human activity is a rapidly developing field, with guidance issued by both the EU and sections of the UN on establishing assessment frameworks. Given the complexity of human activity and the nature of judgements of environmental impacts, such accounting methodology is still developing, but providing new insights, especially as the multipartite concept of sustainability gains ground. The environmental accounts for UK agriculture presented in *Agriculture in the United Kingdom* focused on the use of finite and renewable resources, the levels of damaging emissions to waterways and to the atmosphere, general indicators of phenotypic biodiversity on farmland, and details of public expenditure on environment schemes. A number of pesticides will have new residue levels which apply from 1 July 2001 through implementation of EC Directive 2000/42/EC (see www.pesticides.gov.uk). It was recognised that the finite resources consumed by the agricultural industry included fuels (petroleum, coal, gas), the generation and the direct and indirect consumption of electricity, metals used to manufacture machinery, and the synthesis of agrochemicals. Judicious management of soil and water is essential for sustainability of agriculture. In 1999, the energy used by agriculture represented less than 1% of overall UK energy consumption. It is clear that the Climate Change Levy and the prospects of agrochemical taxes (as well as existing downward pressure on input costs) will lead to increased energy efficiency, and lower emissions, inevitably by widespread cessation of hitherto profitable agricultural activity. Of relevant renewable energy sources, the combination of agricultural biomass, including straw, livestock wastes, and specific crops, particularly those used in short-rotation coppices, attracted attention. Agricultural biofuels were projected to contribute substantially to the policy of the generation of 10% of UK electricity from renewable resources by 2010. Wind farms on suitable agricultural land could offer another potential source of 'green' energy.

Quantification of agricultural emissions is difficult, given the diversity of sources (*e.g.* agrochemical leaching into groundwaters, atmospheric emissions from plant and animal wastes *etc.*). Nonetheless, integrated crop management (ICM) and integrated farm management (IFM) practices giving an holistic farm management system which is designed to balance normal measures of efficiency with minimising environmental impact, are being promoted by Government, especially in conjunction with the Linking Environment and Farming (LEAF) initiative (www.leafuk.org). Related to this is the European Initiative for Sustainable Development in Agriculture,

and *A Common Codex for Integrated Farming* detailing a set of common principles and practices to achieve sustainability. In addition, there are several agri-environment payment schemes to encourage the protection and conservation of the landscape and foster biodiversity (see section on Agriculture and Food). Agriculture's impact on biodiversity must not be regarded simplistically, however. Specialist farmland bird species, in particular, have declined in numbers over the past three decades, presumably as a result of changed agronomic practices, a reduction in the numbers of small-scale mixed farms, loss of habitats, natural population fluctuations *etc.* The rôle of weed species can be regarded positively and negatively in terms of biodiversity, food supplies for heterotrophs, soil structure, marketability of the produce, and visual appearance of the countryside.

Other important reports and documents relevant to agriculture produced by Government included *Environmental Regulations and Farmers* from the Better Regulation Task Force; *A Forward Strategy for Scottish Agriculture and Rural Scotland: A New Approach* from the Scottish Executive; the Rural White Paper *Our Countryside: the future, a fair deal for rural England*; the *BSE Inquiry Report*, and the Competition Commission's report on supermarkets. Helpful websites include www.defra.gov.uk, www.scotland.gov.uk, www.wales.co.uk, www.dardni.gov.uk, and www.foodstandards.gov.uk.

### Plant Biotechnology

In previous editions of the *Report*, besides describing the range and potential of GM crops, the concerns surrounding GM crops and their regulation have been presented in considerable detail, although plant and agricultural biotechnology encompasses huge and dynamic areas of R&D and commercial activity beyond the generation and release of transgenic plants and animals. Nonetheless, in 2000, over 44 million hectares of GM crops were grown commercially in 14 countries. By the end of 2000, over 11 500 field trials of transgenic crops had taken place in 39 countries, including eight LDCs. The USA accounted for over 60% of the GM crop planted area.

Agricultural biotechnology encompasses modern crop and livestock breeding and pathology, aspects of agronomy, veterinary services, propagation, remediation of agricultural wastes and slurries, restitution of agricultural land, storage and processing of agricultural products, diagnostics, nutraceuticals, functional foods, alcoholic drinks and beverages, industrial feedstocks (non-food, see *SCRI Annual Report 1999/2000* pp.46-47), horticultural and aspects of forestry biotechnology, other areas of biotechnology, and service providers. The production of pharmaceutical and related compounds by plants is a fast expanding area of great interest. Series of mergers and acquisitions in recent times have given rise to powerful multinational groups and interlinked groupings, although it is noteworthy at this juncture that many largely national supermarket and retailer chains are substantially larger than some of the more prominent multinational agbiotech companies, and effectively shape their futures. Nearly all the agbiotech companies operate using molecular-genetics-based technologies in common with other biotechnology sectors *e.g.* healthcare, such that there are overlapping IP interests. All are noted for their rapid uptake of new technologies and collaborating with universities and research institutes. It is the advent of the new bioindustries which employ sophisticated scientific, technological, and engineering concepts and processes that are giving rise to quantum leaps in the precision, accuracy, sustainability, and cost-effectiveness of many areas of human endeavour, especially agriculture, environmental protection, and healthcare. All these sectors have yet to reach their zenith. All have been damaged to varying extent by individuals and organisations opposed to various aspects of biotechnology and globalisation, as well as by media accounts of regulatory failures on the control of livestock diseases that adversely impacted on consumers and the taxpayer. Some individuals and organisations have questioned the need for agricultural biotechnology, ignoring global trends in population growth and increasing consumer demands, and the need to mitigate the environmental impacts of food production on a steadily decreasing but precious global area of cultivated and cultivatable land. By about 2020, given continued freedom from catastrophic or cataclysmic events (*e.g.* war, diseases, asteroid strike *etc.*) and modest population growth, food production capacity will revert to being a political imperative. This position contrasts with the present rapid decline in importance and the negative perception of agriculture nationally and internationally.

An area of priority must be plant breeding which has an underpinning rôle in providing improved types of plant to resist the depredations of pests, diseases and adverse environments, to enhance yields, to meet ever-increasing customer demands, to provide livestock feed, and industrial non-food supplies (feedstocks). It is in

essence, even using the most advanced technologies, time-consuming, spanning decades. It requires strategic planning, access to parental material, crossing programmes, selection stages, and finally statutory testing and marketing. For species with long juvenile (ripeness-to-flower) periods, such as many forestry hardwood and softwood species, long-term planning is essential as of now.

Conventional and biotechnologically based plant breeding rely on access to parental material *i.e.* biological diversity. The Convention on Biological Diversity (CBD) specifically called for benefits flowing from the use of biological diversity to be shared with the country of origin of that material. As pointed out by R. Raymond and C. Fowler in *Sharing the non-monetary benefits of agricultural biodiversity*, Issues in Genetic Resources No. 5, 2001 published by the International Plant Genetic Resources Institute, the focus on monetary benefits has led to a downplaying or even disregard for significant non-monetary benefits, and to date there is no agreed definition of 'country of origin'. Non-monetary benefits were seen as access to a wide range of germplasm, improved material and genes, technology, training, information, joint projects, and sharing benefits and the burden of costs. Bilateral agreements between Ecuador and the USA, and networks such as the International Network for Genetic Evaluation of Rice, provide functional examples generating worthwhile non-monetary benefits. To identify the origin and value of individual and sets of genes in the complex genome of any commercial crop species would be a major undertaking, and would seriously impede the exchange of germplasm and the development of new cultivars. Institutions such as SCRI have rôles in maintaining, accessing and releasing germplasm. In 2001, a new international treaty on the use of plant genes was agreed by 116 countries in Rome after seven years of debate; Japan and the USA abstained on the basis that IP rights should accord with WTO rules.

Plants are special. They share characteristics (Table 3) that are fascinating and highly complex. Accordingly, agbiotech applications with respect to plants require specialised facilities and expertise.

Agbiotech is an integral part of the so-called 'knowledge economy'. It is dependent on integrating the skills and concepts of genomics, proteomics, metabolomics, analytical chemistry, information technology, agriculture, horticulture, forestry, and ecology. By deploying an array of fast-developing, generic technologies, it is able to address demands for food and non-food products, for both niche or mainstream markets, bringing in its wake detailed

- Eukaryotic organisms based on a cellular construction. More advanced forms show the phenomenon of alternation of generations.
- Autotrophic mode of nutrition arising from the possession of intracellular chloroplasts that are responsible for photosynthesis.
- Complex carbon metabolism giving rise to special type of squalene cyclisation and production of elaborate cell walls. Considerable cellular and metabolic heterogeneity. Living cells exhibit turgor.
- Lack of cell and organism motility: cells often joined by cytoplasmic bridges, the plasmodesmata.
- Open-ended development of modular growth habit by virtue of retaining apical and lateral meristems.
- Growth patterns subject to interacting influences of the environment with specialised detection systems and biological clocks. Light (spectral composition, intensity, temporal distribution/photoperiod), temperature (day/night and root/shoot differential requirements as well as various chilling and high-temperature requirements), gaseous composition of the atmosphere, magnetic fields, gravity, wind-speed, humidity, rainfall, edaphic (soil) factors, allelopathic and allelomediatory effects, pests, diseases, and grazing all affect growth patterns giving rise to considerable design plasticity, and modification of chemical composition.
- Unique positional signalling systems: no differentiated nervous system.
- Single parenchymatous cells able to generate whole plants (totipotency). The pattern of differentiation can be regulated by defined growth media.
- Reproductive strategies can be sexual and/or asexual, and may involve a juvenile or ripeness-to-flower period. Dormant dispersal units or structures may be produced to distribute progeny in time and space.
- Complex mass transport pathways: the xylem is dead when functional for transport of water and mineral salts: the living phloem transports the products of photosynthesis and nitrogen metabolism. Transport of solutes and growth factors may be apoplastic or symplastic.
- Cellular and physiological differentiation associated with vacuolation and susceptibility to senescence processes. No immune system.
- May have large genome sizes (e.g. *Lilium davidii,* DNA content of $43.2 \times 10^{-12}$g, per genome, and $2\chi=24$, compared with $6 \times 10^{-12}$g, $2\chi=46$, for humans. One picogram ($10^{-12}$g) is equivalent to $0.965 \times 10^{9}$ base pairs).

**Table 3** The general characteristics of plants.

product specifications, properly monitored quality-assurance schemes, improved habitats, lowered inputs, advanced waste processing, and IP protection offering competitive advantage. Current agricultural practice (both conventional and organic) will come under greater scrutiny as its outputs, including those affecting non-monetary goods, will not be able to compete with agbiotech production in the medium-to-long term, regardless of the efforts of certain organisations, governments, or groups of nations to retard the progress of technology. In broad terms, the six major factors suppressing the development of agricultural biotechnology at present are the weakened economic standing of agriculture and its commodities; a general downturn in technologically based investments; a much-reduced emphasis on agricultural and plant sciences in universities; food scares; the vulnerability to anti-technology pressure groups which focus on individual scientists, their organisations, companies, politicians, local and national governments; a risk-avoidance culture; and diminished public-sector investments in the sector. Perhaps the public sector should take the lead in introducing GM crops. Few investors currently consider the vast sizes of the commodity and food markets, the opportunities for wealth creation and improvement in the quality of life, and the societal needs for the products of agbiotechnology. As a consequence of these points, the market has not favoured merged agricultural, pharmaceutical, and environmental companies.

Closely associated with a portion of the agricultural biotechnology industry is the agrochemical industry. Collectively, the global turnover of the sector was *circa* $40bn, and it sustained a strong R&D base. Focused on by the naïve as an unnecessary imposition on agriculture and horticulture, in reality, the use of agrochemicals was instrumental together with improved cultivars and better agricultural engineering in the agricultural revolution that enabled global food production to match the growth in the human population. As the industry developed during the 1980s and 1990s, new products with lowered environmental impacts were released as it became subject to legislative and other controls. In parallel with this, a series of mergers and acquisitions took place leading to stronger vertical and horizontal integration. By 2001, there were only seven international R&D-based agrochemical companies, three of whom were headquartered in the USA (Dow Agrosciences, Dupont, and Monsanto), and the remainder in Europe (Aventis, BASF, Bayer, and Syngenta). Further mergers were mooted. Only Syngenta – the largest of the companies in 2000 - sustains a substantial research centre in the UK, contrasting starkly with the situation three decades ago, when the UK was a major international force in the agrochemical industry, reinforced by a strong public- and private-sector R&D base. As plant biotechnology, newer forms of synthesising and analysing bioactive compounds, and bioinformatics (computational genetics) developed, it was expected that the UK with its pioneering contributions in those areas as well as in agrochemicals would reach a dominant position, extending beyond agrochemicals *per se* into the generation of improved cultivars and wholly novel plants, industrial crops and feedstocks, bioremediation, and novel products for healthcare purposes. This was not to be. Fortunately, the position is recoverable with suitable investments in the public and private sectors, new attitudes and understanding, and crucially, political support.

In 2000, biotechnology companies *per se* generated revenues of nearly $50bn and sales were in the region of $15bn, mainly in the pharmaceutical, bio-medical, agriculture, food technology, environment, energy, chemical, and service sectors. By 2002, it is anticipated that the agbiotech company sector will have revenues of around $2.5bn. As distinct from revenues, the value of the herbicide-tolerant and insect-resistant GM crop market was estimated at $2.6bn, and was expected to reach $6bn in 2005. Ancillary applications of the technology were beginning to be introduced into the treatment of farm and urban wastes, the treatment (mainly phytoremediation) of polluted waters and land, generation of biomass plants to be used as an energy source, and diagnostics and biosensors. Likewise, substantial growth took place in bioinformatics to reach a market estimation of $250m in 2000, and is expected to reach £3bn by 2005. Analytical instrumentation for biotechnology applications was also a substantial market.

In the UK, the issue of GM technology was given prominence by the ongoing Farm-Scale Evaluations (FSEs) of GM herbicide-tolerant (GMHT) crops. These field-level investigations were undertaken at the behest of government and an industry body - the Supply Chain Initiative on Modified Agricultural Crops, and were based on spring and winter varieties of oilseed rape, fodder beet, sugar beet, and forage maize. Orchestrated hostility, campaigns led by some sections of the broadcast and publishing media, and

protests by individuals led to an analysis of the trials by the Agriculture and Environment Biotechnology Commission (see *Crops on Trial. A Report by the AEBC*, 2001) The Commission made 10 recommendations as follows. (i) The programme of FSEs should be completed subject to certain conditions *e.g.* witholding permission for commercial plantings meantime, operating with adequate separation distances from organic crops, and communicating with the public, especially local stakeholders. (ii) The Government should use clear and precise language in its press releases and publications. (iii) Policies should be developed on how to use the results of the FSEs in future decision-making. (iv) An independent review should be commissioned of all information that will complement the results from the FSEs *e.g.* from the Advisory Committee on Pesticides, the Advisory Committee on Releases to the Environment *etc.* (v) Ensure that the level of publicly funded research is such as to ensure an objective independent assessment of the potential impacts of both current practices and new technologies on agriculture and the wider environment. (vi) There should be commitment to an open and inclusive process of decision-making around whether the GM crops being grown in the FSEs should be commercialised, within a framework which extends to broader questions. (vii) Early attention should be given to the framework for post-commercialisation monitoring. Without prejudging the issue of whether GM crops will be approved for commercialisation in the UK, the Government should be prepared to publish and consult widely on its proposals for the post-commercialisation monitoring which would be needed, and for the action to be taken if adverse effects were discovered. (viii) There should be improvement of the understanding of the basis of public views by drawing on the work of social scientists in this field. (ix) Methods should be improved for dealing with risk and uncertainty in relation to the use of biotechnology in agriculture, by ensuring that all the regulatory processes incorporate the principles developed by Lord Phillips in his review on BSE and by Lord May when he was Chief Scientific Adviser to the Government, and that the regulators are publicly explicit about where areas of uncertainty occur in their deliberations and how they have tried to take them into account; and by developing and disseminating examples of best practice. (x) Specific consideration of the future of GM crops should be included in the discussions about the future of agriculture in the UK. The various strategic reviews of farming and food being undertaken by the UK administrations should explicitly address how to promote the co-existence of different forms of farming in the UK. There should then be a wider public debate involving a series of regional discussion meetings to consider what rôle GM crops might have in UK agriculture in the future. The AEBC stated its willingness to contribute to this process.

Missing from the recommendations was an analysis of the economic and social implications in the UK of not introducing commercial GM crops, of delaying the introduction of proven commercial GM crops, and costing the regulatory and review processes. No overt value judgements were offered on the validity or naïvety of the diverse arguments and concerns expressed to the Commission, but the evolution of consensus views was promoted.

Expansion of the global area of GM crops, penetration of the products derived from GM crops into global markets, tourism, consequential analysis of deliberate releases and inadvertant escapes, predictive studies on environmental impacts, development of beneficial gene banking and regenics, new GM crop types of obvious benefit to consumers, and eventual removal of the EU moratorium on the registration of GM crops may offer a boost to the European agbiotech industry, and will hasten decisions on the future of this branch of agriculture, horticulture, and forestry in the UK. The report of the New Zealand Royal Commission on Genetic Modification was an influential landmark analysis which favoured rational thinking for the benefit of society and industry. The tide is surely turning to a more balanced open-minded position, in line with confidence growing in the advisory and regulatory mechanisms.

### Concluding comment

As in previous years, the Institute, MRS Ltd, and BioSS still thrive, producing high-impact globally relevant scientific research and development with unrivalled value-for-money and productivity, and meeting end-user needs. We play a full rôle in Scottish, UK, and international plant and environmental science, launching major scientific initiatives, and we participate extensively in higher educational activities. I thank SEERAD and all our sponsors, public and private, and congratulate and offer my gratitude to my colleagues for their continuing loyalty, forbearance, and outstanding efforts. I thank especially our Chairman, Mr James E Godfrey, for his tremendous support, expertise, and dedication, and the rest of the Governing Body for their commitment and contribution to our development.

# Science Overview

Wayne Powell & John R. Hillman

The scientific reports from the four Heads of Division comprehensively cover the main aspects of the Institute's research activities. This article deals with the more strategic point of how the SCRI scientific portfolio meets the needs of our public- and private-sector sponsors, and society generally. 'How does our science make a difference'? To address this issue, we need not only to examine our scientific and technological competencies, but also to re-calibrate our thinking to take into account fundamental changes in the agricultural industry. Globally, commodity prices are under pressure. There has been a prolonged period of buoyant agricultural production unaffected by massive weather perturbations and by depradations of pests and diseases. Food has never been so plentiful for the bulk of the world's population. In Europe, reforms to the Common Agricultural Policy, particularly those leading to an erosion of production subsidies with more emphasis being placed on environmentally benign production systems, will herald a new era of innovation and opportunity for those organisations with the intellectual agility, managerial flexibility and competencies to respond to the new challenges confronting agriculture in the 21st century. Growing populations will pose new challenges for agricultural productivity, quality and land use efficiency. Our research perspective must, however, also consider the impact of 'society on science' as a major political and economic driver which is shaping government and EU agricultural and environmental policy. This means that scientific and technological opportunities cannot be considered in isolation from societal and political factors. Today, the role of the consumer in democratic societies is paramount, with much more emphasis being placed on safety, quality, authenticity and traceability of our food and industrial feedstocks. Some of the challenges and opportunities facing us are summarised in Figure 1. It is against this background that I wish to consider how SCRI can adapt to the changing face of agriculture, exploit its strong science base, and contribute to the future success of the agricultural and related bio-industries?

Clearly, our research needs to take into account the impact and influence of shifts in economic, political and environmental factors, but we must also be in a position to shape and influence policy by providing new and innovative solutions through the generation of fresh scientific ideas, concepts, technology, products and processes. Scientific innovation and discovery will be the main drivers for broadening the end-user relevance of our research. In parallel with enhancing our strong science base and maintaining an international perspective, we need constantly to con-

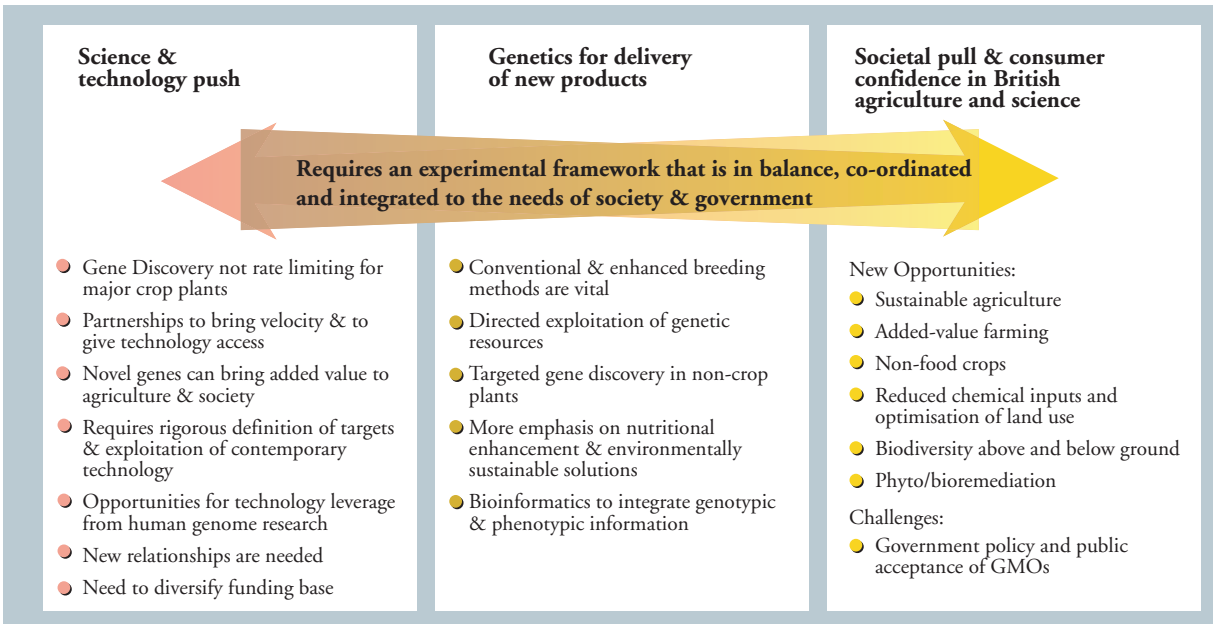


**Figure 1** Opportunities & Challenges: getting the balance right.

nect our research to the food and product supply chain so that we can identify research opportunities and solve problems at multiple points along it. A critical factor will be the deployment of information or knowledge management systems that will allow us to make connections to the various groups contributing to this supply chain. The loss of subsidies is also likely to promote a wider range of farming systems, with more emphasis on added-value farming that utilises natural resources in an environmentally sustainable manner. Some, if not all, sectors of the agricultural industry will need to be more competitive globally and this will require access to, and the introduction of, new technology in a responsible manner. At SCRI, we have access to unrivalled genetic resources and possess the skills and competencies to move from genes to products *via* plant breeding and/or biotechnological routes. Such technology platforms are vital for the delivery of new products from traditional crops for the feed, food, drink and non-food markets. In addition, these skills will be essential for the creation of new niche market opportunities. Success in this arena is very dependent on understanding market needs and translating these needs into breeding and research goals. Under these circumstances, a different strategic overview is needed that recognises the need for calculated scientific risk-taking and exhibits flexibility and adaptability in re-defining our research priorities to reflect changes in political, societal and market forces, and priorities.

From a research perspective, we must continue to embrace genomics and post-genomics technologies together with computational biology to ensure that our research is internationally competitive and is positioned to bring new options and opportunities to agriculture and the bio-industries. The recent completion of the genome sequences of a number of bacterial species and several eukaryotes, including one plant species (*Arabidopsis thaliana*), has profoundly affected the way in which we select and answer questions in biology. The availability of genome sequences opens up a vast range of new scientific opportunities. To capture these, we need to: i) alter the ways in which biological problems are approached; ii) attain a balance between laboratory-based research programmes and computational biology; and, iii) be prepared to change the emphasis of research activity to ensure that new scientific discoveries can be translated into viable new options for agriculture.

Based on these needs, the Institute has recently re-prioritised and consolidated its research into nine programmes that are organized around three themes: Understanding of Basic Mechanisms & Processes; Genes to Products; and, Management of Genes and Organisms in the Environment. Care has been taken to align research programmes to the needs of end-use sectors as identified by the SEERAD strategy document (1999 – 2003), as shown in Table 1.

A major priority for SCRI is to ensure knowledge transfer and exploitation of results emerging from our scientific research to the end-user communities. This is not uni-directional, however, and our research benefits from interactions with the end-user communities

| | Sustainable Agriculture | Environment & Natural Heritage | Nutrition & Human Health | Food & Bioindustries |
|---|---|---|---|---|
| Soil & Environmental Sciences | P 6<br>P 7<br>P 8 | P 3<br>P 5<br>P 6<br>P 7<br>P 8 | | P 6 |
| Plant Science | P 1<br>P 3<br>P 5<br>P 6<br>P 8 | P 5<br>P 6 | P 1<br>P 2<br>P 4<br>P 5 | P 1<br>P 2<br>P 4<br>P 5 |

**P1:** Gene expression, manipulation & transgenics
**P2:** Cell-to-cell communication
**P3:** Host-pathogen interactions
**P4:** Quality, health & nutrition
**P5:** Genome dynamics
**P6:** Ecosystem management & biotechnology
**P7:** Ecological plant interactions at the plant: soil interface
**P8:** Plant-parasite co-evolution
**P9:** Computational biology - cross cutting programme and relevant to all activities

**Table 1** Relationship of SCRI's research programmes to science and end-user domains identified by SEERAD strategy document (1999-2003).

| Themes | Research Thrust | Relevance, added-value and synergy |
|---|---|---|
| **1. Understanding of Basic Mechanisms & Processes** | ○ Functional genomics to validate gene-to-phenotype relationships.<br>○ Mechanisms of gene silencing/disruption.<br>○ Cell-to-cell communication & long distance transport.<br>○ Protein/protein interactions and signalling processes.<br>○ Comparative microbial genomics.<br>○ Quantification and localisation of global gene expression.<br>○ Diagnostics. | ○ Research that underpins gene expression, function, stability and transfer which are crucial for the development of efficient and safe bio-based technologies.<br>○ Create generic technology platforms for delivery of therapeutics and high-value biopharmaceutical products in plants.<br>○ Core competencies and skills aligned to enhance synergy in the post-genomics era.<br>○ Resistance genes for deployment in sustainable agriculture. |
| **2. Genes to Products** | ○ Application of large-scale and targeted gene discovery and profiling approaches to explore & exploit functional diversity in the delivery of new and improved crops.<br>○ Application of genomics technologies to plant breeding.<br>○ Comprehensive diversity studies to identify haplotype gene content.<br>○ Connecting DNA sequence polymorphisms to heritable phenotypic differences with a strong focus on pre-existing, natural variation.<br>○ Primary targets to include product quality and nutritional value. | ○ Focus on genomes as a source of genes to provide options for novel products that can be exploited by both breeders and biotechnologists.<br>○ Provide the resources and technology required to clone and manipulate genes controlling complex phenotypes.<br>○ Devise new breeding strategies that embrace and exploit the tools of contemporary genetics.<br>○ Improved understanding, characterisation and exploitation of plant genetic resources.<br>○ Combined genomic, genetic, biochemical, phytochemical and breeding expertise for the delivery of next generation crops and products for the benefit of consumers and other end-user communities. |
| **3. Management of Genes & Organisms in the Environment** | ○ A universal, individual-based approach linking plant traits to population characteristics.<br>○ Effects of population (genetic) diversity on ecological resilience.<br>○ Interdependence over space and evolutionary time of plants, herbivores and parasites.<br>○ Understanding of plant-parasite interactions and response to the environment.<br>○ Disease epidemiology. | ○ Research underpinning environmentally sustainable production systems, with particular emphasis on conserving and utilising biodiversity and protection of soils.<br>○ Ecological management of genetically modified crops in the environment.<br>○ Integration of research activities from molecules through to genomes and populations and their biological interactions in the environment.<br>○ Integrated pathogen/pest management. |

**Table 2** Conceptual basis for the development of themes and relevance to SCRI's science strategy.

by ensuring that markets are correctly identified and translated into viable research targets.

The organisation and integration of research programmes into Themes (Table 2) reflects our need to change in response to technological advances and to attain a balance between discovery science, as illustrated by genome sequencing projects, and traditional hypotheses-driven research groups. Changes are also required to ensure that our research is connected to the food supply chain and recognises the need to move beyond the simple 'farm gate to the dinner plate'concept. Table 2 summarises the main research foci of each theme together with relevance, added value and our vision to broaden the end-use relevance of our research to include the production of high-value biopharmaceutical products.

The timely delivery of these research goals is dependent on investment of resources. Fortunately, our successful bid for supplements to our grant-in-aid, the so-called 'outer core' component, will reinforce strongly our capability and competency in selected areas in each of the three Themes. The ways in which the outer-core programmes are integrated with our core activities are designed to 'use modern biotechnological and information science tools to create plants, especially crops, that are capable of deliver-

ing added-value products in an environmentally sustainable manner'. These goals also contribute to the creation and development of cross-disciplinary teams. Our future success is also dependent on access to cutting-edge technology. During the past year, we have identified two priority areas for capital equipment investment: large scale genotyping/sequencing and metabolic profiling and proteomics. The purchase of an ABI 3700 system, together with a PSQ 96 Pyrosequencing system, provides state-of-the-art technology for gene discovery and single nucleotide polymorphism (SNP) detection. Two new mass spectrometers (ThermoQuest LCQ-DECA and ThermoQuest TEMPUS-TOF) have also been purchased to increase substantially our capabilities and sample turnover in both high-throughput metabolic profiling and proteomics. A Q-Bot (Genetix) robotics system for colony picking and arraying technology has also been purchased. The picking system is the fastest on the market, with a capacity of up to 3,500 picks per hour. The Q-Bot has two arraying facilities, gridding on membranes to a maximum density of 23k clones per 22cm filter, and microarraying onto glass slides to 14k spots per slide. This is only the third Q-Bot to be installed in the UK and the first in Scotland.

A vital aspect of our future science strategy is the need to develop partnerships to bring velocity and, in some cases, to allow technology access. A good example of this is provided by our relationship with Large Scale Biology, USA. Outer-core funding is designed to extend and broaden our relationship with LSB, particularly in the area of viral vector development in barley and cereal functional genomics. The production of recombinant proteins in plants *via* viral vectors represents an opportunity to create biopharmaceuticals relevant to clinical medicine. Part of our strategic intent, therefore, is to explore with LSB research and commercialisation partnerships, methods that will allow delivery of SCRI's science and technology to a wider range of end-users.

The recently formed strategic alliance with the Waite Institute, Adelaide, Australia also provides an example of partnerships between organisations with complementary expertise and is designed to make our research more competitive globally. In this case, we are exploring options for joint funding from various agencies to develop new approaches to the localisation of quantitative traits based on association mapping. These examples of strategic alliances, together with our role as co-ordinating centre for the International Triticeae Mapping Initiative, reflect the needs of modern biology where new relationships are needed to accelerate and integrate our R&D effectiveness.

The interface between research and post-graduate training is a traditional strength of the Institute that merits renewed investment. Post-graduate students and visiting scientists are a valued and integral part of the academic life of SCRI. Generations of students and visitors have benefited from SCRI's unrivalled facilities, resources and competencies of our staff. Part of our vision, therefore, is to establish a recognised post-graduate school at the Institute that actively markets our unique range of facilities and expertise. These developments reflect our commitment to train talented postgraduates and to nurture our relationships with universities in the UK and overseas to ensure that SCRI plays a pivotal role in the generation of future scientific leaders.

In conclusion, our future success will be built around four inter-related elements: innovation, internationally competitive research, partnerships to develop new opportunities and attract inward investments, and the need to realign our research priorities to deliver added-value options for agriculture and the related bio-industries. We must continue to meet successfully our demanding performance indicators and unrivalled value-for-money research productivity. Finally, we must recognise that information technologies are transforming our society and we are in the midst of an information revolution. Our future will be dependent on enhancing our visibility and using many forms of communication beyond conventionally printed scientific articles to reinforce our identity and purpose.

# Electron Microscopy at SCRI

I.M. Roberts

As you read this, it is sobering to realise that there has been a continuous electron microscopy service at SCRI for over 43 years, making it a research presence that started before most of the current staff were born. It also means that, because this was a new technology in its infancy, this Institute was at the forefront of the development of electron microscopy in biology, particularly in the study of plant viruses. Initially SHRI, as it was then called, borrowed time on an old EM 2 electron microscope that was the first EM in Scotland, (Fig. 1). This was supplanted in 1958 by a second-hand Metropolitan Vickers EM3A microscope that boasted a 'fitted' binocular for focusing, and a large luminous faced clock, just in case you were so engrossed with the work that you forgot that it was time to go home, or, more likely, unable to see your watch in the complete darkness! This electron microscope had a few unusual features. Its first user

Figure 1 EM 2 electron microscope (c.1958).

here was a Dundonian called Jimmy Cathro (of whom more later), and he, like almost all early electron microscopists, topped six feet in height. Unfortunately, the console of this machine was rather too low to the ground, either to sit comfortably or to view the screen through the binoculars. His answer…. raise the whole microscope to a more suitable height, which he did by standing it on layers of wooden disks, actually the 'holes' cut from lavatory seats by a local manufacturer, as shown in the accompanying picture (Fig. 2). The other 'unusual' feature

Figure 2 Metropolitan Vickers EM 3A electron microscope (1960).

was that this EM had two massive radiation leaks, one through the gun, and the other through the camera chamber that effectively bathed the operator's lap in unmentionable amounts of radiation that was thought to be damaging to the male reproductive organs. However, since its last operator went on to father two healthy children, this seems to have been an unfounded fear.

Such was the impact of the EM on the Virology research, that in 1961, the A.R.C Visiting Group authorised the purchase of a new EM which was installed in 1962, a German machine called the Siemens Elmiskop I which cost £11,000 (Fig. 3). At

Figure 3 Siemens Elmiskop I electron microscope (1962 – 1980).

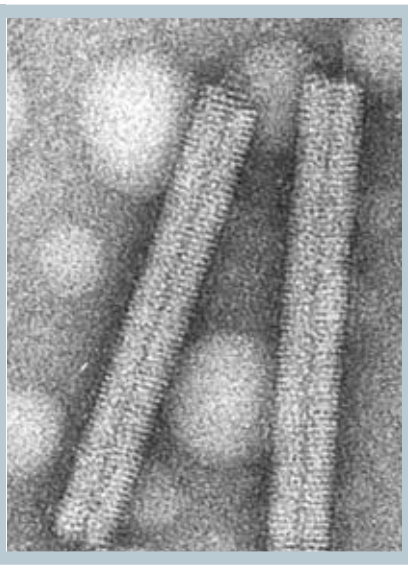

Figure 4 *Tobacco rattle virus (TRV)* particles in uranyl formate negative stain, imaged using the Siemens Elmiskop, and showing the 2nm helical periodicity.

the time, this was the 'Rolls Royce' of electron microscopes. It was capable of resolving 2nm routinely and some of the early pictures taken by Jimmy Cathro on this microscope are still classics and were used in text books. To do justice to this fabulous new equipment, an old pea shed was converted to form the first SHRI Electron Microscopy suite. Not long after the installation of this electron microscope, the National Radiological Protection Board (NRPB) visited the site to establish the safety (or otherwise) of this new radiation source. Imagine, therefore, the considerable concern when they found that a focused beam of X-rays was being emitted from a flaw in the electron gun casing, and was passing uncomfortably close to the Farm Manager's chair in his office next door. Lead sheeting was immediately glued on to the gun, thus permanently curing the problem. One interesting aspect to the purchase of this EM was that, in those far off days, considerable pressure was put on scientists to 'buy British'. At that time, the only UK manufacturer of electron microscopes was the AEI company whose microscopes were 'Morris Minors' compared to this German 'Rolls Royce', and realistically were not serious opposition. To actually purchase this foreign machine, the Institute therefore had to pay a 30% cost penalty to Customs and Excise, before it could be imported. So much for scientific freedom! Nonetheless, the fact that you had a 'Siemens' meant that you were envied by everyone else for the next ten years or more. Time would, however, prove that this was a wise investment, because it performed to its original specification for the 18 years it served the Institute. It comfortably resolved the 2nm helix of *Tobacco rattle virus (TRV)* (Fig. 4), kept SCRI at the forefront of electron microscopy, and helped to establish its international reputation for plant virus research under Bryan Harrison. The Siemens Elmiskop I, which moved into the old Laboratory Service workshops in 1968 when they were converted into the present EM laboratories, left SCRI in 1980 in bits, as spares for another 20 year old machine operating at the Moredun Institute, Edinburgh, complete with part of the then service engineers thumb, the legacy of a mishap with a high voltage valve and an earthing strip.

When Colin Cadman became the second Director of SHRI in 1965, one of his first changes was to split Virology and create the fledgling Zoology section under Charles Taylor, thus establishing a co-operation and rivalry which continued for the next 30 years or so. This resulted in the acquisition of the Institute's second EM, a Jeol Superscope for Zoology in 1967, thus setting the precedent of having two Departments, each with its own EM unit and technicians. This new EM was unusual in that the column was angled like the barrel of a cannon and you sat in front looking down the 'muzzle' (Fig. 5), and the electron beam was so poorly screened that it could sometimes melt the copper support grids of inexperienced operators. One wonders what it did to the operator, but like the present incumbent, he not only survives, but thrives. Its poor performance and difficulty of use thus led to the purchase of a new EM for Zoology, the

Figure 5 Jeol Superscope electron microscope (1967 – 1970).

Figure 6 Hitachi HS 8 electron microscope (1970 – 1985).

Hitachi HS8 (Fig. 6) in 1970, and this gave good service for 15 years until its replacement in 1985, when this microscope was put into an electron microscope collection in an Edinburgh museum. Prior to the arrival of the Hitachi microscope, Zoology made use of the Siemens Elmiskop, usually after normal working hours, and this EM produced the first ever micrographs of spherical virus particles located at a specific retention site within its nematode vector (Fig. 7). Such was the rivalry in the quest for scientific scoops in those early days, that, because we (Virology) had the only darkroom used to develop the photographic plates, the 'opposition' would sometimes develop

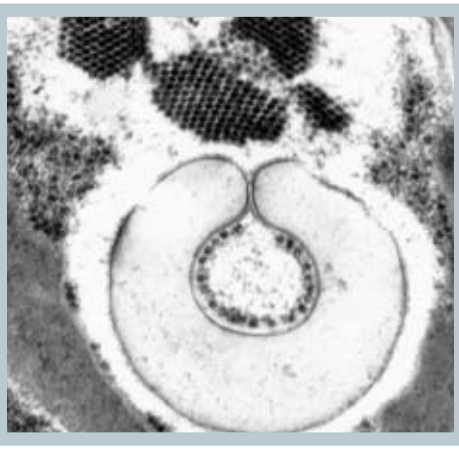

Figure 7 Spherical particles of *Raspberry ringspot nepovirus (RRV)* attached to the lumen of the odontostyle of the vector nematode *Longidorus macrosoma.* Small crystalline clumps of virus lie free in the foregut.

theirs after hours, in case sensitive results became common knowledge!

Along with the rest of the biological science community, electron microscopy moved ahead swiftly with new developments in staining, ultramicrotomy and imaging, nowhere more rapidly than in the field of virology. A second Virology EM was therefore bought, ostensibly as a replacement for the existing microscope, but this was reviewed in the light of the volume of work. This brought the number of EMs to three, thus making SCRI unrivalled in Scotland and only surpassed by the John Innes Institute, Norwich, which had four. This latest acquisition was a Philips EM 301G, (Fig. 8) the new 'leader of the pack' and it was installed in 1973 in rooms specially constructed for it. In those halcyon days, it was common Institute

Figure 8 Philips EM 301 G electron microscope (1973 – 1990).

policy to allow you to choose your own colour scheme, and the new Philips was surrounded by dull yellow and deep purple, ideally suited to rapid dark-adaption of the eye. However, this colour scheme did not meet with universal approval, notably by one Tony Murant who maintained he 'wouldn't be seen dead in there', thus evoking the instant retort by one of the female technicians, 'that's why we chose it!'. This microscope was fitted with the first eucentric goniometer stage (hence the 'G' in the name, and now a standard stage for all new EMs) which allowed the operator to examine a specimen under tilt conditions, and thus permitting 3D reconstruction from a 2D image. To mark the auspicious occasion of its installation, a cocktail party was held in the new refur-

bished EM suite, hosted and supplied by Bryan Harrison, and enjoyed by all (except Zoology). At the time, this electron microscope had the highest routine performance specification using a conventional specimen stage, and was guaranteed to give a resolution of 0.34nm on site using graphitised carbon as a test specimen. In fact, our new microscope did considerably better, actually achieving 0.17nm, the half-lattice spacing of graphite, as can be seen from the micrograph in Figure 9.

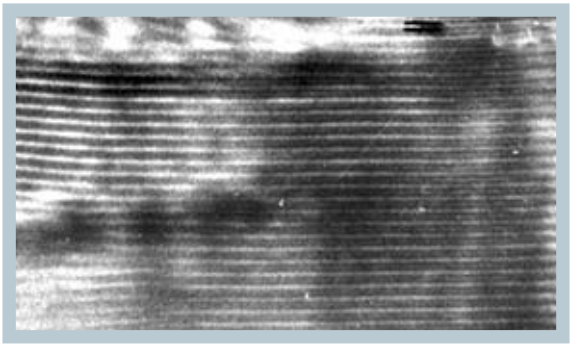

Figure 9 Graphitised carbon test specimen for electron microscope resolution, showing the 0.17nm half-lattice spacing of the crystals.

Throughout this period of often frenetic and competitive EM activity, additional equipment essential to the units was purchased. This included new photographic enlargers, printing equipment and also new ultramicrotomes, bringing the number of these machines to three. However, these new microtomes were not without their problems. Manipulating these minute sections required very fine points, so it became (and remains) a common practice to 'acquire' eyelashes from the ladies in the department. It soon became clear, however, that not all eyelashes were of the same standard and so the girls with dark hair were the most sought after, one instance when natural blondes were not the preferred choice! Another common problem was static electricity which created charges on the sections and the specimen block. This showed the true dedication of the female technicians who removed (or should I say changed) their nylon underwear and stockings, in an attempt to cure the problems. A new vacuum coating unit, somewhat unkindly called 'the black coffin' by some members of staff, also came into use. This was designed and built on site in 1970 and for many years sported a small white label inscribed 'manufactured by Roberts, Robertson & Co.' The company referred to was one Aileen K. who supplied Ian and Walter with coffee and biscuits during the

Figure 10 Jeol 100 S electron microscope (1980 – 1997).

construction period, and who smilingly ignored the strong language when things did not always go according to plan. This vacuum coating unit was in constant service until October 2000, when it was replaced by something more visually appealing.

When the Siemens microscope reached the end of its working life the intention was to replace it with a new Philips microscope. At that time there was intense rivalry between Philips and Jeol as manufacturers, and all sorts of enticements were offered to prospective buyers, primarily by Jeol. Thus it was that in 1980 SCRI got two electron microscopes for the price of one, a Jeol 100S transmission microscope like the previous ones (Fig. 10), and the Jeol T200 our first (and only existing) scanning electron microscope (Fig. 11),

Figure 11 Jeol T 200 scanning electron microscope (1980 – present).

Figure 12 Jeol 1200 EX electron microscope ( 1985 – present).

thus bringing the total to four. This SEM was improved by the purchase of a cryo-transfer stage in 1985, which allowed examination of fully hydrated specimens with minimum preparation, at temperatures as low as –140°C, and expanded its usefulness for the examination of specimens such as fungi, nematodes, mites, and other soft tissues. The Jeol 100S transmission microscope was particularly easy to use, and thus was ideally suited to training new users or visitors. It gave good service until 1997, when the electron microscope suite was completely refurbished, at which time it was decommissioned to leave only the two better transmission microscopes, and the scanning microscope.

Figure 13 Philips CM 10 electron microscope (1990 – present).

In 1985, the Zoology department replaced its aging Hitachi with a Jeol 1200EX (Fig.12), which initially had a STEM (scanning transmission and analysis) unit attached. This EM was extremely versatile and was suitable to biological and physical applications. The Philips 301G was sold to one of the Oxford colleges, and replaced, in 1990, with a Philips CM10, a dedicated biological electron microscope (Fig. 13). Thus, together with the 1200EX and the SEM, SCRI had an impressive electron microscope facility in terms of equipment and its versatility. With the renovation of the microscopy laboratories, the disposal of one 'spare' electron microscope in 1997, and the unification of the facility as an Institute service under the Cell Biology banner, SCRI is extremely well placed to extend its expertise and reputation in this field for many years to come.

Figure 14 Jimmy Cathro, Electron microscopist 1960-1968.

A review of the electron microscopy facilities at the Institute would be incomplete without some mention of staff. The first electron microscopist at SHRI, as it was then, was Jimmy Cathro, as mentioned earlier. He was a professional glass blower and had been in charge of Laboratory Service before moving to Virology to operate the electron microscope. He was a true perfectionist and having taught you how to make the perfect Pasteur pipette, proceeded to teach you how to make glass animal and bird ornaments, or, seasonally, coloured glass baubles for the Christmas tree. In his search for perfection, Jimmy Cathro also found a unique solution to the problem of obtaining suitable glass for making ultramicrotome knives.

Since the best knives were achieved with 'strain free' glass, and this was not yet commercially available, we made several trips to the Dundee tram's graveyard in the mid 60's to collect the remains of the windows in large metal pails. This glass, having rattled around the streets of Dundee for several decades was essentially strain free and supplied the unit with excellent knives for some considerable time. His genius, however, was sometimes flawed, and on one notable occasion, he reluctantly sought out the Director to ask for a replacement anglepoise lamp, because he 'couldn't get the bulb out'. In truth, this piece of equipment gave him an electric shock, whereupon he threw the offending article against a wall (accompanied by a few well chosen words), and then proceeded to systematically kick it around the EM room until it was in bits, the lamp cover buckled but with the bulb still intact inside! He could also be slightly eccentric at times, sometimes sitting at the microscope wearing a crash helmet lined with lead, ' to protect him from stray radiation'. As mentioned earlier, Jimmy was also over 6 feet tall, a feature common to many of the early breed. The reasons for this was that the early models of electron microscopes were mechanically aligned, thus necessitating the adjustment of the electron gun c.6 feet off the ground while sitting down and looking through the binoculars. This was only possible if you were above average height, or possessed gorilla-like arms. The aforementioned Aileen K., who was all of 5 feet tall, had to stand on a stool, reach the high controls with one hand while bending and peering down one ocular from the side! The prerequisite for tall operators disappeared in the mid seventies, with the advent of electrical alignment controls. Jimmy Cathro was electron microscopist for 10 years, and since he left in 1968, there have been only five staff changes in the Units. Following the recent early retirement of Walter Robertson after 30 years in Zoology there remain two 'dinosaurs' who have accumulated over 60 years between them. This 90 year continuing service record of electron microscopy is probably unequalled anywhere. Much of this long service has to do with the nature of the job, experience counting perhaps for more than anything else, because by nature it is highly skilled, labour intensive, and generally slow with regard to the time required to produce results. It takes a minimum of 2 years to become proficient in this line of work, and the learning curve continues steeply thereafter. Like the real dinosaurs, the present incumbents are of course irreplaceable, but the Institute should be looking forward to alternatives to ensure the future of this facility.

It is difficult to put into context the contributions of electron microscopy to the scientific research of the Institute, in the UK, and also world wide. SCRI has been responsible for training scientists from all corners of the globe, and staff have set up electron microscope laboratories in countries such as Peru, Pakistan, and India, thus establishing fruitful collaborations, many of which exist still. Although for most of their working lives the microscopes have remained within only two Departments, they have contributed significantly to the scientific reputation of SHRI and SCRI. It is also probably fair to say that no other single piece of scientific equipment has been so influential to the science of the Institute. Within Virology alone, more than 20 percent of publications since 1962 have had some electron microscopy input and, including those from Zoology and external collaborations, this

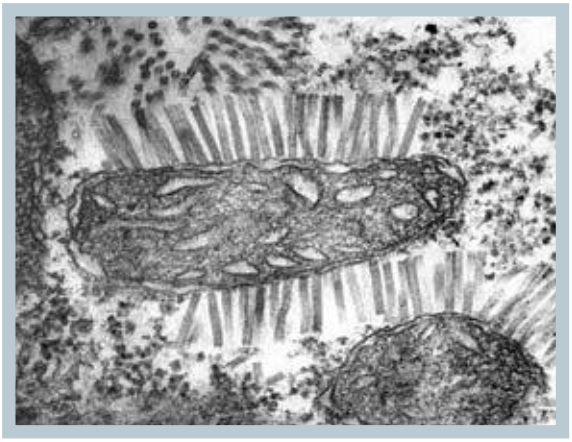

**Figure 15** Particles of *Pepper ringspot tobravirus (PRSV)* attached to the outer membrane of a mitochondrion in a leaf cell.

amounts to c. 200 publications, some of which have been scientific landmarks. As a science in its own right, many papers were themselves milestones, either in technical innovation or in scientific discovery, and set the pattern of recognition and reputation which exists to this day. Among the many notable discoveries were the first association of a plant virus with mitochondria (Fig. 15), and the first detection of a plant virus at specific sites within the food canal of vector nematodes (Fig. 7). Complementary studies of virus/vector combinations provided much new information on the ultrastructure of the feeding apparatuses of certain nematodes, aphids and mites, subtle differences in the structure of the cuticle between different species of nematodes within the same family, and the first evidence of a chemoreceptor gustatory

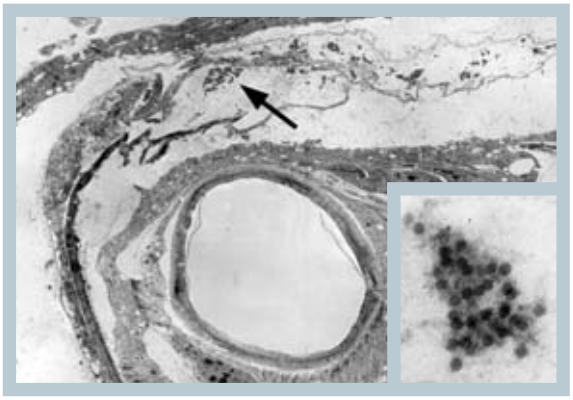

Figure 16 Median section through the head of the aphid *Cavariella aegopodii* showing the oesophagus and food contents. Arrow indicates the specific site of location of virus particles (inset).

organ in a nematode. Numerous other studies of ultrathin sections of virus infected leaf, plant or insect material gave us *Rhabdoviruses, Badenaviruses, Closteroviruses* and others in diseases of soft fruits and their vectors, mycoplasmas in potatoes infected with potato witches broom disease, and *Nepoviruses* in tubules passing through plasmodesmata. Of major significance was the discovery for the first time of the location of two semi-persistent viruses, *Parsnip yellow fleck (PYFV)* and *Anthriscus yellows (AYV)* at specific sites within the feeding apparatus of their vector aphid *Cavariella aegopodii* (Fig. 16). Elsewhere, studies on the leaf architecture and the types, numbers and the location of plasmodesmata have also helped in our understanding of the role plasmodesmata play during leaf development, and in virus movement and cell-to-cell signalling, complementing other studies using green fluorescent protein (GFP) and the confocal laser scanning microscope (CLSM).

In other work with GFP, we were the first to illustrate that this protein could be incorporated into the capsid of a plant virus, *Potato virus X (PVX),* without adversely affecting its assembly or replication in cells (Fig. 17), thus demonstrating its potential for protein

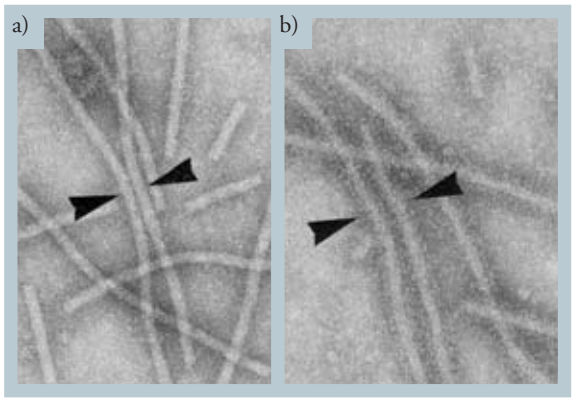


Figure 17 Negatively stained particles of *Potato virus X (PVX )* (a) and the additional coat of GFP protein (b) which does not affect virus assembly. Arrowheads illustrate the marked difference in the centre-centre spacing of adjoining particles.

overcoat technology and its implications for the production of therapeutics. These discoveries, together with those mentioned elsewhere, illustrate the wide range of techniques which are available, and which have done much to help our understanding of the interactions between plants, virus pathogens, and their vectors. Several new technical advances also had their origins in SCRI, including the Heat Pen for removing the cutting compression from ultrathin sections, a grid holder which later formed the basis of the grid block for modern automatic staining modules, and a method for coating glass knives with a thin film of tungsten, an advantage which had to wait almost 20 years for the new technique of cryo-sectioning to make full use of it. Aspects of virus architecture were also unveiled in the determination of the structure of the disk aggregates of *Tobacco rattle virus (TRV)* and the handedness of its helix, and in the first illustration of the surface structure of a *Nepovirus viz.* the T=1 capsid morphology of particles of *Tomato ringspot virus (TRSV)* by freeze drying and high resolution shadowing with uranium metal (Fig. 18). This latter discovery resulted from the design and construction, and ultimately the commercial production, of a freeze-drying module. This Institute was also at the forefront of the developments in immunoelectron

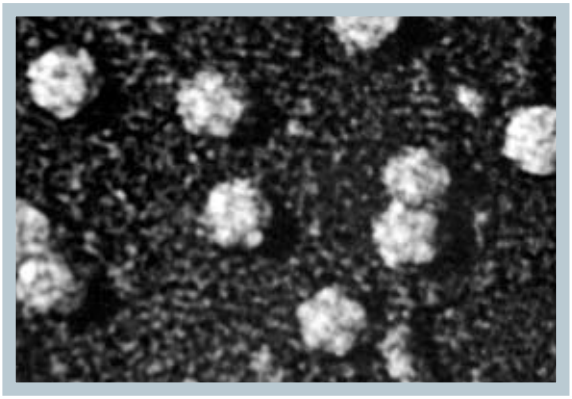

Figure 18 Particles of *Tomato ringspot nepovirus (TRSV),* after freeze-drying and high resolution shadowing with uranium metal. The T=1 capsid structure is clearly seen.).

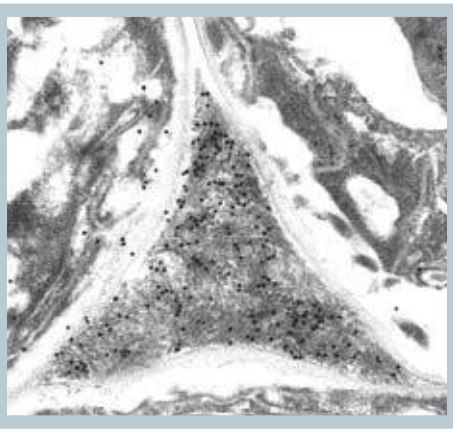

Figure 19 Immunogold labelling of particles in the oesophageal lumen of the vector nematode *Paratrichodorus anemones* confirming the identity of the tobravirus *Tobacco rattle (TRV).*

microscopy, where serology and electron microscopy were combined to aid the detection and identification of viruses in extracts of leaves, roots, petals etc. and in insect vectors such as nematodes and aphids. Variations of these techniques therefore gave 'firsts' for the detection of *Potato leafroll luteovirus (PLRV)* in a single aphid, *Raspberry ringspot nepovirus (RRV)* in a single nematode, as well as determining more precisely the serological relationships between *Luteoviruses* and between *Geminiviruses.*

Complementary to these immuno-techniques for viruses in extracts is immunogold labelling (IGL). This technique involves conjugating or tagging specific antibodies with small (5-20nm) gold particles, thus allowing the identification and/or localisation of

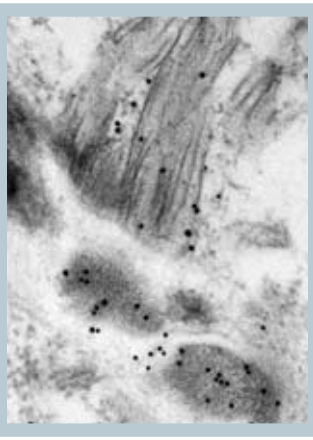

Figure 20 Immunogold labelling of *Pea seed-borne mosaic virus (PSbMV)* infected tissues, confirming the presence of the virus coat protein in the plasmodesmal cavity and on the cylindrical inclusions (CI).

viruses or viral proteins in negatively stained extracts, or in ultrathin sections of cells. An application of this technique allowed us to prove for the first time that the rod-shaped (putative) virus particles found in sections of vector nematodes (Fig. 19), were indeed those of the tobravirus they transmitted. In collaborative work with the John Innes Institute, IGL was a key feature in the studies of the location and distribution of *Pea seed-borne mosaic virus (PSbMV)* at the infection front in peas. In this study, IGL confirmed the role of the cylindrical inclusion (CI) in cell to cell movement, and further showed that, while both virus coat protein and that of the CIs could be found associated with the cell walls and plasmodesmata, only virus coat protein was present in the plasmodesmal cavity (Fig. 20). IGL was also instrumental in determining specific epitope sites on the surface of the

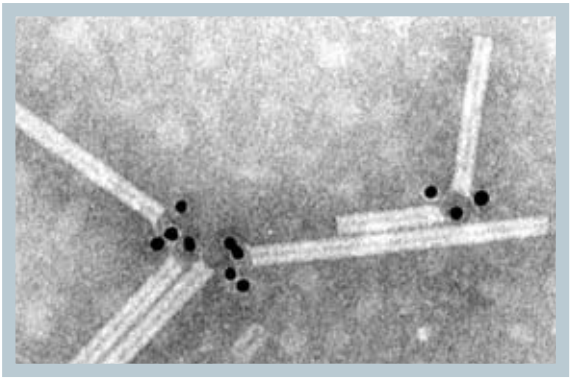

Figure 21 Immunogold labelling of negatively stained particles of *Potato mop-top virus (PMTV).* The gold particles have been conjugated to a monoclonal antibody that recognises an epitope at one end of the virus particle.

virus capsid of *Potato mop-top virus (PMTV),* by direct conjugation of monoclonal antibodies to a gold probe (Fig. 21), and convincingly demonstrated that different monoclonal antibodies recognised epitopes at different sites on the virus particles. A particularly effective application of this technique was in studies of the expression of the coat protein gene of *Potato leaf roll virus (PLRV).* In these studies, IGL conclusively demonstrated that the quasi-crystalline structures found in the nuclei of insect cells expressing this gene consisted of isometric particles, indistinguishable from those of PLRV. A fundamental part of the *in situ* hybridisation (ISH) technique to detect RNA in sections also makes use of antibodies conjugated to gold particles. In very recent work, this has shown that the nucleic acid of a plant virus (*Tobacco mosaic virus, TMV*) co-localises in leaf cells with the non-structural

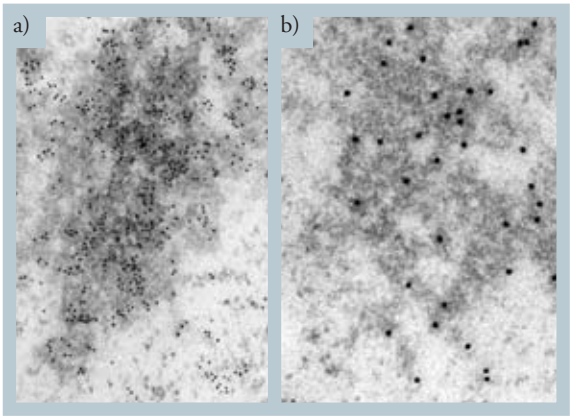


Figure 22 Co-localisation of the movement protein of *Groundnut rosette virus (GRV)* with RNA of *Tobacco mosaic virus (TMV)* by (a) conventional immunogold labelling of the coat protein, and (b*) in situ* hybridisation (ISH) of the RNA..

long-distance movement protein encoded by the unrelated *Groundnut rosette virus (GRV)* (Fig. 22).

Scanning electron microscopy (SEM) has also played a significant role in the Institute's research and has led to some novel findings. In particular, the low temperature studies of mites which infest blackcurrant species, and are responsible for the transmission of blackcurrant reversion virus, has provided new information on the taxonomy of these creatures, and showed that subtle differences in the pattern of folds on the insect's head shield could be used to give unequivocal identification (Fig. 23). Other notable SEM observations include the finding that dry *Botrytis* fungal spores dusted on the surface of rose petals (Fig. 24) could germinate and penetrate the surface, in the absence of water droplets, and that sporulating colonies of powdery mildew (*Blumeria graminis* f.sp. *hordei*) often originated from more than one conidium, even at low inoculum density (Fig. 25).

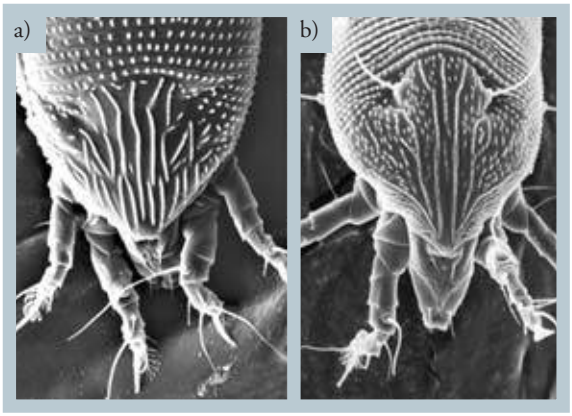


Figure 23 Scanning electron micrographs of (a) *Cecidophyopsis selachodon* and (b) *Phylocoptus gracilis*, showing the differences in the head shield structure which can be used for identification purposes.

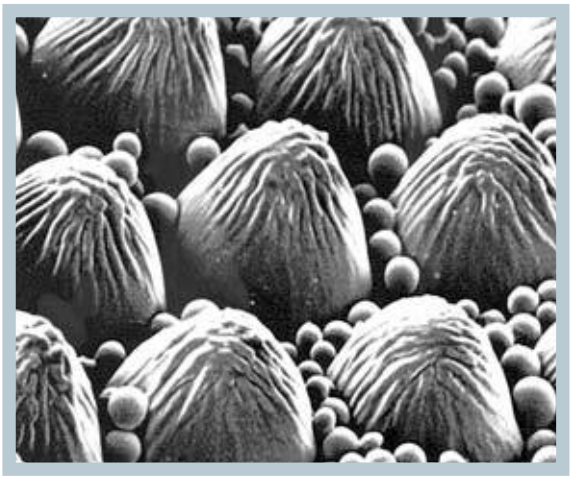

Figure 24 Scanning electron microscope image of the surface of a rose petal which has been dusted with the dry fungal spores of *Botrytis* (small spheres).

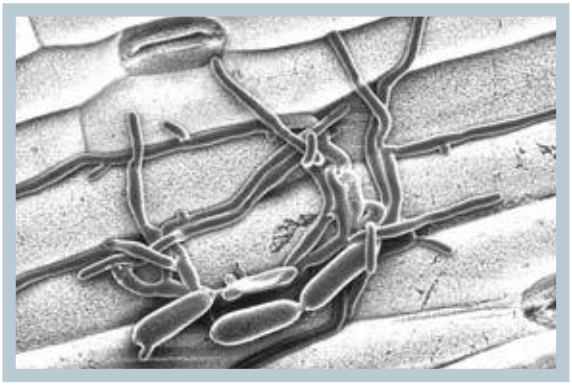

Figure 25 Powdery mildew (*Blumeria graminis* f.sp. *hordei*) colony on the surface of a barley leaf..

As an Institute, SCRI is rare in having, as well as the TEM and SEM microscopes under the same collective 'roof', a wide range of imaging facilities including NMR microscopy, FTIR microscopy and also state-of-the-art CLSM microscopes, so creating possibilities for many research areas as yet unexplored. Electron microscopy is not the dying art that some would have us believe, but an expanding, vibrant and versatile science that, in addition to its own specialist field, plays a complementary role to many other imaging techniques now available. To plagiarise a current rather well known advertising slogan, 'the future is bright, the future is electron microscopy!'.

# Genetics

John W. S. Brown, John E. Bradshaw, Gordon C. Machray & Robbie Waugh

*The year 2000 was the first full year of the reorganised Genetics Division. One of the main aims of the reorganisation was to bring the different Genetics groups closer together to share and benefit more fully from the breadth of knowledge of plant genetics and molecular biology, and to make implementation of common approaches and technologies more efficient.*

Success in forging closer associations is illustrated by the establishment of a new molecular marker lab in Applied Genetics, the start of a programme on the molecular characterisation of *Rubus* and *Ribes* germplasm, and the formulation of a new programme to characterise the Commonwealth Potato Collection genetically and phenotypically. All three areas are supported by increased interactions and collaborations among scientists from the different Units within Genetics. Similarly, gene expression during grain development and germination, based on cloning and sequencing of Expressed Sequence Tags (ESTs) and eventually on microarrays, run in parallel in the Units of Gene Expression and Genomics. The importance of Single Nucleotide Polymorphisms (SNPs) as simple markers for following traits in crop improvement, for mapping ESTs, and for association mapping of traits, will increase in parallel with gene discovery programmes. SNP discovery and implementation are being co-ordinated across all three research Units. Interactions were further enhanced by strategy meetings, particularly on barley and potato research, involving a number of scientists across the Division. Collaborations with the Pathology, Biochemistry and Cell Biology, and Plant, Soils and Environment Divisions in areas such as pathogen genomics, malting barley, and root structure, continue to increase as programmes develop and new programmes are initiated. The forthcoming year will also see the transfer and integration of two plant research groups from the University of Dundee into the Genetics Division and we look forward to this opportunity for closer collaboration.

Apart from a number of scientific successes described in the Unit sections below, scientists in the Genetics Division are responsible for a number of 'firsts' in plant research. These include the successful establishment of physical mapping techniques such as HAPPY and radiation hybrid mapping for the first time in plants, new exciting results in the area of homologous recombination, the characterisation of plant intron signals, and the theory and practice of linkage and QTL analysis in autotetraploids (with BioSS).

In terms of external funding, the IGF programme with SCRI, IACR, and JIC on grain development in barley (jointly funded by SEERAD and BBSRC) was a major success for the Division and Institute, and research began in mid-2000. Further significant funds were obtained for chromosome manipulation,

biodiversity, blackcurrant breeding and potato breeding. Significantly, SCRI now has breeding contracts for improved processing and quality traits from the major potato and blackcurrant processors in the UK. In addition, the international reputation of many scientists in the Genetics Division has been demonstrated by the organisation of, and outstanding contributions to, international meetings. Early in 2000, a barley genomics meeting at SCRI ('The Dundee Workshop') saw the gathering of scientists involved in cereal genomics from around the globe which catalysed the reorganisation of the International Triticeae Mapping Initiative (ITMI) and the location of the ITMI Head Office at SCRI. The 8th International *Rubus* and *Ribes* Symposium was held in Dundee in July 2001 and the XVIth EUCARPIA Congress in Edinburgh in September 2001. In parallel with the EUCARPIA Congress, there was an ITMI Workshop on Association Genetics.

The year 2001 will see large increases in the generation of DNA sequencing and genotyping data at SCRI, due to the increasing number of programmes using genomics approaches. This has placed more emphasis on automating processes to achieve higher throughput. SCRI is investing in more robotics and this investment will have to continue to meet the demands of genomics, proteomics and metabolomics. The Institute's DNA Sequencing and Genotyping Facility, run by the Unit of Gene Expression, underpins all of the DNA work of the Institute, and its importance is reflected in the increasing number of sequencing and genotyping samples, exceeding 40,000 in 2000 and likely to exceed 80,000 in 2001. Again, to meet the necessity for increased capacity, the Institute has recently purchased a new capillary DNA sequencing machine. More importantly, however, is the need for Bioinformatics services and support across the Institute. This is required to handle and analyse a variety of sources of data generated at SCRI and to mine and utilise the vast compendium of data being generated world-wide. A key challenge in the immediate future is to resource a Bioinformatics infrastructure which will meet the needs of the Institute for the next decade.

Unit of Applied Genetics During the year, we produced a document outlining our vision for plant breeding at SCRI, based on applying the new sciences of genomics, proteomics and metabolics to crop improvement, whilst recognising the different end-user communities and their needs, and hence the most appropriate commercialisation routes. Our aim is to connect genome science to plant breeding in a way that allows the novel utilisation of our unique germplasm collections and the development of innovative approaches to the production of crop plants with improved, novel and value-added properties. We want to extend our breeding targets beyond traditional products such as barley malt, and potato crisps and French fries, to improved nutritional value and anti-carcinogenic properties that can have a positive impact on human health, and to altered biosynthetic pathways that result in new products for a range of processing industries. This is already happening in our commercially-funded soft fruit breeding programmes and their underpinning research, where there is increased emphasis on fruit quality and nutritional traits. We are also conscious of the need to train a new generation of young scientists in the plant breeding methods of the future.

SCRI now interacts with the majority of major seed and processing companies in the UK in its mandate crops, and the termination in March 2000, by mutual agreement, of the Consortium Agreement (for barley and potatoes) of 1989 will allow us, through Mylnefield Research Services Ltd (MRS), to seek new marketing opportunities for wholly externally-funded plant breeding activities. The global perspective of our industrial partners in potato and blackcurrant breeding for processing will open up wider opportunities for SCRI germplasm.

During the year, after careful consideration, seed potato production was transferred from rented land at Blythbank Farm (one of the Roslin Institute farms) near West Linton, to rented land at Balruddery Farm, near Invergowrie, from 1 April 2001. Blythbank had proved a good site when potato breeding was based at Pentlandfield from 1955 to 1989. However, the move to Balruddery and modification of the potato store on site at SCRI will make our operations more efficient and convenient.

SCRI has been a leader in genetic marker development and application for a number of years and this is the area of immediate impact in our plant breeding programmes. AFLP markers linked to the *Ce* gall mite resistance gene in *Ribes nigrum* have now been confirmed, and deployment strategies are under development. The source of the major gene resistance to *Rhynchosporium* in the barley cultivar Livet has been identified through the use of molecular markers as being derived from Digger. An allele of one of SCRI's library of SSRs is associated with the resistance and is

also found in the newly recommended cultivar Pewter. This allele, therefore, appears to have value as a diagnostic marker for the resistance gene. The locus is independent of the *Rh* complex on chromosome 3H and, hence, there is the opportunity to pyramid the resistance genes. We have also established in barley that a β-amylase SSR is diagnostic for variation in enzyme thermostability. In potato, a major QTL for *Solanum vernei*-derived resistance to the white potato cyst nematode (*Globodera pallida*) was located on chromosome 5 using AFLP markers, as part of a SERAD flexible funded project between the Genomics, Applied Genetics, and Mycology, Bacteriology and Nematology Units. These AFLP markers were converted to an easy-to-use PCR marker which is now being used to explore the efficacy and efficiency of molecular marker assisted selection in the glasshouse-grown seedling generation of our potato breeding research programme.

In the longer term, plant breeding at SCRI will benefit from the expansion in genomics and functional genomics research at SCRI. For example, the barley IGF programme being led by Dr Robbie Waugh, will underpin our priorities for crop improvement for the malting and distilling industries, combined with in-built disease resistance for benign methods of control, which are highly relevant to sustainable agriculture and the environment end-users.

Unit of Gene Expression The Gene Expression Unit covers a wide range of research activities involved in understanding how plant genes and gene families are expressed at the transcriptional and post-transcriptional levels, plant transformation, and the development of new technologies for gene function analysis. Major areas of research in the Unit of Gene Expression are RNA processing, gene expression of isolated gene families (e.g. splicing factors and invertase genes), gene expression during barley germination and malting, and the development of transformation methods for targeted gene manipulation to examine gene function.

RNA processing research has concentrated on utilising the potato mini-exon splicing system to analyse *cis*- and *trans*-acting factors in plant pre-mRNA splicing. Characterisation of the mini-exon system, discovered in the Unit of Gene Expression, was published in *RNA*[1] early in 2000. The sensitivity of the system to mutation of key sequence signals has allowed the first detailed characterisation of plant intron branchpoint and polypyrimidine tracts. In collaboration with Profs. Witek Filipowicz (Basel) and Andrea Barta (Vienna), this system is now being utilised to examine the role of plant RNA binding proteins and splicing factors in intron recognition and removal. Further studies on intron splicing in collaboration with Prof. Artur Jarmolowski (Poznan), focus on AT-AC introns. These are rare introns which are removed by a secondary splicing machinery and may be important in the regulation of expression of genes containing them.

A second area of research is the analysis of small nucleolar RNA genes in *Arabidopsis*. The RNA processing lab has led plant snoRNA research for a number of years and, with the availability of the *Arabidopsis* genome sequence, it has now been possible to identify many new snoRNA genes. Of key interest is the finding that most snoRNA genes are organised in polycistronic gene clusters which are distributed throughout the *Arabidopsis* genome. Many gene clusters are duplicated and sequence changes in related genes provide information on the evolution of this gene family. The characterisation of these genes has involved mapping of modification sites on *Arabidopsis* ribosomal RNAs, and has led to the identification of a number of novel plant snoRNAs.

The analysis of the components and mechanisms of RNA processing represents basic science underpinning our understanding of plant gene expression. To exploit our knowledge of these essential processes in gene expression, an applied project to target and knockout splicing functions was established in 1998, with funding from SEERAD and BBSRC through the DTI-LINK Cell Engineering programme, and in collaboration with a UK Biotechnology company. The programme aimed to generate tolerance or resistance to nematode pests in potatoes by disrupting nematode feeding sites. One of the selected gene targets has now been shown to confer tolerance to these important pests, paving the way for further detailed studies and identification of new targets.

Besides the utility of the potato invertase mini-exon in splicing studies, invertase genes have been of interest due to their role in carbohydrate metabolism and the variety of expression patterns exhibited by this gene family. The specificity of the expression of one member of the invertase gene family was demonstrated in a joint study with the Units of Cell Biology and Plant Biochemistry on the development of the potato tuber. Discrete expression was observed in the apical hook region of the elongating stolon and associated with the

apical bud region of the mature tuber. This multidisciplinary study was published recently in the *Plant Cell*[2].

Highly parallel gene expression analyses are the focus of studies on the malting barley grain. From an ever-expanding catalogue of gene sequences (ESTs) generated at SCRI and elsewhere, we have manufactured an initial microarray of a thousand elements to examine temporal and developmental expression of this gene set during malting. The same gene set is being exploited for SNP discovery with subsequent genetic mapping of the SNPs on standard mapping populations. These sequences are also being located on a physical map of the barley genome using radiation hybrid technology. Our development of this technology in plants, the first such achievement world-wide, significantly enhances the ability to link physical and genetic maps of the barley genome, and hence will facilitate the move from phenotype to a determination of the gene(s) which control it.

Research on the manipulation of gene expression in transgenic plants has also led to exciting new discoveries in the past year. This work has developed in, and takes cognisance of, a social and regulatory framework that provides important drivers for the research. We are developing novel selection systems coupled to efficient protocols for the transformation of monocots. These will replace antibiotic and herbicide selection systems that will be phased out. For the model dicot, tobacco, we have developed highly efficient male germ line transformation protocols that dispense with the need for any selection at all. Using these protocols, we have shown for the first time that gene targeting, at a frequency allowing knockouts, knockins, and allele replacement, is feasible in higher plants. This research has major potential for application in the preparation of new generations of transgenic plants that address quality and disease-resistance attributes.

Genomics Unit Last year, two exciting new three-year programmes of work were initiated in the Genomics Unit which firmly place our research in an international context. The first of these is to develop high throughput, high resolution physical mapping in crop plants, using an innovative approach known as HAPPY mapping. Over the past year, we have demonstrated that HAPPY mapping is a powerful, physical mapping approach which could have an important role to play in several current or planned projects in the Institute. The second and larger project is the 'Cereal Community Resources' programme funded jointly by BBSRC and SEERAD through the Investigating Gene Function (IGF) initiative. This £3.1 M initiative covers parallel programmes on barley, at SCRI, and wheat, at the John Innes Centre in Norwich (Dr Graham Moore) and the Institute for Arable Crops Research (IACR) in Long Ashton (Dr Keith Edwards). The project has brought five new members of staff to the Unit and is focused on the development of 'biological resources' which will be exploited by SCRI, national and international research communities. There are three basic components in the barley programme. The first is a gene discovery project where we intend to sequence upwards of 40,000 ESTs, largely from the developing grain, and deposit the information into local UK (UKCropNet) and large international databases (dbEST, ITEC). The data we generate will complement information emerging from parallel wheat and barley gene discovery programmes being carried out in the USA, Canada, Japan, Germany, Australia, France and Finland, and will result in a world-wide collection of around 750,000 Triticeae ESTs in the public domain. These EST sequences will represent the majority of the genes present in the cereal genome and act as an entry point into detailed biological discovery. To this end, we are currently involved in discussions with the wider Triticeae genomics community about the development of a microarray expression platform which will unify and integrate barley/wheat expression studies globally. In parallel with the lab-based activities of the gene discovery programme, the second class of resources we have initiated are automated procedures for managing and analysing the vast amount of data being generated, and establishing searchable, informative databases for its long term storage and annotation. This area of Bioinformatics is becoming increasingly important to the work carried out in the Unit and the Institute, and will play a vital role in the way scientific information is utilised and exploited by both local and remote users. It is an area set to expand dramatically over the coming years. The third component of the IGF program, which will serve to link the ESTs to biological function, is the generation of chemically mutagenised barley populations. Mutant populations are a powerful resource which can be exploited to determine gene function by reverse genetics approaches through the identification of gene knockouts and allelic variants of target genes. Mutant plants, in which a target gene has been disrupted, can assist in unravelling the role that the gene plays in the biological process being studied, through phenotypic analyses and gene expression studies. A challenge for

us in the coming year will be to develop a robust and sensitive detection platform which allows us to quickly and accurately identify mutations in target genes.

Funding has also been secured to use molecular methods to study biodiversity and population genetics in a bryophyte (*Anastrophyllum joergensenii*), a pteridophyte (*Athyrium distentifolium*) and two angiosperms (*Koenigia islandica* and *Arabis petrea*) (in collaboration with the Royal Botanic Garden, Edinburgh), and an analysis of linkage disequilibrium in barley. In addition, a divisional effort has been initiated to discover, characterise and map Single Nucleotide Polymorphisms (SNPs) in the barley genome.

While these represent the directions in which the Unit is moving, existing projects are also now coming to fruition. For example, the EU-funded Ultra High Density (UHD) linkage mapping project in potato has progressed well and we now have segregation data for some 10,000 markers. In conjunction with two potato Bacterial Artificial Chromosome (BAC) libraries constructed over the last year, the UHD map is being exploited to build a genetically anchored physical map of a small region (2-10 cM in genetic length) of the potato genome. It is also beginning to impact upon our SEERAD and BPC funded tetraploid genetics projects by allowing us to anchor what were previously anonymous markers to defined positions on the potato genetic map, providing rapid assignment of chromosome segments to defined regions of the potato genome.

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# A genome based approach to improving barley for the malting and distilling industries

R.C. Meyer, J.S. Swanston, G.R. Young, P.E. Lawrence, A. Bertie, J. Ritchie, A. Wilson, J. Brosnan[1], S. Pearson[1], T. Bringhurst[1], G. Steele[1], P.R. Aldis[2], M. Field[2], T. Jolliffe[2], W. Powell & W.T.B. Thomas

**Background** Up to 60% of the Scottish barley crop is used in malting for brewing and distilling. The distilling industry alone uses some 500,000 tonnes per annum, with the total malt purchases in Scotland exceeding 800,000 tonnes. Scotch Whisky is the fifth largest British export and the leading food and drink commodity, earning over £2 billion per annum. Malt whisky can only be made from malted barley and is at the premium end of the market. High spirit yield is probably the main quality requirement of the malt whisky distilling industry, because a 1% increase in spirit yield would lead to a saving of approximately £1.1 million in distilling production costs. Spirit yield is the product of hot water extract (i.e. the total soluble component following malting) and the fermentability of the extract, since not all solubilised components are fermentable. The peak level of fermentability is achieved earlier in the malting process than the peak level of extract, and malting has to be optimised to produce the maximum spirit yield. A further requirement in some malt whisky distilleries is for varieties that do not produce epi-heterodendrin (EPH), a glycosidic nitrile occurring in germinating barley. A breakdown product of EPH can react with ethanol, catalysed by copper in stills, to produce the putative carcinogen ethyl carbamate (urethane).

The development of genetic finger-printing techniques in human genetics has led to applications of the various types of molecular markers, especially in the rapid creation of genetic maps of an organism. The advantage of such maps is that regions controlling complex characters, such as malting quality, can be identified as Quantitative Trait Loci (QTL). This knowledge can then be applied in a targeted manner to improve plant characters for a specific end-user need. As noted above, fermentability is a key character for the distilling industry but its analysis is difficult to carry out in plant breeding and genetical studies. It is, however, an ideal character for exploiting molecular marker methods in plant breeding. Within a MAFF funded Agri-Food LINK project, the most significant QTL that we identified from the Derkado x B83-12/21/5 mapping population accounted for around 6% of the phenotypic variation in the character. The Derkado QTL allele reduced fermentability by just under 0.5% and off-setting this decrease would give an extra 3 litres of spirit yield per tonne. Applied over the whole malt whisky industry, this would translate into an extra production of 1 million bottles annually from the same quantity of malted barley.

On the basis of their phenotypic performance, we used two lines from the mapping population as donors of high fermentability to initiate a programme to produce first backcross (BC1) inbred lines. Our initial strategy was to use marker-assisted selection to transfer the QTL enhancing fermentability into a Landlord genetic background, as it was a promising new cultivar but

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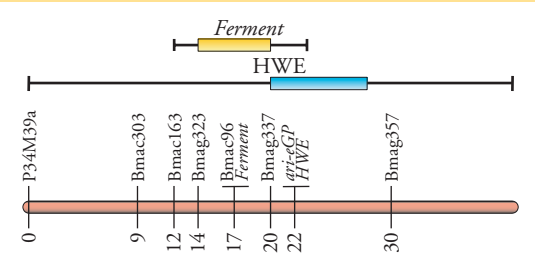


**Figure 1** Detailed map of target region of 5H showing location of fermentability and hot water extract QTLs, their 1 LOD confidence intervals (bars) and regions over which a significant effect was detected (lines).

with scope to improve its fermentability. It became apparent, however, that the QTL was linked in coupling with the *ari-e*GP dwarfing gene on chromosome 5H. An increase in the minimum sieving size in trading Scottish grain meant that cultivars with this dwarfing gene were no longer commercially viable as the gene was associated with small grain. Furthermore, we found that the fermentability QTL was also linked in repulsion to a hot water extract QTL at the *ari-e* locus (Fig. 1).

The development of the breeding population is outlined in Figure 2. The donors both carried B83-12/21/5 alleles at all loci within the target region, so it

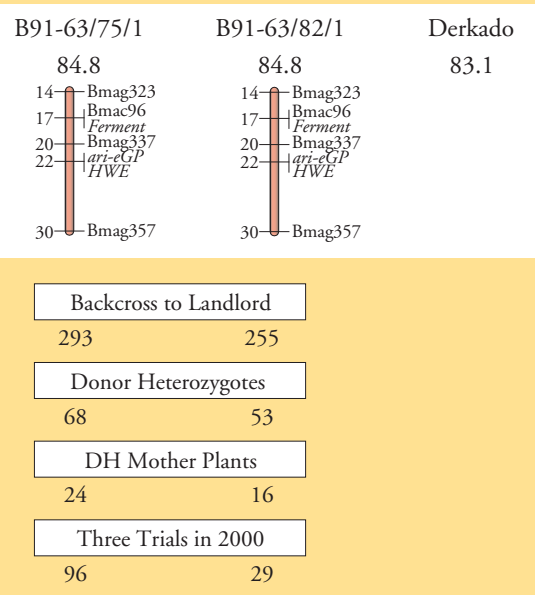


**Figure 2** Derivation of Population used to test the effect of the target fermentability QTL in another genetic background. Values under the genotypes are the mean % fermentabilities from four trials conducted from 1995-7.

was necessary to generate recombinants between the fermentability QTL and *ari-e* to develop lines that had high levels of fermentability and extract, and were also agronomically suitable for Scotland. There were not the resources within the project to generate a large enough population with sufficient recombinants from which we could hope to select a line with commercial potential. We therefore changed our strategy to a more random one by testing all the BC1DH lines that we developed. This strategy would have the added bonus of enabling further genome-wide testing of the location of regions controlling fermentability. We therefore tested 125 BC1DH lines in large plot (7m$^2$) trials at commercial density, with and without fungicide at SCRI, and fungicide treated trials near Sleaford and Docking, in 2000. The plots were harvested with a small plot combine and their yields recorded. Cleaned and sieved samples from all the plots, except those from the Sleaford site, were retained for analysis of the malting quality characters, hot water extract and fermentability.

## Results

Overall, the mean yield of the BC1DH lines was less than that of Landlord, which may reflect the presence of both the *ari-e*GP and *sdw1* dwarfing genes in a number of lines. Whilst the minimum yielding line from the BC1DH population was significantly lower than Landlord, the highest yielding line was not significantly greater. Despite delaying malting until dormancy had been broken, the samples from the SCRI trials generally malted poorly with very low extracts, leading to abnormal overall mean values for many of the malting characters. Landlord itself performed

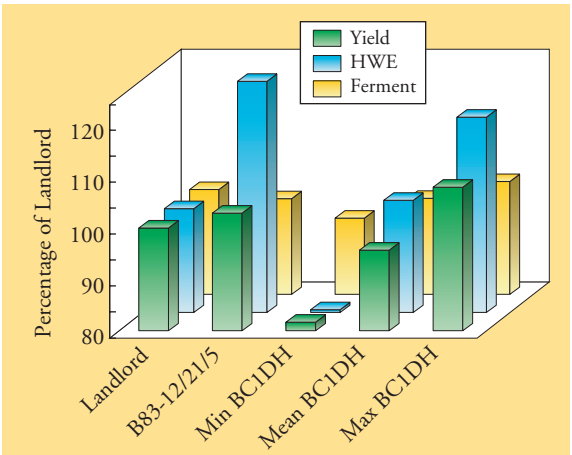


**Figure 3** Summary of the BC1DH population for Yield, Extract and Fermentability from performance in trials grown in the year 2000.

poorly as it and many other samples were under-modified. In contrast, the parents of the donors, Derkado and B83-12/21/5, malted normally and thus B83-12/21/5 was significantly better than Landlord, even though it was only of moderate malting quality. The lines with the lowest extract and fermentability were both significantly worse than Landlord and, whilst there were lines that were higher than Landlord, only the extract difference was significant (Fig. 3). The means of the BC1DH lines from the two SCRI sites show that extract was very low. If the lines were under-modified, one might expect a higher mean fermentability. The fact that fermentability is also relatively low indicates the presence of significant amounts of unfermentable material, under which circumstances one might not detect many differences in fermentability. Fermentabilities from the Docking results were also low but samples were more typical of commercial malts, despite some evidence of over-modification.

We found that germinations of Landlord and Chariot, a parent of Landlord, were low and concluded that some environmental factors present at SCRI in 2000 induced some genotypic differences in germination, leading to poor micro-malting performance. A water sensitivity test of some of the samples from the SCRI trial treated with fungicide showed that germination was still very poor some 10 months after harvest and gave a correlation of >0.9 with hot water extract. Both Landlord and Chariot have been found to be very susceptible to *Ramularia* infection but there is, as yet, no evidence that the disease inhibits germination. The results do highlight the problem of using a backcrossing strategy to meet a commercial target. The choice of a newly recommended cultivar as the recipient parent for the major part of our backcrossing programme was correct as, if successful, the cultivar would still be relevant at the time of release of the backcross line. The problem is that malting and agronomic information about such cultivars is limited, despite a large amount of yield trial data, and problems only become apparent when the cultivar is grown on a large scale. A safer system would be an adaptive backcrossing scheme in which one changed the parent at each stage. Previously mapped SSRs would be of great advantage in genotyping such a population, as one would have a good chance of separating out not only the donor alleles but also the different recipient alleles, due to their multi-allelic nature.

The genetic fingerprints of the BC1DH lines entered into trials were established by surveying them with 44 previously mapped Simple Sequence Repeat (SSR) markers, which were selected to sample the whole barley genome as well as the target QTL. In addition, allelic differences at the *sdw1* and *ari-e*GP loci were established from observations of the juvenile growth habits of the plots. We wished to detect whether or not the donor QTL chromosomal segment altered the expression of fermentability in the recipient. We coded all the genotypic data as being either donor or recipient in origin and compared the means of the different genotypes observed in the target region. We could then classify all 125 lines as having either donor or recipient alleles at the fermentability and extract QTLs on chromosome 5H. We predicted, therefore, that donor alleles at the fermentability QTL should result in an increase in the character, whereas donor alleles at the extract QTL should decrease extract.

The effect of the donor segment can be seen in the summaries of results presented in Table 1. Donor alleles at both the fermentability and extract QTLs have very similar effects upon the characters that were measured on the 2000 trials. The similarity of the response is to be expected as the two QTLs are closely linked. The results are generally consistent for each character as well. As expected, donor alleles significantly decreased yield and extract. The differences were more pronounced at the SCRI sites but the poor malting performance of samples in these trials may have exacerbated the differences in extract. Allelic differences at the fermentability QTL did show an increase in the character associated with the donor alleles from the results from the Docking site but the effect was not significant. Donor alleles at the HWE QTL not only significantly reduced extract but also significantly increased fermentability, and the same

| Site | Differences at 5H QTL | Yield | Ferment | HWE |
|---|---|---|---|---|
| Docking | Ferment | **-4.0** | 0.7 | **-1.2** |
| Docking | HWE | **-3.1** | **1.1** | **-1.6** |
| SCRI-F | Ferment | **-12.2** | 0.1 | **-8.1** |
| SCRI-F | HWE | **-12.0** | 0.5 | **-9.6** |
| SCRI+F | Ferment | **-12.0** | -0.7 | **-11.2** |
| SCRI+F | HWE | **-11.9** | -0.5 | **-12.7** |

**Table 1** Differences between means of BC1DH lines grown in three trials in the year 2000 and classified according to whether they possessed donor or recipient alleles at the fermentability and HWE QTLs on chromosome 5H. Data are expressed as the donor minus recipient means expressed as a percentage of the recipient and those in bold type are significantly different.

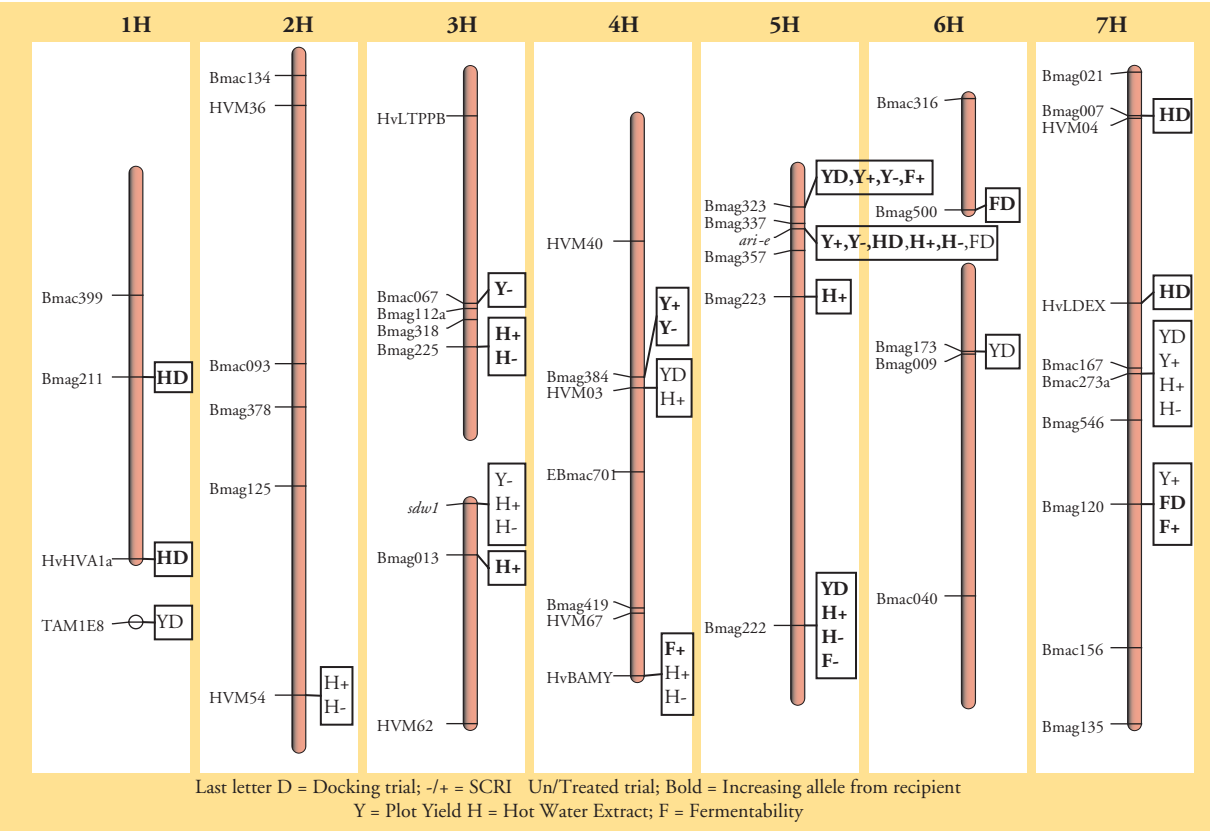


**Figure 4** Location of SSR and morphological markers in relation to the genetic map of the mapping population together with significant marker associations detected by multiple regression analysis of data collected from the year 2000 trials of BC1DH lines.

pattern can be seen in the results from the SCRI untreated trial, although the increase was not quite significant ($P$=0.06). In the SCRI treated trial, donor alleles apparently reduced fermentability but this difference was not significant and could be an artefact of poorer malting performance compared to the untreated trial. The general effect appears to be that the fermentability and extract QTLs are more closely associated than was apparent from the mapping study but we would need more recombinants and malting results from Scottish trials to verify this finding.

We also used forward stepwise multiple regression analysis to identify markers that acted together in statistically significant associations with yield, extract and fermentability. Applying this analysis to the phenotypic and genotypic data collected on the BC1DH means from each of the SCRI and the Docking trials, revealed a number of significant associations. Each character at each site was associated with at least one marker and there were 12 cases where results agreed between at least two of the trials and more confidence can be attached to them. Seven of these 12 marker / trait associations involved extract, with donor alleles at four associated with an increase. Another four of the 12 marker / trait associations where results agreed between two or more sites were for yield, with donor alleles producing a significant increase at one. For fermentability, results were in agreement at just one locus, Bmag120 on 7H with donor alleles significantly decreasing the character. Whilst donor alleles at *ari-e* were significantly associated with an increase in fermentability from results obtained from the Docking site, donor alleles at a nearby locus (Bmag323) were significantly associated with a decrease in fermentability at the SCRI treated site in 2000. No significant associations of fermentability with markers in the target region were detected from the results of the untreated trial at SCRI by either multiple or single marker regression. Donor alleles at *ari-e* did produce an increase in fermentability but the effect was far from significant.

## Conclusions and end-user relevance

The one trial that malted normally was grown outside the target environment but did produce evidence of

increased fermentability due to the presence of donor alleles in the target region, and there was some indication of corroborating evidence from the untreated trial grown at SCRI. The data did indicate, however, that the fermentability QTL might be associated with the *ari-e*GP dwarfing gene. Further work is necessary to establish whether or not this is so, as deleterious effects of the dwarfing gene, such as high screenings and reduced extract, mean that it is no longer viable in a commercial cultivar.

Other evidence revealed that fermentability was controlled by a number of genes, each of small effect, and highly subject to modification by the environment. Detecting this QTL in another genetic background requires most of the other increasing loci to be present and using marker assisted selection for just the target QTL means that many of the other increasing alleles are eliminated by chance. This is not just a problem for the current project, but also applies to other characters of low heritability with a number of controlling genes. In such cases, there is no alternative but to generate large populations, use marker-assisted selection to form a pool of 'improved' lines and rely on phenotypic selection to pick out the best lines.

Whilst the cultivar Golden Promise carried the *ari-e*GP gene and was used in great quantitities by maltsters and distillers, it was never regarded as a top-class malting quality cultivar. The fermentability QTL studied in this project either represents the action of an anonymous gene or *ari-e*GP. There is evidence that the gamma-ray mutation of Maythorpe to produce Golden Promise resulted in an increased rate of modification. Such a gene, taken from a moderate malting background, may lead to excessive modification in a good malting quality background and this is a possible weakness of the anonymous approach used in the current project.

There is opportunity to manipulate natural variation in fermentability, however, and the targeting of specific genes of known function may well be a better means of improving barley for use in distilling in the short term. For instance, natural variants of β-amylase with improved stability at extraction temperatures may improve fermentability. As starch breakdown continues into the fermentation stage in a distillery, enhancing the release of the enzyme limit dextrinase during fermentation would be another potential approach that increases fermentability. Putting natural variants of these two enzyme systems together may well provide a further means of improvement. Results from functional genomics programmes could provide better overall understanding of the genetics of complex traits such as malting quality and eliminate some of the problems associated with the single gene approach that we adopted within this project. With functional genomics, one can attempt to establish how various candidate genes interact to produce a given phenotype. After gathering such information from a range of cultivars and associating it with malting quality data, it will be possible to identify targets to manipulate in order to improve performance for specific malting attributes.

# A 1260 point genetic linkage map of potato chromosome 1: paving the way for Ultra High Density genetic linkage maps in crop species

D. Milbourne, E. Isidore, H. van Os[1], G. Bryan, H. van Eck[1], F. Rousselle-Bourgeois[2], E. Ritter[3], J. Bakker[4] & R. Waugh

With the paradigm shift towards high throughput characterisation of genes and their expression patterns inherent to the 'genomics revolution', the ability to relate simple and complex phenotypes to their underlying genes is becoming increasingly important. High-density linkage maps are indispensable tools for this task and, in addition, such maps have formed the basis for more in depth, genome-wide characterisation approaches such as physical mapping and whole genome sequencing projects for an array of model organisms. The utility of any genetic linkage map as a platform for all of these purposes is largely a function of both its density and accuracy. A primary goal of our group is marker saturation of the potato genome by the construction of an AFLP-based ultra high density (UHD) map containing approximately 10,000 markers. This represents a tenfold increase in the current coverage of 1000 markers for the combined tomato/potato RFLP-based map developed at Cornell University.

Despite the high throughput nature of AFLPs, time and financial constraints limit the population size upon which this type of experiment can be performed. Population size is important in determining the resolution of a map; the larger the population, the greater the number of meioses upon which the map can be based, and the greater the number of markers which can be ordered. Thus, lower resolution maps have greater numbers of markers that map to the same genetic location. We refer to this as the 'bin' concept (Fig.1), in which groups of co-segregating markers are represented on a map as a single co-segregation bin. This bin is defined by a 'bin signature', which is the common segregation pattern of all markers in that bin.

A second concept central to the creation of UHD maps is the realisation of the disproportionate effect of low levels of error in the segregation dataset used. Inclusion of an erroneous datapoint will result in a difference between the true and calculated position of a marker. The significance of this factor in creating a UHD linkage map can be illustrated by considering the creation of a linkage map of a single chromosome consisting of 1000 markers in a population of 100 individuals, with a marker scoring accuracy of 99%.

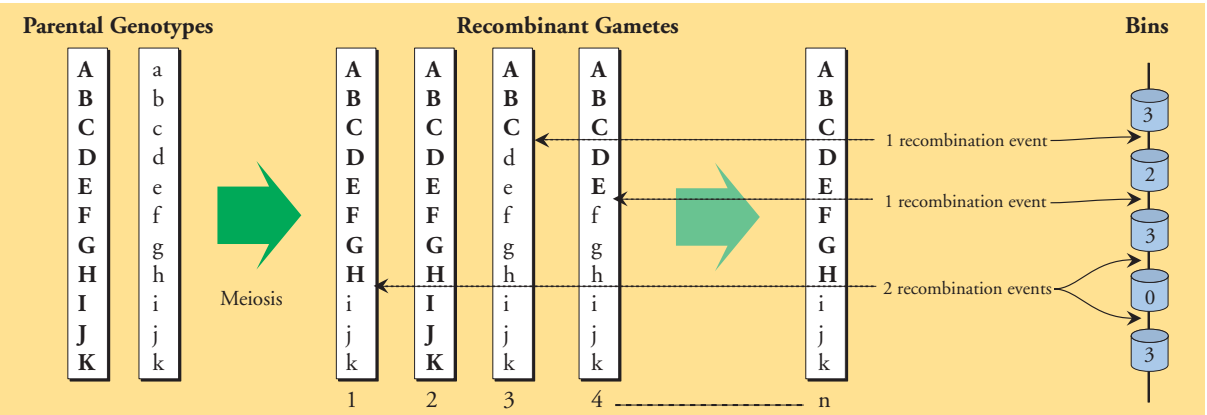


**Figure 1** The Bin Map Concept. A completely heterozygous, diploid parental chromosome undergoes meiosis to produce recombinant haploid gametes. Following the segregation of marker alleles (upper and lower-case letters) in progeny derived from these gametes allows the generation of a linkage map. Markers that are never separated by a recombination event (in a population on *n* progeny individuals) co-segregate, and are placed in the same co-segregation bin. When more than one recombination event occurs between two consecutive markers, empty bins (bin 4 above) are placed on the map to represent every recombination event which cannot be visualised.

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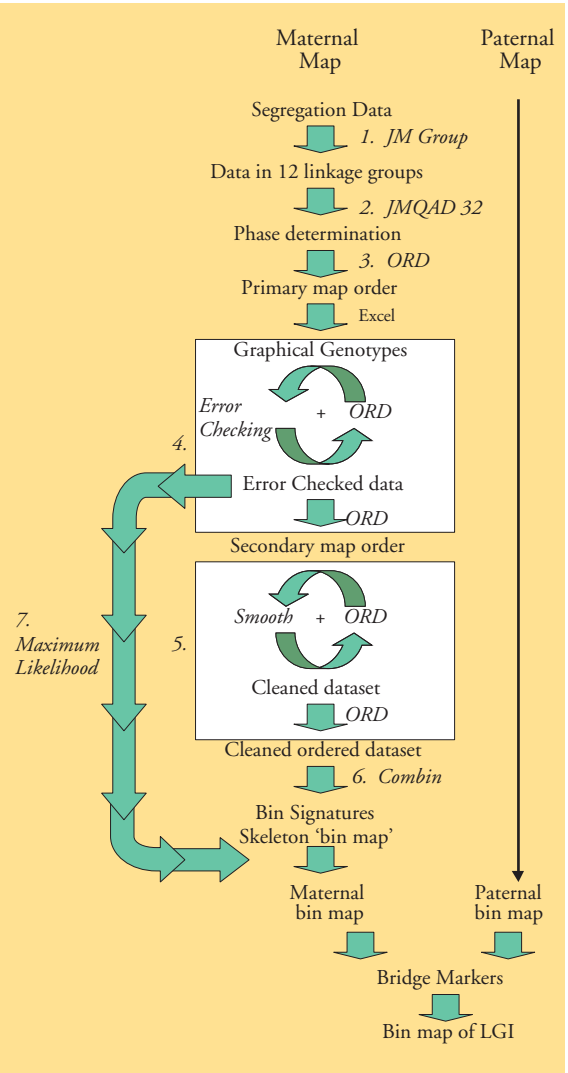


**Figure 2** Steps involved in the creation of the 1260 point linkage map of chromosome 1.

The fact that each erroneous datapoint can introduce two false recombination events (a so-called single marker double recombinant) means that there exists the potential for 2000 false recombination events to be introduced into the dataset. This is an order of magnitude greater than the total number of recombination events expected in a population of 100 individuals, assuming 1-2 crossovers per chromosome. The consequence of using such data in currently available mapping software is the generation of vastly inflated maps with nonsensical marker orders.

From the above, we concluded that there are two pivotal requirements for creating UHD genetic linkage maps. The first of these is a system for rigorously and systematically identifying and correcting errors in the marker segregation data. The second is the development of a mapping model that allows the use of the most reliable data to calculate a framework map into which the remaining data can be fitted without upsetting the model. An important factor in this strategy is the fact that, in very large segregation datasets, the most reliable data is easily identified as that for which there is a level of redundancy. Multiple co-segregating markers reinforce the confidence in the accuracy of the shared segregation pattern of those markers. One potential model for a UHD map is the generation of a robust linear map, consisting largely of 'bins' of co-segregating markers and any non-redundant markers which can be incorporated into the linear map without conflict. Anomalous markers not resolved by error checking can be placed subsequently in the bin into which they fit best, without perturbing the overall map order. Thus, markers in a bin fit either perfectly, in complete agreement with the bin signature, or deviate from the bin signature by a number of recombination events. Another advantage of this model is that the quality of the resulting map can be verified by assessing the overall proportion of data that fits well into the model.

In order to achieve the UHD map of potato, we have deployed 400 AFLP primer combinations on 130 individuals of the $F_1$ progeny of a cross between two highly heterozygous diploid *Solanum tuberosum* genotypes, referred to as SH (maternal parent) and RH (paternal parent). To explore the implementation of the above model, an interim dataset of 6756 segregating markers generated by 234 primer combinations was analysed. Due to concerns about genome coverage, three restriction enzyme combinations, differing in the rare (6bp) cutting enzyme were used. As a result, 1278 of the markers were *PstI/MseI* based AFLPs, 1759 were *SacI/MseI* based, and 3719 were *EcoRI/MseI* based. To facilitate chromosomal identification, segregation of a small set of previously mapped SSR markers was also analysed in the population.

The segregation data were divided into maternal (genotype: abxaa, 2682 markers), paternal (genotype: aaxab, 2223 markers) and biparental (genotype: abxab, 1851 markers) datasets. Step 1 (see Fig.2) in the process was the use of the GROUP function of the mapping package JoinMap v2.0 to split the marker segregation data into 12 linkage groups corresponding to the 12 chromosomes of the potato genome. Chromosomal identities were assigned to the linkage groups on the basis of the SSRs and locus specific AFLP markers. The linkage group identified as Chromosome 1 was chosen to illustrate the principles

outlined above because it was the most extensive linkage group, containing a total of 1260 markers (627 maternal, 420 paternal and 213 biparental). The maternal and paternal datasets of Chromosome 1 were subsequently subjected separately to the process outlined in Figure 2.

Steps 2 and 3 involve determination of marker phase and map order using the JMQAD32 function of JoinMap and a newly developed programme called ORD, which calculates the marker order using an algorithm that minimises the number of recombination events. To identify potential errors, marker segregation data were sorted into a primary map order calculated by ORD and displayed as colour coded graphical genotypes in an Excel spreadsheet (Step 4, Fig. 2). Graphical genotypes are a representation of the recombined parental chromosomes in the progeny, and allow the identification of individual marker datapoints acting as single marker double recombinants (singletons). These singletons are potential marker scoring errors, and once identified, can be rechecked on the original AFLP autoradiograms, and corrected if necessary. This was done once for the entire dataset, and the marker order was recalculated in ORD using this more accurate data. In theory, this process could be repeated several times, but in practice, its time consuming nature allowed only two iterations, producing an improved secondary map order. Removal of remaining singletons was automated using a computer programme called SMOOTH (Step 5, Fig 2) which institutes an algorithm that calculates the probability of each marker datapoint being 'true' on the basis of flanking markers in the secondary map order. Markers that are not supported by observations at flanking markers are replaced by missing values, and a new marker order is again calculated using ORD. This process was repeated several times, gradually decreasing the stringency threshold allowing markers to be nominated as singletons. This cleaned ordered data set was then used to construct maternal and paternal maps of Chromosome I using a programme called ComBin (Step 6, Fig. 2). In ComBin, co-segregating markers are placed in `bins` to remove redundancy in the data set. Subsequently, the bins are 'threaded' like beads on a string (i.e. linearly organised), with adjacent bins differing by a single recombination event. When two adjacent bins were separated by more than one recombination event, a number of empty bins equal to the number of recombination events separating the markers were placed on the 'skeleton bin map'.

The accuracy of the skeleton bin map was verified by fitting the original marker data (after data checking but before cleaning with SMOOTH) into the skeleton bin map on the basis of the highest LOD score between markers and the bin signatures (Step 7, Fig. 2). Chromosome I consists of 90 maternal bins and 93 paternal bins (Fig. 3). The 627 maternal markers fitted into 66 bins, leaving 24 bins empty. The 420 paternal markers fitted into 49 bins, leaving 44 bins empty. The 210 biparental markers and three SSR loci were used to link the two parental maps as bin bridges (again on a maximum likelihood basis), giving a final map of 1260 markers. We estimated a residual singleton rate of 1-3% per marker per primer combination after two rounds of graphical genotype checking. Thus, we chose a threshold of a 3% deviation from the bin signature to determine whether markers fit well into the bins. Overall, 75% and 80% of the maternal and paternal markers respectively fit into bins within a range of 0 to 3% recombination, indicating that the model holds up well for the majority of the data. As the remaining 22.9% of markers outside the threshold do not perturb the map order, they can be retained in the dataset. This is an important aspect

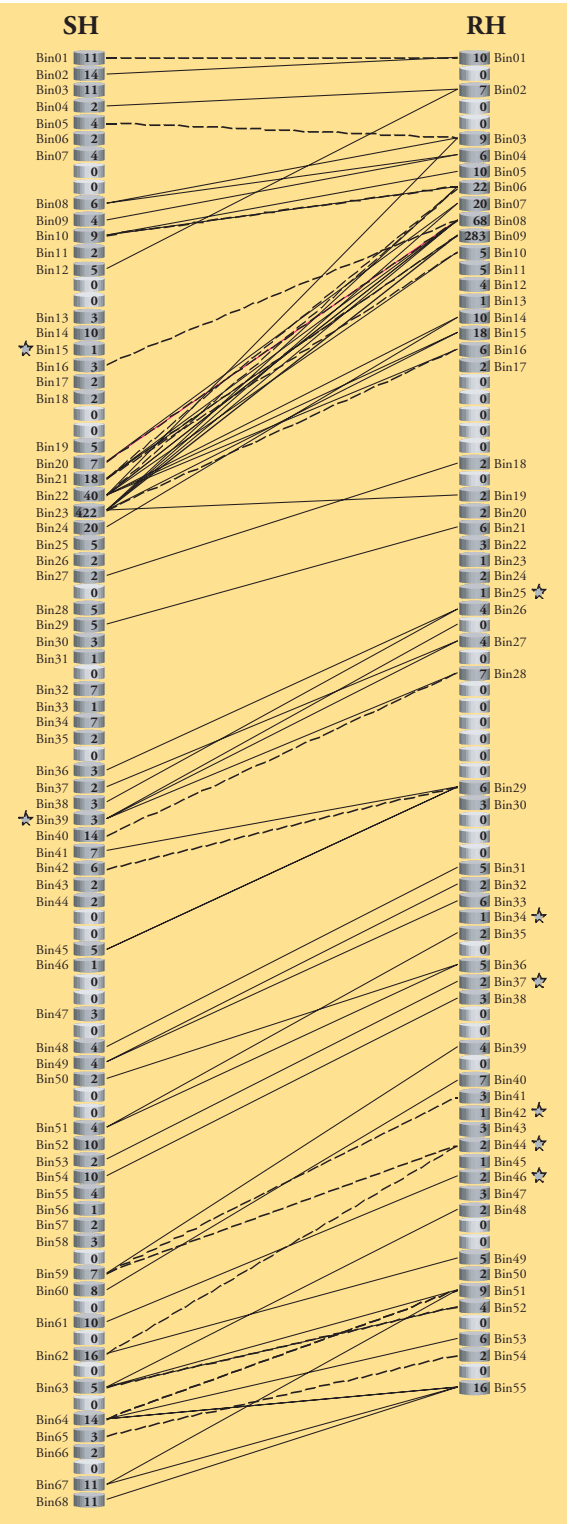


**Figure 3** Skeleton bin map of chromosome I of potato. SH and RH are the maternal and paternal maps respectively. Lines between the two maps represent the allelic bridges (<ab x ab> markers and SSRs) between the two individual maps. Number in each bin is the number of 1:1 markers.

of the strategy as subsequent studies may be able to use (or even resolve the true position of) these markers. As well as residual scoring error, technical and biological phenomena such as PCR mispriming, co-migrating independent bands and methylation may all contribute to markers fitting badly into bins. The last of these is a testable hypothesis, since if methylation was responsible for markers not fitting well into bins, we would expect to observe a higher proportion of badly fitting *PstI* markers in comparison to *SacI* or *EcoRI*, due to the methylation sensitivity of *PstI*. We therefore compared markers that deviate from the bin signature at greater than 3% recombination. Approximately double the proportion of *PstI* markers are observed in this class (30%) compared to *EcoRI* and *SacI* markers (15%), indicating that methylation does play a role.

Both gaps (empty bins) and significant clustering (bins containing many markers) are evident on the map (Fig 3). The presence of empty bins might be considered surprising on such a high density map and probably represent regions with high levels of recombination or an absence of polymorphism. The largest bin in both parental maps contains approximately 50% of the markers, and this probably represents an area of suppressed recombination around the centromeric regions observed in many maps. Interestingly, when the distribution of the three enzyme combinations is analysed independently, the centromeric clustering is far less pronounced for *PstI* based markers compared to the *EcoRI* and *SacI* based markers. This is probably due to the fact that the methylation sensitivity of *PstI* favours the targeting of these markers to under-methylated, euchromatic (gene-containing) regions which tend to be located toward the ends of the chromosome.

In conclusion, we have developed a mapping model that will allow the rationalisation of 10,000 segregating markers into an ultra high-density genetic linkage map of the potato genome, resulting in the densest genetic map of any crop plant species to date. Unlike previous genetic linkage maps,the model allows the assessment of the robustness of any marker on the map by virtue of how well it fits the bin in which it has been placed. We are currently developing strategies that will allow the deployment of this map as a generally applicable resource for several uses including rapid local physical mapping, positional cloning, and development of markers for accelerated breeding programmes.

# High density, high throughput physical mapping in plants

A.B. James, G. Bryan, P.H. Dear[1], M. Thangavelu[1], L. Ramsay & R. Waugh

Genetic linkage maps have been constructed for many plant and most crop species. By correlating the pattern of inheritance of trait information in a meiotic mapping population with that of individually mapped genetic markers, many monogenic and polygenic traits have been located to specific regions of the plant genome. The major impetus for meiotic mapping was the discovery of extensive, yet easily visualised, variability at the DNA level, which could be used as markers in most natural populations. Over the last 20 years, this approach has been extremely valuable and productive. However it suffers from a number of limitations. Firstly, it relies on recombination occurring between polymorphic loci during meiosis, thus excluding the ability to map chromosomal regions that are identical by descent. Secondly, the ability to order the entire complement of genes on a plant chromosome is unlikely since the probability of recombination events occurring over ever shorter distances becomes vanishingly small. Meiotic mapping is further compounded by the presence of recombination 'hot-spots', and segments of suppressed recombination. The result is a poor correlation between genetic and physical distances.

The advent of physical mapping, based on sequence tagged site (STS) or expressed sequence tag (EST) content analysis of large insert clones such as YACs or

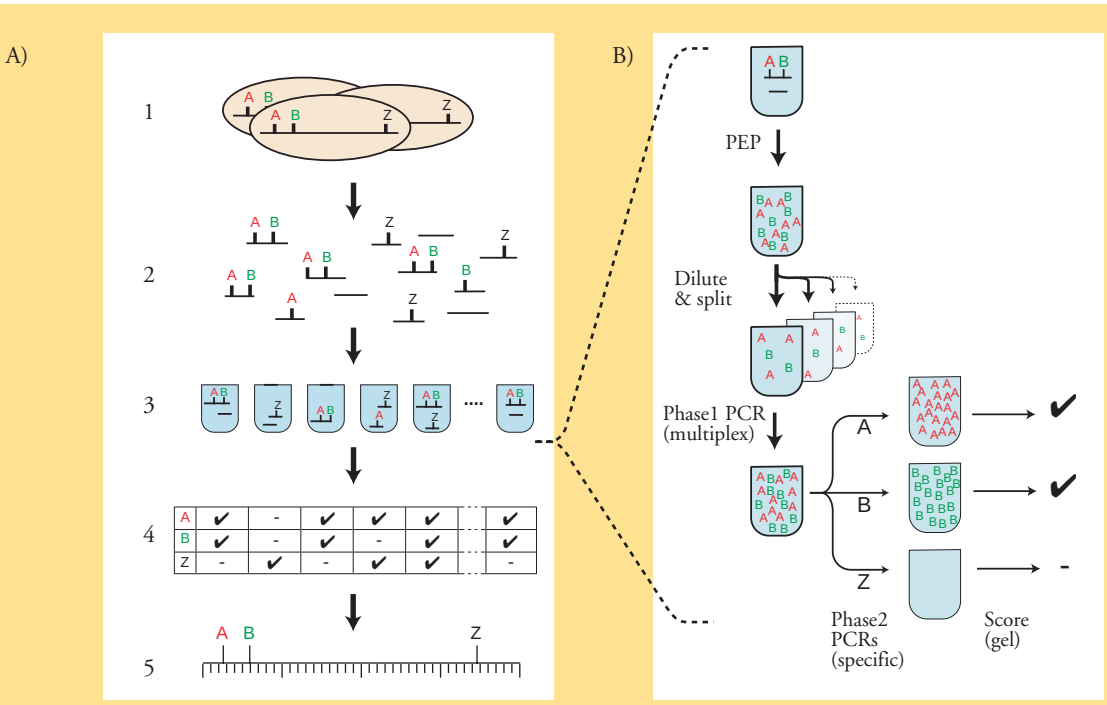


**Figure 1** Principle of HAPPY mapping and marker typing. (A) Overview. DNA carrying STS markers (A, B, Z) is extracted from cells (1) and broken randomly to give a pool of fragments (2). These are dispensed at limiting dilution into a series of aliquots - the mapping panel (3). The panel is screened by PCR to produce a table (4) showing the marker content of each aliquot. Linked markers (A, B) are found to co-segregate; remote markers (B, Z) do not. Co-segregation frequencies reflect marker-to-marker distances, allowing a map (5) to be computed.
(B) Expanded view of marker screening using a 3-step PCR. The protocol is illustrated for one aliquot of the mapping panel. All DNA in the sample is first pre-amplified >100-fold using PEP. This material is diluted and split into sub-fractions for multiple rounds of screening. One sub-fraction is amplified in a multiplex PCR for many markers (Phase1). The products of this reaction are then diluted and split again, and screened for individual markers (A, B, Z) in turn using hemi-nested primers (Phase2). Results are scored on gels, determining the marker content of the aliquot.

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BACs, has circumvented many of these drawbacks. In particular, it precludes the requirement for polymorphic genetic markers. Using this approach, 'contigs' of overlapping cloned fragments are assembled to span the entire genome in question, or the particular region of interest. A genome-wide STS content physical map of *Arabidopsis thaliana* has been constructed and maps of several crops species, including rice and sorghum, are being prepared. Physical mapping in this manner is generally regarded as enhancing the molecular genetics of the particular organism, since it serves as an archive of genomic information. Furthermore, integration of physical maps with genetic maps is extremely valuable for map-based gene isolation, comparative genome analysis, and as sources of sequence-ready clones for genome sequencing projects. However, mapping chromosomes or genomes in this way is limited by the cloning process on which it relies; regions recalcitrant to cloning lead to uncloseable gaps, while rearranged or co-ligated fragments, or repeated regions larger than the size of the clones, can lead to distortions. Hence, it has been proposed that physical maps are most effective if built over an independently constructed STS 'scaffold'.

HAPPY mapping[1] has been developed as an *in vitro* physical mapping technique that addresses the problems associated with other *in vivo* methods, such as Radiation Hybrid (RH) mapping. HAPPY mapping's utility has been demonstrated by mapping human chromosome 14 at high resolution[2], the complete genome of the parasite *Cryptosporidium parvum*[3], and chromosome 6 of *Dictyostelium discoideum*[4]. The technique involves breaking intact genomic DNA at random, segregating the fragments into aliquots (the 'mapping panel') each of which contains less than one genome's worth of DNA

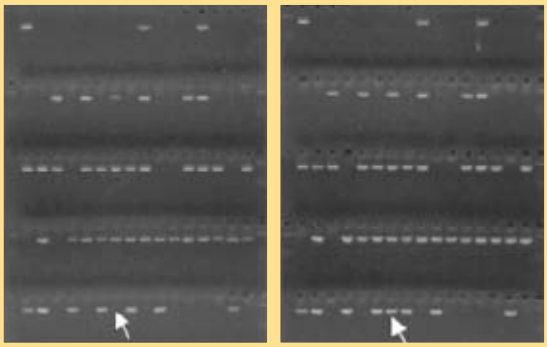

Figure 2 Typing two physically linked PCR-STS markers on a High resolution HAPPY mapping panel of barley. Only a single 'recombinant' is observable between the two markers which are separated by 10kb on the barley genome.

fragments (the *in vitro* analogue of a radiation hybrid cell), and measuring the frequency of co-segregation of markers among the aliquots (Fig. 1). Closely linked markers are rarely separated by an intervening break and therefore tend to co-segregate. In this way, it is analogous both to classical genetic linkage analysis, which measures the frequency of recombination between markers during meiosis, and to RH mapping. HAPPY mapping, however, possesses all the advantages of RH mapping (no requirements for polymorphisms; flexible resolution of markers depending on the size of DNA fragments utilised), but none of the drawbacks - it is immune to artefacts caused by the biological activity of the DNA fragments, to cloning artefacts, or to the effects of chromosome structure. In addition, constructing and screening a HAPPY mapping panel is relatively simple, and only small quantities of DNA are needed.

As proof of principle, we have constructed a high resolution HAPPY

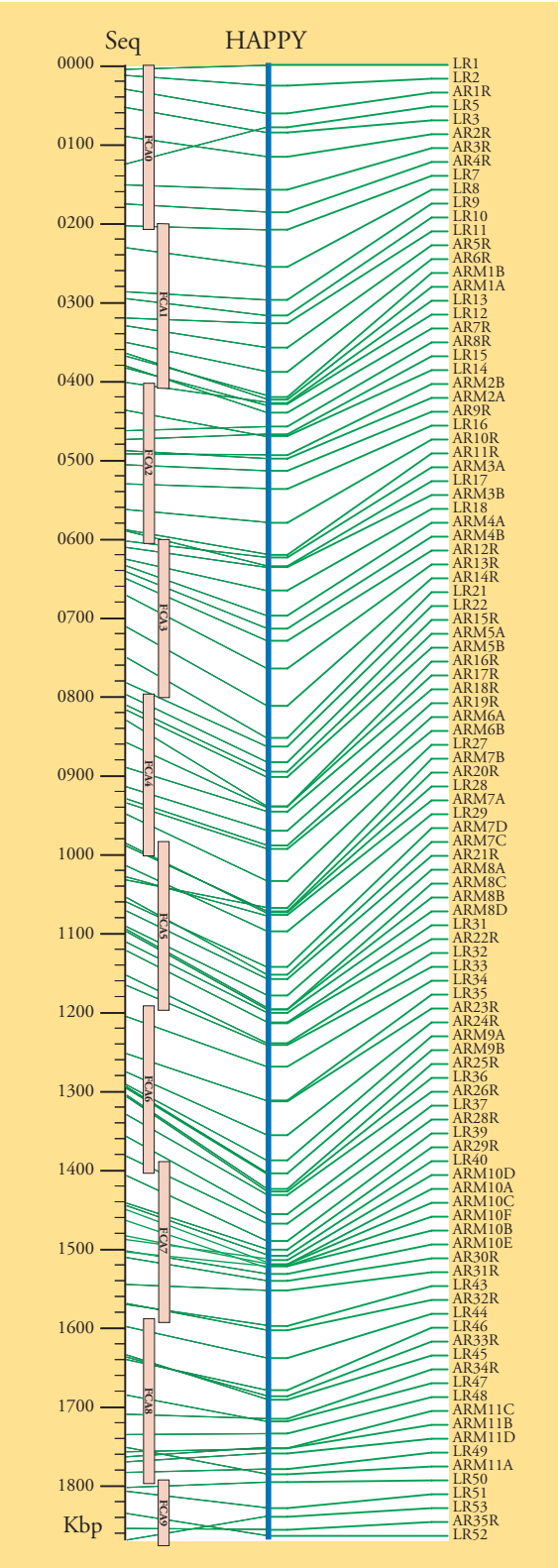


**Figure 3** Comparison of HAPPY map, physical map and nucleotide sequence. The mapped positions of 107 markers (named at right) are shown on the HAPPY map (central heavy line) and nucleotide sequence (left).

map of a 2.0 Mbp section of *A. thaliana* chromosome 4. This map, offering an average marker to marker distance of 16 kbp, spans approximately 10% of the chromosome and approximately 1.4% of the entire *A. thaliana* genome. In order to construct the map, a HAPPY mapping panel was prepared comprising 80 aliquots of genomic DNA broken at random, with each aliquot containing less than one genome's worth of DNA. The panel was analysed for the presence of PCR amplified STS marker sequences spanning the sequenced FCA locus of *A. thaliana* chromosome 4 (~2.0 Mbp) (Fig. 1). Analysis of the frequency of co-segregation of 107 markers enabled the order and physical distance between markers to be estimated and compared with the true physical distance (Fig. 2). The map revealed a good overall correspondence with the true physical map; the order of all but 16 markers (< 15%) was as given in the sequenced FCA locus and the errors in the positions of all but one of these markers were well within the margins of error expected from the mapping panel. We have also shown that these local inversions could be corrected by re-typing the markers on a shorter range mapping panel comprising smaller DNA fragments. Having demonstrated the potential of the method in *Arabidopsis,* we have also started to look at crop plant genomes which, physically, can be significantly larger. Preliminary experiments carried out to compare physical maps of the *mla* locus in barley cultivars, with different introgressed *mla* disease resistance specificities (genome size c. 40x that of *Arabidopsis*), indicate that HAPPY mapping will be a useful 'comparative physical mapping' tool. This obviates the need to construct large insert DNA libraries (e.g. BACs) for many separate accessions, followed by comparative structural analysis. In conclusion, we believe that HAPPY mapping panels could be developed for any plant species and utilised as a high resolution physical mapping resource by the plant research community.

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# Radiation hybrid technology in plants

J. Wardrop, J. Snape[1], W. Powell & G.C. Machray

Radiation hybrid (RH) mapping is an extremely powerful technique developed to facilitate the construction of high-resolution physical maps of the human genome. A number of mammalian RH mapping panels are available to the scientific community for research purposes, and the transfer of this technology from mammalian systems to plant systems will greatly aid progress in the physical mapping of plant genomes.

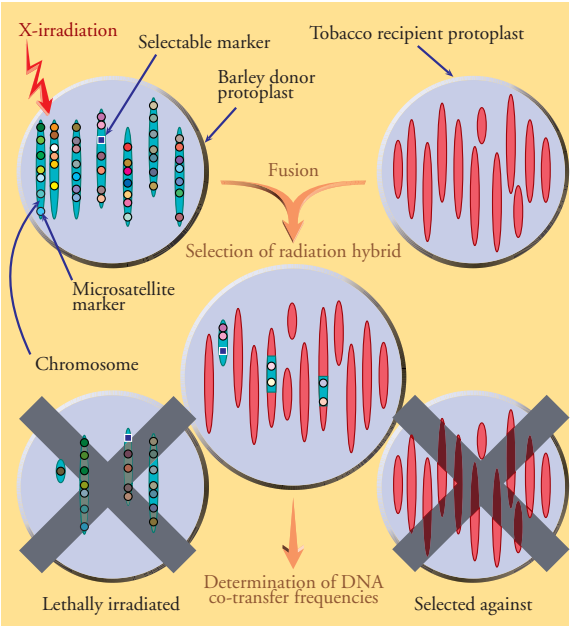


**Figure 1** Irradiation and fusion gene transfer.

RH mapping is based on irradiation and fusion gene transfer (IFGT) in somatic cells (Fig. 1). Donor cells are exposed to lethal doses of irradiation which fragment the chromosomes. These fragments are then rescued by fusion with suitable recipient cells. Characterisation of resulting cell lines and subsequent mapping is achieved by assessment of co-retention frequencies for molecular marker alleles originating from the donor material and the use of statistical methods to calculate the order and distance between marker loci.

There are a number of reasons why RH panels are considered valuable tools for mapping programmes. Markers used do not rely on polymorphism to produce maps, and a simple plus/minus assay is used to assess retention or elimination of marker loci within cell lines. Problems associated with the presence of recombination hotspots during conventional mapping are avoided by utilising irradiation to induce random breakpoints within chromosomes. A further advantage of using RH mapping panels is that, by altering the irradiation dose to which donor cells are exposed, map resolution may also be manipulated. Elevated levels of irradiation treatment will induce increased chromosome fragmentation and, thus, allow the construction of high-resolution maps. Additionally, a relatively small number of lines are required to produce a mapping panel. In mammalian systems, RH panels typically number between 80 and 100 lines.

## Generation of a barley whole genome radiation hybrid panel

**Donor and recipient material** Young, transgenic barley cell suspensions were used as a source of donor protoplasts. Incorporation of a selectable marker (herbicide resistance) within the donor genome allowed selection of putative hybrid material by manipulation of culture conditions. Tobacco, mesophyll-derived, protoplasts were utilised as recipient cells, as this species has well established protoplast fusion and culture protocols. A dicotyledon fusion partner was also chosen to ensure that molecular marker analysis of resulting putative hybrid material avoided false positive results which may occur due to homology between the donor and recipient genomes.

**Irradiation and fusion gene transfer** X-irradiated donor material was used in asymmetric somatic hybridisation experiments to produce a panel of radiation hybrids, exhibiting partial genome transfer, suitable for mapping procedures. X-irradiation treatment was provided by a linear accelerator and protoplasts

[1]John Innes Centre, Norwich

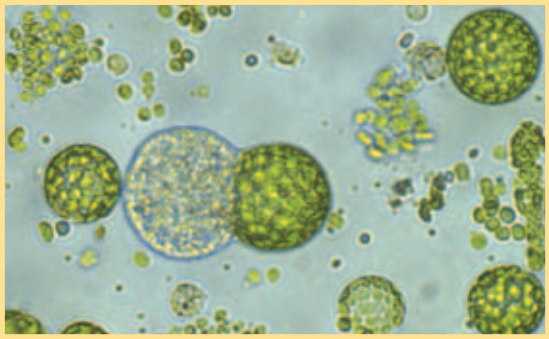

Figure 2 Barley donor (colourless) and tobacco recipient (green) protoplast fusion.

were exposed to varying doses of irradiation (0-100 Gy), prior to fusion, to induce chromosome fragmentation. Protoplasts were then mixed and aligned in an AC field where fusion was facilitated by the application of a DC pulse (Fig. 2).

Protoplast fusion assessments have revealed that an X-irradiation dose of 50 Gy resulted in the most efficient throughput of material. Experiments, where protoplasts were exposed to irradiation levels exceeding 50 Gy, were less efficient, generating resistant cell lines at a frequency too low to be suitable for the construction of mapping panels.

### Characterisation of radiation hybrid cell lines

**PCR screen for selectable marker** All cell lines, which survived culture upon herbicide selection medium, were assessed by PCR for the presence or absence of the selectable marker. A screen of five putative hybrid cell lines revealed four herbicide resistant lines which are PCR positive for the selectable marker (Fig. 3).

**Co-retention of microsatellite marker loci within cell lines** PCR-positive RH cell lines were further characterised using 37 barley-specific microsatellite markers offering genome-wide cover. Cell lines which retain the marker, as in RH 23 and RH 33 (Fig. 4), are scored 1 and those where the marker loci has been eliminated, as in RH 61 (Fig. 4), score 0. This allows

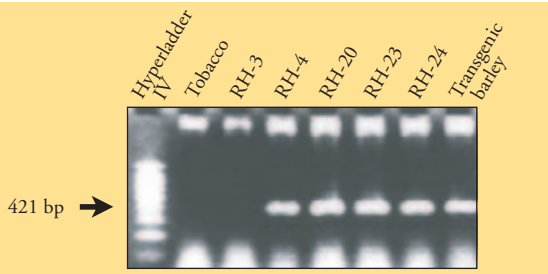


**Figure 3** PCR-based validation of radiation hybrid cell lines.

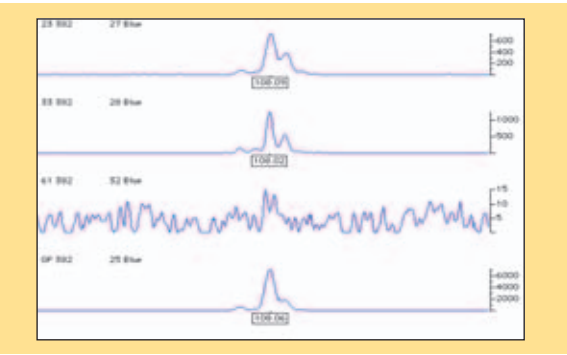

**Figure 4** Microsatellite marker characterisation of radiation hybrid cell lines (RH 23, 33, 61 and control barley from top to bottom).

us to build up a picture of which parts of the genome have been retained in individual cell lines. Retention frequency varies among cell lines but marker data collected reveals that barley DNA is retained within the tobacco genome at a frequency similar to that of currently available human RH panels. Assessment of the microsatellites chosen for characterisation of the RH lines has also revealed that certain genomic regions are more frequently retained than others but that, across the panel, it is possible to secure retention of most of the genome for whole genome mapping purposes.

### Conclusions

This research has established a protocol for the generation of the first whole genome radiation hybrid panel in barley and this panel is currently being exploited to physically map a group of barley-specific microsatellite markers.

Ongoing work on this project includes further validating the panel using previously mapped markers. Two approaches are being undertaken to achieve this goal. Firstly, PCR screens are being carried out for closely linked pairs of markers that have been physically mapped using BACs. Both markers should always be either present or absent in RH lines. Secondly, work carried out at JIC on physical mapping has revealed an RFLP marker mapping closely to the transgene. This marker should be retained at a frequency similar to the selectable marker (100%) and will also further validate the panel.

Exploitation of this technology is focusing on further production of RH lines to be used concomitantly with the current 50 Gy RH panel for high density mapping of single chromosomes using ESTs produced at SCRI. Future work will also focus on studying cell lines using fluorescence *in situ* hybridisation to visualise barley chromosome fragments retained within the recipient genome.

# Progress towards transformation of fibre hemp

L. MacKinnon, G. McDougall, N. Aziz & S. Millam

**Introduction** The last decade has seen the reintroduction to the UK of a crop with a history of triumphs and tragedies; today its very name provokes visions of a drug culture, fuelled by its prohibition in Britain for over 70 years. Historically, cultivation of *Cannabis sativa* L. (hemp) originated in China around 2700 BC, where its properties as a medicinal plant were first discovered. Cultivation then spread across Asia and through Europe, arriving in the UK some 2000-2200 years ago, by which time it had become widely cultivated as it had so many uses. Hemp fibres were found to be durable and were used in clothing, sailmaking and papermaking. Notably, the first copies of the Bible were composed of hemp paper. Oils from hemp seeds were used for a wide range of purposes, from cooking to cosmetics, and extracts of hemp were used to treat a wide range of ailments. Queen Victoria is rumoured to have used hemp on a regular basis. This widespread industrial use of hemp continued to the early 20th century, until cheap and plentiful imported jute and cotton made hemp uncompetitive. Around this time, as the legitimate uses of hemp declined, the misuse of hemp as a hallucinogenic agent became more apparent and many countries began to outlaw the cultivation of cannabis. In 1928, an act was passed that finally prohibited hemp cultivation in the UK.

Hemp cultivation continued in other European countries, such as The Netherlands and France, and in many Russian states. European hemp breeding programmes produced hemp cultivars for fibre production that were low in the psycho-active constituents, cannabinoids. Eventually, cultivars with ultra-low (<1% wild type) cannabinoid levels were available, which reawakened UK interest in hemp. In 1994, a new hemp-licensing scheme allowed the crop to be cultivated again in the UK and its reintroduction began. Over recent years, hemp cultivation has been increasing steadily (Fig. 1). This renaissance is also attributable to the need for renewable non-food crops in the UK, as outlined by the UK Biodiversity action plan.

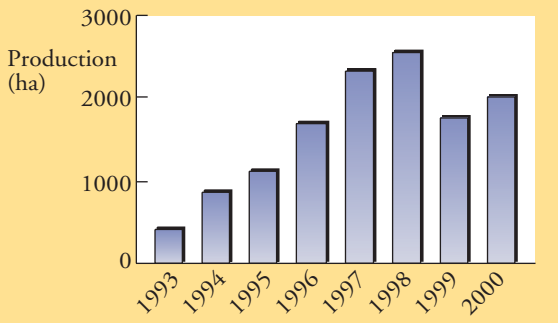


**Figure 1** The UK production of hemp over recent years.

**Why grow hemp?** Hemp is a particularly environmentally friendly crop and can be grown on soils poor in nutrients, with no additions of fertilisers. Routinely, it needs no herbicide or pesticide inputs and can be grown on set-aside land. It has been found to be useful in crop rotations as it suppresses three major soil pathogens, reducing the need for pesticide use in subsequent crops.

Hemp has a short growing season, reaching maturity in only 100 days, which makes it a useful alternative annual crop, and there is a current EU subsidy of 100 euros per tonne of hemp produced in Europe.

In addition to the environmental aspects, hemp is a multi-use crop and can provide fibres for paper, board and textiles; oil from the seeds; and provide stem residues (straw), which are valued as non-edible bedding for animals.

One of the main applications of hemp is for fibres for the papermaking industry, and there has been a great deal of research in this area. Unlike other fibres such as cotton, which require considerable processing and strong chemical treatments prior to use, hemp fibres are obtained by retting in water and can be bleached prior to use with hydrogen peroxide, which produces only water as a by-product. In addition, there is scope for hemp fibres to be used in the high quality textile industry.

**Why transform hemp?** Despite its many natural advantages, hemp could be improved further as a valuable multi-use crop. There is a need for new hemp products so that the crop can achieve long-term competitiveness, even as an alternative or rotational crop. The use of genetic transformation to produce novel products is a possible route. Additionally, although hemp suppresses certain soil pathogens, as discussed above, it is susceptible to infestation by *Botrytis cinerea* and significant losses, which are not prevented by pesticide use, can result. Transformation of hemp with existing genes that increase plant resistance to fungal infection may be an answer to this problem.

The properties of the fibre could be improved to match consumer requirements. For example, transformation of hemp with genes involved in lignin biosynthesis, could be used to upgrade fibre quality and widen potential end-uses in the paper and textile industries. Genetic modification of the oil profile of hemp seed, as achieved for oilseed rape, could find high-value niche markets.

Such improvements in hemp quality could be achieved by conventional breeding techniques. However, conventional breeding is a very slow process, which could take decades, especially for *Cannabis* which has not been intensively bred. An additional problem is that the existing, legally acceptable fibre hemp cultivars, have been bred to be low in cannibanoids and breeders must ensure that this trait is not bred out.

**Towards the transformation of hemp** There has been very little work on the biotechnology of hemp[1] and therefore basic research was required to establish tissue culture and regeneration systems suitable for the crop. Two French hemp fibre cultivars (Fedora 19 and Felina 34) were selected for use in this project. Both have low cannabinoid contents and are genetically similar, but Felina is an early maturing genotype. These cultivars were obtained from an accredited source (Hemcore) and are grown under Home Office licence.

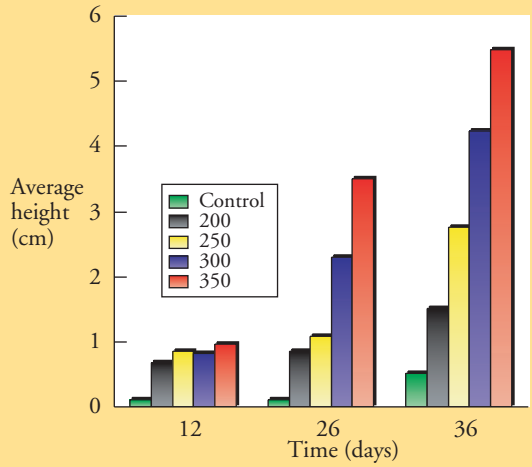


**Figure 2** The average height of Felina shoot tips grown under varied concentrations of cefotaxime.

**Establishment of tissue culture and regeneration systems** The establishment of effective tissue culture and regeneration systems is an essential prerequisite of genetic transformation for any plant species. A large series of experiments was undertaken to establish sterile plants in tissue culture, regeneration of explants, and shoot tip regeneration. Effective protocols have been established for growing hemp seedlings *in vitro*, with the different varieties having different media preferences. Callus and roots were easily obtained from hemp explants but shoots were not readily produced. Thus, alternative transformation strategies were devised and, as a result, a method of transformation involving shoot tips was developed from methods previously reported in cotton[2] and petunia[3].

It was found that shoot tip regeneration was markedly improved by the addition of antibiotics, specifically cefotaxime (Fig. 2), and subsequent shoot tip growth was aided by reduced light treatment.

**Susceptibility of hemp to infection by the genetic transformation agent *Agrobacterium tumefaciens*** The most common and convenient means of introducing genes into plants is to use the bacterium *Agrobacterium tumefaciens* as the vector. However,

Figure 3 Stem section of *C. sativa* infected by wild-type *Agrobacterium.*

since the ability of this bacterium to infect hemp had never been reported, experiments were undertaken to confirm its potential as a transformation agent. Over 50% of hemp plants exposed to this bacterium exhibited the classic Crown gall (Fig. 3), which confirmed that genetic transformation of hemp using this vector should be possible. An efficient transformation system for hemp has now been developed.

*Botrytis*-resistant hemp As mentioned previously, hemp is susceptible to infection by the fungus, *Botrytis cinerea*. Previous work at SCRI has shown that polygalacturase inhibitory proteins (PGIPs) can convey resistance to this disease[4]. PGIP genes were introduced into hemp, along with herbicide resistance as a selective marker of transformation. The efficiency of our transformation method was comparable to other crop efficiencies. Initial molecular analysis has confirmed that the transformed herbicide-resistant plants also contain the PGIP gene. The PGIP positive transformed plants have been tested for resistance to *Botrytis* and preliminary results (see figure 4) are encouraging.

Figure 4 Plants 1-4 are transformed with an empty vector control plasmid, plant 5 transformed with PGIP genes. Photo taken 3 days post-inoculation with *Botrytis cinerea.*

Conclusions A reliable and effective transformation system has been developed. This is the first report of genetic transformation in hemp. This preliminary work will enable more targeted approaches to gene transfer in this valuable crop species in the future and enable the range of uses of this versatile and historically significant plant to be enhanced.

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# SnoRNA gene clusters

J.W.S. Brown, G. Clark, G. Thow & C.G. Simpson

Small nucleolar RNAs (snoRNAs) are found in the major sub-compartment of the nucleus – the nucleolus. In the nucleolus, ribosomal RNA (rRNAs) is transcribed, processed, and complexed with ribosomal proteins to form ribosomal subunits for translation. SnoRNAs are involved in the processing of rRNAs. Apart from specific cleavages to form the 18S, 5.8S and 25/28S rRNAs, the two major types of modifications are 2'-O-ribose methylation and pseudouridylation, each of which occurs at around 100 sites in rRNAs in plants and animals. These modifications are thought to fine tune the structure of the ribosome, making the translation process more efficient. The sites of 2-O-ribose methylation in rRNAs are determined by a class of snoRNAs called box C/D snoRNAs. These snoRNAs contain two terminal conserved sequences, box C and D, and each contains either one or two sequences complementary to rRNAs which determine the methylation site via base pairing of the snoRNA to rRNA (Fig. 1a).

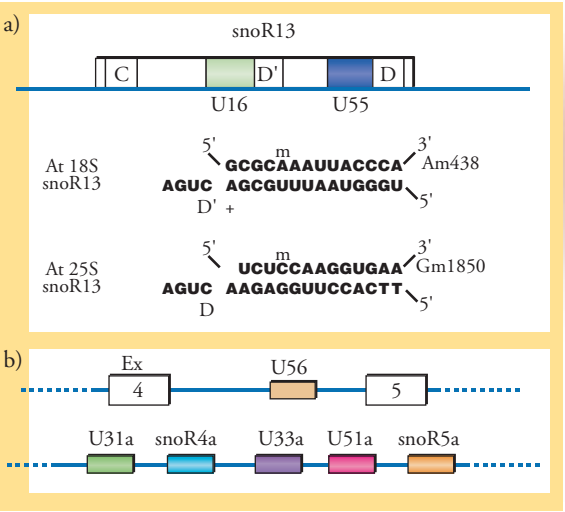


**Figure 1** Structure and function of box C/D snoRNAs. (a) Schematic structure of box C/D snoRNAs showing the terminal box C and D sequences, an internal D' sequence and sequences complementary to rRNAs (shaded boxes). SnoR13 contains two regions which base-pair with specific rRNA sequences and guide methylation at position Am438 in 18S rRNA and Gm1850 in 25S rRNA. (b) Intron-encoded and polycistronic snoRNAs from *Arabidopsis*. Large open boxes – exons; small boxes – different snoRNA genes.

The way in which the majority of snoRNAs are expressed differs in the three eukaryotic organisms in which they have been most widely studied. The main modes of snoRNA gene organisation are intron-encoded and polycistronic (Fig. 1b). Intron encoded snoRNAs are found in introns of protein coding genes and are processed following splicing. Polycistronic snoRNAs consist of closely linked clusters of genes which are transcribed together as a precursor snoRNA from which they are processed. In vertebrates, there are no cases of polycistronic snoRNA genes and the majority are intron-encoded. In yeast, there are five polycistrons and the majority of the remaining snoRNA genes are intron-encoded. In contrast, in plants, the vast majority of snoRNA genes are polycistronic[1] and there are only three examples of intron-encoded plant snoRNAs to date.

We have mapped methylation sites in plant rRNAs and have searched the *Arabidopsis* genome sequence for snoRNA genes using complementary sequences. To date, 24 snoRNA gene clusters each containing 2-5 snoRNA genes have been discovered. Some of the clusters are related, containing the same genes, and are found in different positions in the *Arabidopsis* genome. The organisation of the different gene clusters illustrates a number of mechanisms of gene and genome evolution. Clearly, there are local gene duplications of either single genes (Fig. 2a), single genes

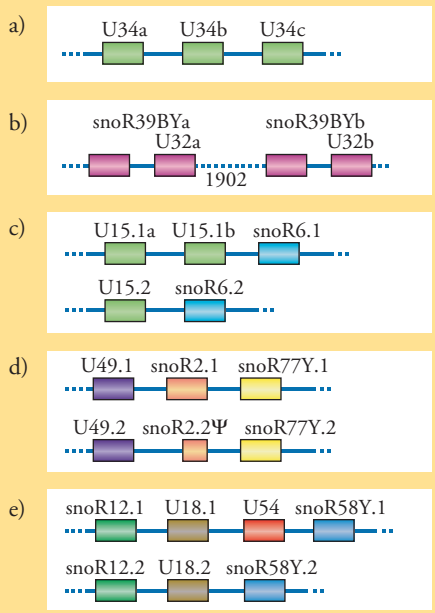


**Figure 2** *Arabidopsis* snoRNA gene clusters showing: (a) a homogeneous gene cluster; (b) locally duplicated pairs of genes; (c) gene clusters at different chromosomal locations with single gene duplication; (d) gene clusters at different locations with partial gene loss forming pseudogenes; and (e) gene clusters at different locations showing gene loss. The genes are shown by coloured boxes.

within a cluster (Fig. 2c), or pairs or clusters of genes (Fig. 2b). The presence of related gene clusters on different chromosomes and chromosome areas (Fig. 2c-e) could reflect duplication events, including polyploidisation, dispersed by chromosomal translocations or inversions. In some cases, mutation has led to loss of conserved sequences, leading to pseudogenes (snoR11 in Fig. 2d) and other related clusters suggest that genes have been lost completely from clusters (U54 in Fig. 2e). At the level of gene sequences, different alleles exhibit different degrees of sequence variation. The sequence variation can take the form of base substitutions, insertions or deletions.

The evolution of snoRNAs from *Archae* and yeast to higher eukaryotes is thought to have occurred through a series of gene duplications, mutation and selection, both in terms of ability to associate into stable snoRNPs, and functional advantage of the modifications to the ribosome. Thus, some sites of modification are conserved among yeast, plants and vertebrates while others are novel. rRNAs of different organisms differ in their rRNA expansion segments (regions of rRNA which are not conserved among species). The expansion segments can influence rRNA/ribosome structure such that the effects of particular modifications can be variable. This leads to differences in the modification patterns of rRNAs among different organisms.

In plants, polyploidy is an important influence of genome structure and gene evolution. It is estimated that around 70% of plant species have undergone hybridisation events during their history. Gene duplication through polyploidisation and intra- and inter-chromosomal rearrangements means that plants can often tolerate gene mutations which in single genes would otherwise be disadvantageous or detrimental and lost from the gene pool. Although there are examples of protein-coding gene variants with different structural or expression properties, the large number of snoRNA genes, in the box C/D snoRNA gene family, and the non-protein coding nature of the genes makes these genes an excellent model for analysing gene evolution mechanisms in plants.

### Reference

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# New potato cultivars

**Sebastian** is a new, general purpose ware (table use) potato, which produces a good yield of long-oval, attractive parti-coloured (red-eyed) tubers, added to National List in 2000. It was originally selected by our commercial partners because of its superior performance against cv. Cara in the Canary Islands and Southern Spain, and so offers an opportunity for export of seed to similar environments, where part-coloured varieties such as King Edward and Cara are grown for export back to the UK for the fresh, early ware market. However, its improved resistance to blackleg and virus Y, allied to its resistance to the golden cyst nematode (Ro1), plus a degree of partial resistance to the white cyst nematode, may also make it attractive to UK ware growers as well.

**Thyme** is also destined for table use. Added to the National List in 2000, it is an early maincrop producing an attractive yield of oval/round, white skinned tubers with cream flesh. Although susceptible to the golden cyst nematode (Ro1), its partial resistance to the white cyst nematode (*G. pallida* Pa2/3) and high resistance to powdery scab, will offer English ware growers a variety superior in these respects to current popular varieties. It is also reasonably resistant to both internal and external damage.

| | **Sebastian** | **Thyme** |
|---|---|---|
| Origin : | 8890AB42 x Cara | Cara x SCRI 12380ac2 |
| Year of cross : | 1982 | 1985 |
| Maturity | Maincrop | Early Maincrop |
| Wart | Resistant | Susc. |
| Late blight (f) | 3 | 4 |
| Late blight (t) | 6 | 5 |
| Gangrene | 5 | 3 |
| Common scab | 4 | 3 |
| Powdery scab | 5 | 7 |
| Virus: | | |
| PVY | 8 | 7 |
| PLRV | 4 | 4 |
| PCN: | | |
| *G. rost.* | 9 | Susc. |
| *G. pall.* | 3 | 5 |
| Blackleg | 9 | 5 |

Sebastian

Thyme

# *Plant biochemistry and cell biology*

Howard V. Davies, Roberto Viola & Karl J. Oparka

*The key disciplines within the Division i.e. phytochemistry, cell biology, molecular biology and biochemistry, are central to the delivery of SCRI's goals. The alliances being fostered within the Division and across Divisions are now providing real added value in terms of publication quality, scientific depth and opportunities for income generation. Sustaining and developing further these collaborations will be an important consideration in coming years to achieve SCRI's primary goals – the delivery of novel science of relevance to the consumer and to industry.*

## Developmental processes

**Meristem activation** The life cycle of the potato tuber is being used as a model developmental system to study the activation/deactivation phases of shoot apical meristems. The mechanisms controlling the meristem's activation status play a fundamental role in the establishment of dormancy and apical dominance. Physiological studies have indicated that the activation and deactivation status of meristems correlate with the presence and absence, respectively, of molecular trafficking between the meristem and the rest of the plant. Biochemical markers of tuber apical meristem activation status have been identified and have been used to guide the generation of gene libraries, enriched in sequences that are up-regulated in the early stages of dormancy release. Over 300 different sequences have been obtained. *In situ* hybridisation techniques have enabled the precise patterns of gene expression to be determined. A transgenic approach is being applied for the functional characterisation of candidate genes.

**Fruit ripening** The biochemical characterisation of carbohydrate metabolism in ripening strawberry fruits has been completed. A key role for mitochondrial citrate synthase and mitochondrial malate dehydrogenase in the regulation of fruit acidity, and hence flavour, has been established and the corresponding cDNAs have been cloned. Additional studies have focused on the effects of ethylene upon the quality of ripe fruit and ethylene has been shown to accelerate fruit deterioration. A productive collaboration initiated with the Molecular LASER Physics Department of Nijmegen University (the Netherlands), has provided unique insights into patterns of ethylene evolution

from ripening strawberry fruits. This work has proceeded in parallel with a search for novel genes involved in the ethylene-sensitive deterioration processes. To identify these genes, Suppressed Subtractive Hybridisation (SSH) has been successfully employed and *c.* 800 clones have been isolated for direct sequencing.

### Biochemistry

**Leaf urea metabolism** Urease and urease genes have been cloned from potato and urease activity successfully up- and down-regulated using a transgenic approach. $^{15}N$ labelled urea has been used to determine nitrogen turnover and partitioning in control and transgenic lines. The outcome is that the rate of metabolism of foliar applied urea can now be manipulated in both directions. Opportunities now exist to test the impact of variable urease expression on N use efficiencies. The literature indicates that N losses due to volatilisation can be significant using foliar applied urea. However, data obtained with the transgenics indicate that losses due to volatilisation are relatively small (10-15%) and do not correlate with urease activity, which itself generates the ammonium prone to volatilise.

**Regulation of barley malting quality** A project has been initiated aimed at defining the role of specific enzymes and enzyme inhibitors involved in the dissolution and breakdown of starch, cell wall and protein components of barley grains during the malting process. The activity of the starch debranching enzyme, limit dextrinase, has been defined during the malting process in two good quality and two poor quality cultivars and evidence for the presence of specific limit dextrinase inhibitors has been obtained. The nature of these inhibitors is being investigated. The timescale and global levels of arabinoxylan-degrading activities (endoxylanase, exo-xylosidase and arabinosidase) have been surveyed. The solubilisation of arabinoxylans during malting appears to be unrelated to the total endoxylanase activities of the malts. This may be due to the expression of endoxylanase isoforms with different kinetic properties or different levels of specific endoxylanase inhibitors in the good and poor quality varieties. The global proteolytic enzyme activities of the good and poor quality malts were qualitatively and quantitatively similar. Thiol and aspartic proteases contributed the majority of the total proteolytic activity and similar patterns of protease expression were noted during the malting of the good and poor varieties. The global proteolytic activity also matched the profile of protein solubilisation in the different malts. Polypeptide profiling of malting barley has been undertaken. Initial pilot studies on cell wall-associated proteins have been carried out and stage- and cultivar-specific proteins have been identified.

### Phytochemistry

**Glycoalkaloids** The effects of post-harvest stress and light exposure on the glycoalkaloid content of long-day adapted tubers have been investigated. The expected increases in solanidine-based glycoalkaloids, α-solanine and α-chaconine occur in *Solanum phureja* but, in contrast to the behaviour of *S. tuberosum* cultivars, a number of *S. phureja* lines accumulate an additional glycoalkaloid. A combination of liquid and gas spectrometric techniques, together with traditional thin layer chromatographic methods, were used to identify this compound as α-solamarine, a tomatidenol-based glycoalkaloid with solatriose sidechain. Little information is available on the toxic properties of this compound but it has been reported to occur in a limited number of *S. tuberosum* cultivars.

**Volatiles** A method, utilising porous polymer entrainment combined with thermal desorption-gas chromatography-mass spectrometry, has been developed for the identification of volatile compounds which are likely to be a major source of flavour compounds. Preliminary results utilising this method have shown consistent results. The majority of products identified are derived from lipids and/or amino acids. This methodology is currently being used to compare *S. tuberosum* and *S. phureja* cultivars which differ distinctly in organoleptic properties.

**Antioxidants and lipids** Research into plant antioxidants is continuing apace as part of the core research programme and in conjunction with a SEERAD grant and funds from a European Northern Periphery Programme (NORTHBERRY project). Studies on the soluble antioxidants in raspberry, strawberry and blackcurrant have shown that the derivation of antioxidant activity is distinct for each species. In blackcurrant, vitamin C is the dominant antioxidant whereas in strawberry, the polyphenolic compounds, catechins and anthocyanins, predominate. The main soluble antioxidants in raspberry remain uncharacterised but experiments suggest that they are glycosylated phenols. Extensive studies have revealed limited variation in soluble antioxidant potential between strawberry genotypes but significant variation in raspberry and even more so in blackcurrant. Examination of wild blackcurrant and raspberry species indicates even greater variation. This could be utilised to boost the

antioxidant status of cultivated germplasm. Investigations into non-soluble, or cell wall-associated antioxidants, suggests that a significant proportion of the antioxidant potential of soft fruit, in particular blackcurrant, has been unaccounted for. These antioxidants interact with the wall through both covalent and non-covalent binding. For a full report see p. 94.

Comparisons of the fatty acid composition of 36 SCRI blackcurrant genotypes indicated that the contribution of γ-linolenic acid (GLA) generally varied between 11-19%, although three genotypes had values of 22-24%. Such levels have not been reported previously for blackcurrant oil. The study showed the potential for developing blackcurrant genotypes with 'added value', particularly as blackcurrant seed is a by-product from juice production. GLA is a nutritionally important fatty acid present in the seed oils of a restricted number of plant taxa and is beneficial for a range of conditions including atopic eczema and rheumatoid arthritis.

Rape plants in which β-ketoacyl-acyl carrier protein reductase (a component of fatty acid synthase) is down-regulated, using an antisense approach, have reduced seed and leaf fatty acid contents. The effects on complex leaf membrane lipids were examined in two mutants. Compared to the wild-type, there were significant differences particularly in one mutant, but the nature of the differences depended on leaf maturity. In young leaves, the concentrations of all lipids were reduced. In mature leaves, the concentrations of most lipids were reduced but phosphatidylglycerol was unchanged whereas phosphatidylethanolamine increased. The fatty acid profiles of individual lipids were mainly similar to the wild-type, particularly in mature leaves. However in young leaves, linoleic acid was reduced in all lipid classes and trans-3-hexadecenoate was substantially higher in plastidic phosphatidylglycerol.

## Cell Biology

**Plasmodesmata and virus movement** The structure/function relationships of plasmodesmata continue to form the research focus of the Unit of Cell Biology. Previously, it was shown that plasmodesmata in developing leaf tissues change from simple to branched forms during the sink-source transition, a conversion associated with a massive down-regulation of plasmodesmatal conductance. The basic differences in plasmodesmata continue to be studied to establish the 'division of labour' that occurs among plasmodesmatal types. Techniques have been developed, based on confocal laser scanning microscopy, that determine accurately the size-exclusion limits of plasmodesmata connecting different tissues within the plant. Studies show that plasmodesmata differ in their permeabilities depending on both physiological and developmental cues. An almost universal feature of plant virus movement is the modification of plasmodesmata to allow passage of the viral genome between cells. Two significant features of the movement process are plasmodesmal 'gating' (transitory increase in the plasmodesmal size exclusion limit) and RNA trafficking (passage of the viral RNA as a ribonucleoprotein complex through the plasmodesmal pore). The behaviour of plasmodesmata during virus infection is being studied by reverse genetics and by incorporating Movement Protein-Green Fluorescent Protein (MP-GFP) fusions into viral genomes to study the subcellular behaviour of MPs *in situ*. Biolistic approaches have helped in these studies by allowing the MP-fusions to be introduced into cells in the absence of other viral gene products. Recently, the gene for a red fluorescent protein (DsRed) was cloned from the non-bioluminescent coral, *Discosoma*, increasing the fluorescent 'palette' of proteins available to cell biologists (see below).

**Biosource collaboration** The major collaborative venture with Biosource Genetics Corporation (now Large Scale Biology) is entering its third year. The major thrust of this programme is to improve the stability and movement of plant viral vectors for novel protein production in plants. As part of the joint venture, the role of MPs in the viral movement process is being studied using a unique combination of molecular virology, cell biology and non-invasive imaging techniques. One aim has been to produce robust viral vectors, based on *Tobacco mosaic virus* (TMV). Enhanced cell-to-cell movement and stability have been specific targets for vector improvement. Recently, the technique of DNA (gene) shuffling, a PCR-based method for *in vitro* recombination of either single randomly mutated genes or gene families, has been used to evolve enzymes and proteins with novel specificities or enhanced activities. As part of the Biosource collaboration, the MP gene of TMV was shuffled to compensate for losses in activity incurred by foreign gene expression. A full report on this novel approach to viral vector improvement can be found on p. 103.

To study the subcellular basis of improved viral vector movement, fluorescent proteins were fused to wild-

type (*wt)* MP and to the improved shuffled (*shuff)* MP. A direct comparison of the *wt*- and *shuff* MPs was made possible by fusing the MPs to alternative fluorescent proteins (DsRed *versus* GFP) for subcellular localisation. A more detailed report can be found on p. 107. Studies of the behaviour of viral MPs also provide important clues as to the endogenous mechanisms of protein trafficking between plant cells. A popular model for macromolecular trafficking (including that of viral MPs) invokes the microtubule cytoskeleton as an intracellular scaffold along which protein-RNA complexes are transported from their sites of synthesis to plasmodesmata. Because the *shuff*MP was able to enhance TMV movement without associating with microtubules, it was decided to examine the movement of a fluorescently tagged TMV through cells treated with the microtubule-depolymerising agent, colchicine. In these experiments, transgenic plants expressing a GFP-tubulin fusion were used to determine colchicine concentrations at which microtubules were completely depolymerised, without affecting other cell functions. TMV moved unimpeded through the colchicine-treated cells demonstrating that microtubules are not required in the viral movement process. These critical observations force a re-evaluation of current models of macromolecular transport in plants, and the Cell Biology/Biosource Groups are currently developing alternative models to explain the targeted movement of macromolecules through plasmodesmata.

# Antioxidants in soft fruit

D. Stewart, N. Deighton & H.V. Davies

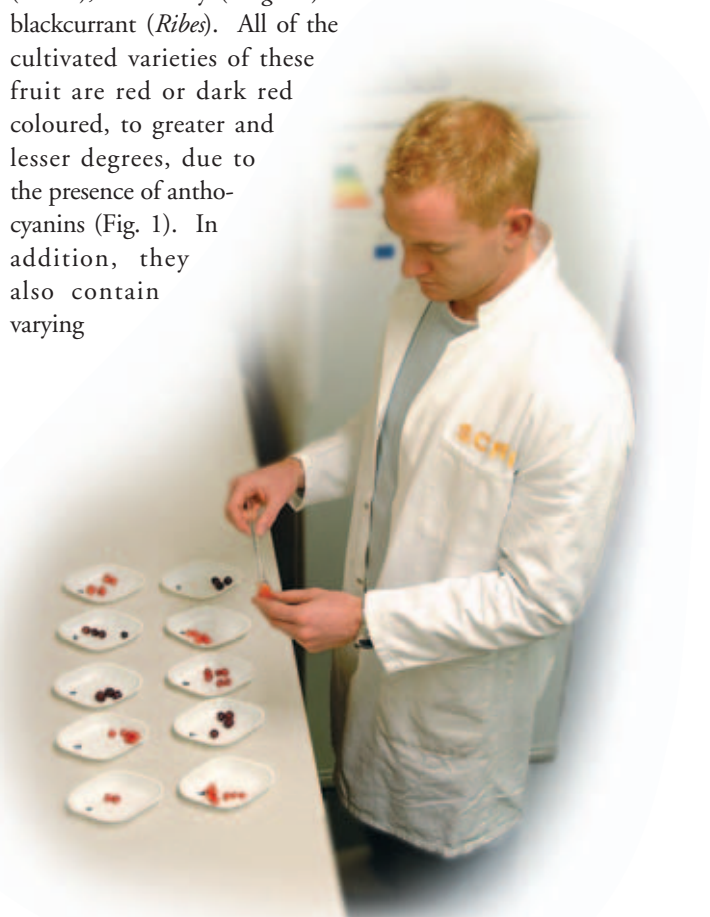
## Introduction

Now that the ability to produce sufficient quantities of food has largely been met in the developed world, the emphasis in agriculture and its associated research has shifted towards issues such as reduced production costs and more perceptible benefit to consumers through increased product quality and nutritive value. Within this latter area, a diverse group of plant compounds called 'antioxidants' has been the focus of intense research and associated economic and consumer interest. This is because it has been shown that oxidative stress, caused by an imbalance of pro-oxidants and antioxidants in the body, significantly increases the incidence of many degenerative and age-related diseases such as coronary heart disease (CHD), in particular atherosclerosis[1], and many cancers including those of the mouth, stomach and colon[2]. In addition, there is increasing evidence that changing one's diet to increase the intake of food relatively high in selected natural antioxidants, such as plant polyphenols, vitamin C, *etc.* (Fig. 1), can reduce the incidence of such diseases. Indeed, such a preventative, government-sponsored study[3], was initiated in 1972 in North Karelia, Finland, a region in which the diet was very similar to that which currently prevails in Scotland. In essence, the study aimed to reduce the consumption of saturated fat and double the fruit intake *per capita*. The researchers found that, over a period of 20 years, mortality rates caused by CHD and stroke were reduced by more than 50% in men and women. Those caused by cancer were reduced by 35% in men and 11% in women[3].

The underpinning mechanisms by which plant antioxidants exert their influences is best approached by bringing together the skills and knowledge of clinicians and nutritionists, with those of natural plant product chemists and plant breeders.

## Soluble antioxidants

**Antioxidant capacity** Scottish soft fruit production is confined predominantly to genotypes of raspberry (*Rubus*), strawberry (*Fragaria*) and blackcurrant (*Ribes*). All of the cultivated varieties of these fruit are red or dark red coloured, to greater and lesser degrees, due to the presence of anthocyanins (Fig. 1). In addition, they also contain varying



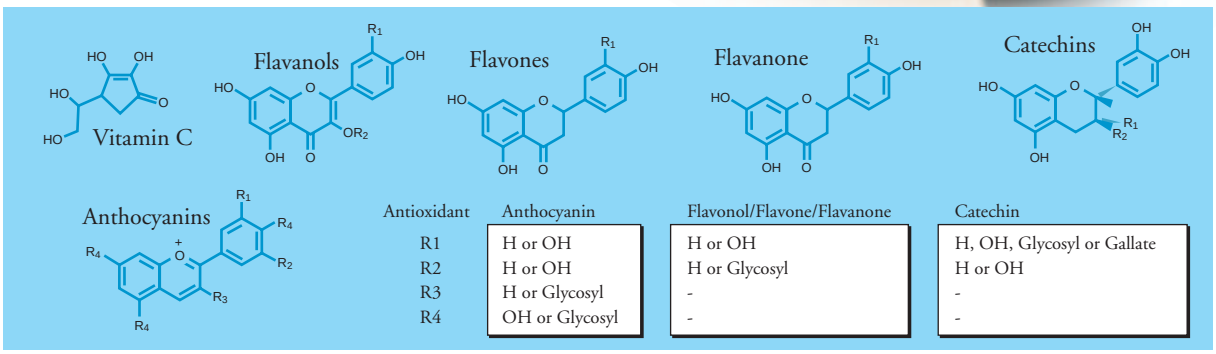



**Figure 1** The general chemical structures of vitamin C, anthocyanins, catechins, flavonols, flavone and flavanones.

amounts of catechins and flavonoids, compounds with structures similar to anthocyanins (Fig. 1). The intake of such compounds has previously been linked to reduced susceptibility to CHD[4]. At SCRI, extracts have been prepared from over 100 varieties of soft fruit and these have been analysed for their antioxidant capacity (ability to exert an antioxidant effect) using two independent *in vitro* methods (Trolox Equivalent Antioxidant Capacity (TEAC) and Ferric Reducing Ability of Plasma (FRAP)). Furthermore, their composition with regard to established antioxidants (phenols, anthocyanins, vitamin C, *etc.*) has been determined.

Overall, blackcurrant extracts have the greatest antioxidant activity, followed by raspberries, with strawberries marginally lower (Fig. 2). In general, and within species, varietal variation in antioxidant potential is minimal in strawberry but greater in raspberry. For example, a wild raspberry species (*R. caucasicus*, ex Russia) displayed an antioxidant potential almost double that of any of the cultivated varieties. Blackcurrant showed the greatest variability with regard to antioxidant potential. Some of the wild species, such as *Ribes sanguinium,* exhibit antioxidant levels more than double that found in the cultivated varieties.

As part of a collaborative EU project termed 'NORTHBERRY'[5], the SCRI fruit antioxidant study has been widened to include a selection of soft fruit species native to, or popular in, Scandinavia. These included rowan (*Sorbus aucuparia*), sea buckthorn

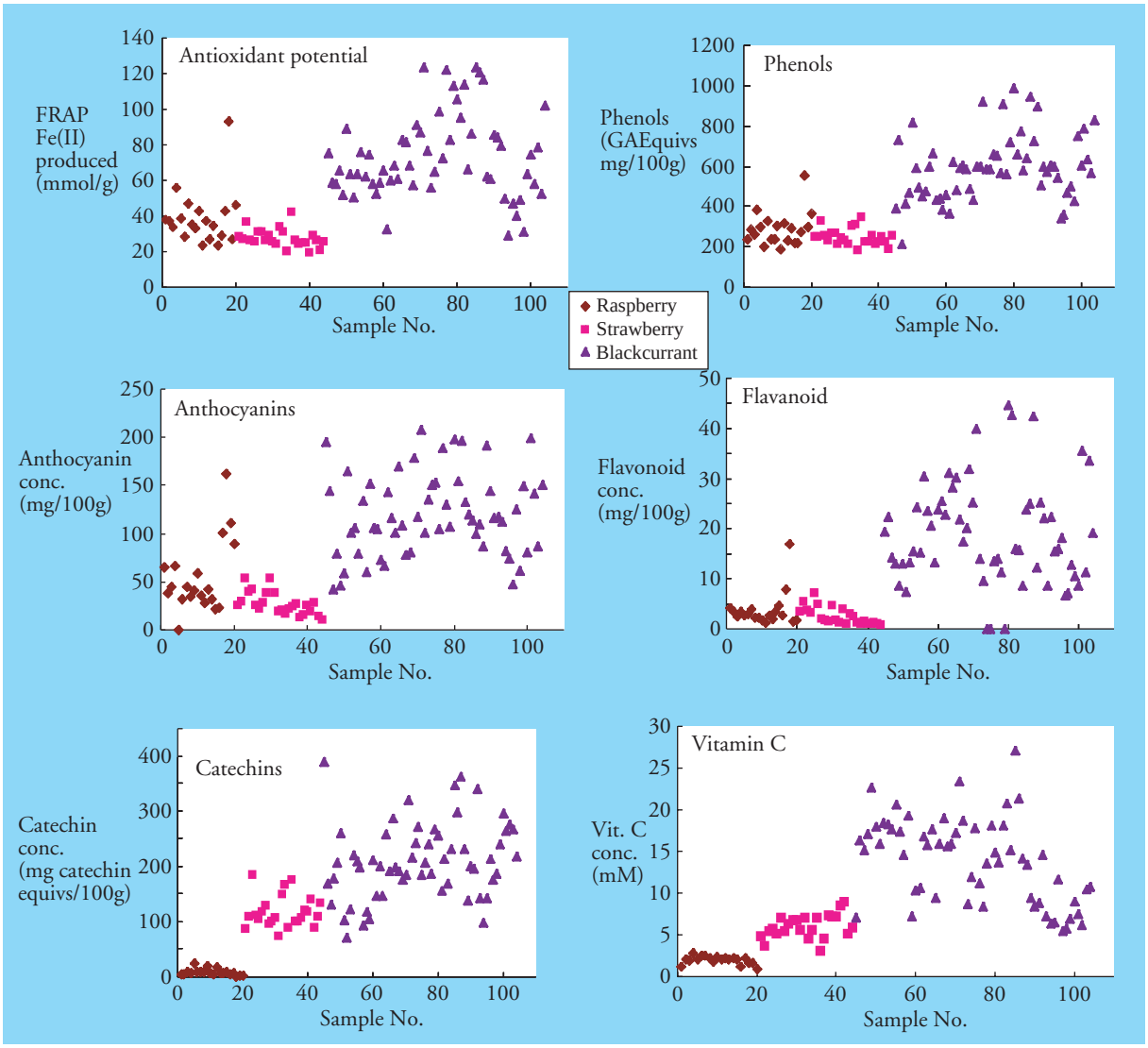



**Figure 2** Species and varietal variation of antioxidant activity (FRAP), and anthocyanin, catechin, phenol, flavonoid and vitamin C contents of strawberry, raspberry and blackcurrant extracts.

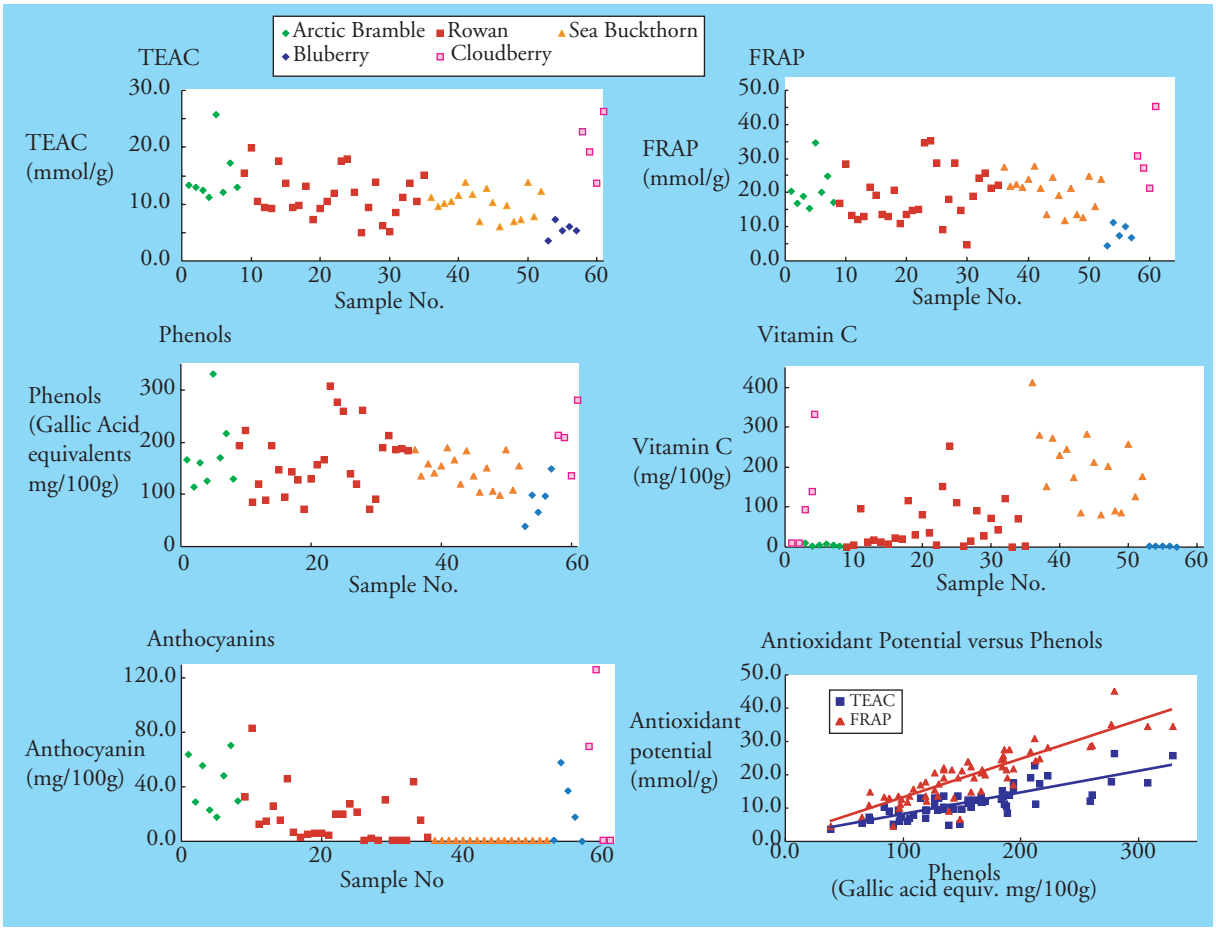



**Figure 3** Species and varietal variation of antioxidant activity (TEAC and FRAP), and anthocyanin, phenol and vitamin C contents of extracts from fruit derived from the NORTHBERRY project.

(*Hippophae rhamnoides*), blueberry (*Vaccinium spp)*, arctic bramble (*Rubus articus*) and cloudberry (*Rubus chamaemorus).* This study has also allowed different Rubus species to be compared e.g. *R. idaeus* (raspberry), with *R. articus, R. chamaemorus and R. caucasicus.*

Significantly, blueberry, one of the current favourite sources of fruit antioxidants in the USA, proved, by FRAP assay at least, to be a less effective source of antioxidants than blackcurrants (Figs 2 & 3). Overall, blackcurrant displayed a three-fold greater antioxidant potential than blueberry.

Great care must be taken when comparing the antioxidant potentials within and between species, since the relative rankings can change depending on the method used to measure such potential. For example, the FRAP assay indicates that the antioxidant potential of raspberry (*Rubus idaeus*) is greater than that of arctic bamble (*R. articus*) and cloudberry (*R. chamaemorus*). However, using the TEAC assay, cloudberry has the greatest overall antioxidant potential. This highlights the fact that methods used to assess antioxidant capacity measure, or are sensitive to, different chemical moieties and their relative concentrations within plant extracts. Indeed, the variable rankings of antioxidant potential between raspberry (*R. idaeus*), arctic bamble (*R. articus*) and cloudberry (*R. chamaemorus*) with the use of a different method of measurement, may well reflect the different chemistries of the Rubus species. Both raspberry and arctic bramble have low levels of vitamin C, whilst cloudberry generally contains higher levels. The anthocyanin and total phenol contents also exhibit a range of values. Phenol content is notably higher in raspberry than in either cloudberry or arctic bramble. When the values for total phenols are compared against antioxidant capacities, be they derived from TEAC, FRAP or even spectroscopy-based (EPR) methods, a good relationship exists between the two parameters (Fig. 3). Total phenol content can be measured relatively easily and could form the basis of a screen for soluble antioxidant capacity in fruit.

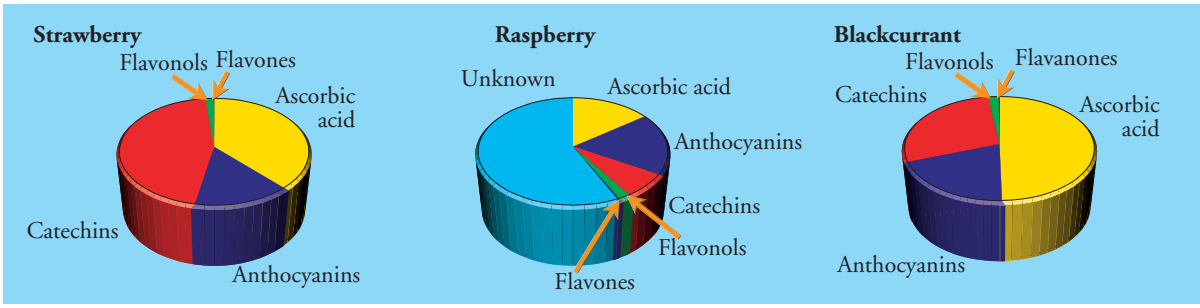



**Figure 4** The relative contributions of components to the total antioxidant potential of strawberry, raspberry and blackcurrant extracts.

Composition of soluble antioxidant capacity in soft fruit The contribution made to total soluble antioxidant potential by vitamin C content is variable. For the various fruit(s) studied, the values are; blackcurrant 50%, strawberry 40% and raspberry 15%. Mass spectrometry indicates that the soft fruit analysed contained many polyphenolic compounds (Fig. 1). Anthocyanins accounted for about 20% of the antioxidant capacity of raspberry, blackcurrant and strawberry, whereas the contribution from flavonoids was minimal (<5%; Fig. 4). The contribution from catechins (Fig. 1) was least in raspberry (5-10%), greater in blackcurrants (25-20%) and greatest in strawberries (40-50% of the total antioxidant potential). Perhaps the most interesting feature is that more than half the soluble antioxidant capacity of raspberry remains unaccounted for. The direct relationship between total phenols and soluble antioxidant capacity suggest that the unknown fraction is probably phenolic and most likely glycosylated phenolics. Clarification of this is in progress.

Cell wall-associated antioxidants

The soft fruit studied are clearly excellent sources of antioxidants. However, virtually all published studies on plant antioxidants have confined themselves to soluble compounds and juices. Given that whole fruit is commonly eaten and that phenolics are ubiquitous in plant cell walls, it is possible that a significant proportion of the absolute antioxidant capacity of fruit is unaccounted for. Preliminary experiments have been undertaken to determine the presence of antioxidants associated with, or covalently bound to, the cell wall and the contribution they make to the total antioxidant capacity of the fruit.

Non-covalently bound cell antioxidants Anthocyanins were optimally extracted with medium polarity solvent (methanol and ethanol; Table 1). Extraction of phenols was directly related to solvent polarity with 2-3 times more phenols being extracted by water than by acetone. This suggests that the phenols are present as glycosides. Natural variation was evident, with levels of phenols greater in the blackcurrant cultivar Ben Alder than in cv. Ben Lomond. The importance of the non-covalently bound anthocyanins, and hence the importance of whole fruit consumption, can be demonstrated by comparing the relative proportions of soluble antioxidants in the juice and that present in whole fresh fruit. By assuming that the cell wall (pulp) accounts for ~30% of the fresh fruit weight (R. Brennan, pers. comm.) and that the remainder is juice with a specific gravity of 1.2, then 100 g fresh weight of blackcurrant fruit provides

| Variety: | Ben Alder | | | | | Ben Lomond | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Extraction solvent: | Water | Methanol | Ethanol | Acetone | Juice* | Water | Methanol | Ethanol | Acetone | Juice* |
| Total Anthocyanins (mg $100g^{-1}$)* | 0.0 | 1624.0 | 1330.0 | 93.0 | 608.6 | 15.0 | 733.0 | 610.0 | 117.0 | 539.9 |
| Total phenols (mg $100g^{-1}$)* | 2224.0 | 1906.0 | 919.0 | 924.0 | 673.8 | 1327.0 | 1051.0 | 452.0 | 572.0 | 533.9 |
| Ascorbic acid (mg $100g^{-1}$)* | nd | nd | nd | nd | 63.9 | nd | nd | nd | nd | 55.5 |
| Ascorbic acid (millimolar) | nd | nd | nd | nd | 3.6 | nd | nd | nd | nd | 3.1 |
| TEAC (mmol $g^{-1}$)* | 115.1 | 122.2 | 80.1 | 64.3 | 66.4 | 64.8 | 59.8 | 32.9 | 24.4 | 61.1 |
| FRAP (mmol $g^{-1}$)* | 203.1 | 212.0 | 140.5 | 107.3 | 118.6 | 113.6 | 101.4 | 56.1 | 43.1 | 110.8 |
| % of TEAC contributed by ascorbic acid | nd | nd | nd | nd | 5.4 | nd | nd | nd | nd | 5.1 |

* The values for the corresponding juices are expressed as (mg $100^{-1}$) nd - not determined

**Table 1** Composition and antioxidant capacities of solvent extracts of blackcurrant cell walls.

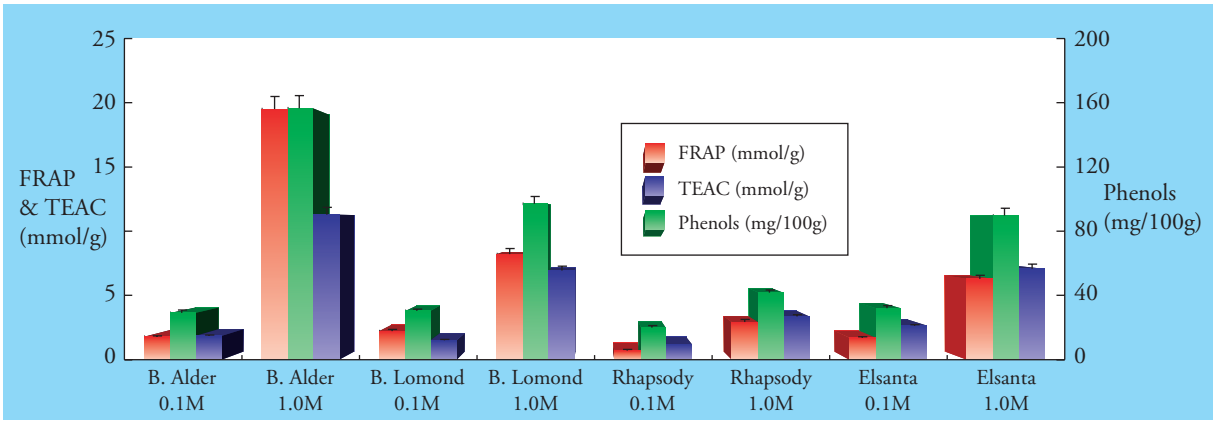



**Figure 5** The antioxidant ability (TEAC and FRAP) and phenol contents for sodium hydroxide extracts of cell wall preparations of two blackcurrant and strawberry varieties.

355 mg anthocyanin in the juice and 487 mg anthocyanin associated with the cell wall!

Covalently bound cell wall antioxidants The levels of covalent, ester-bound, alkali-extractable phenols and TEAC and FRAP activities were uniformly less in 0.1 M than in 1.0 M NaOH extracts of both blackcurrant and strawberry varieties, although the distinction is less significant for strawberry (Fig. 5).

Although the levels of alkali-extractable phenols are only 10% of those extracted by water alone (Table 1), they follow a similar pattern to the non-covalently bound (methanol extractable) phenols and are greatest in the Ben Alder extracts.

Our studies indicate that soft fruit are undervalued as a source of natural antioxidants. Our research has shown that previous studies on fruit (and possibly vegetable) antioxidant capacity may have significantly underestimated the absolute antioxidant potential, since significant antioxidant activity remains associated and/or covalently bound to the cell wall. Also, given that covalently bound molecules contribute to a proportion of the overall antioxidant capacity, enzymes and metabolic pathways must exist which are responsible for their occurrence. These processes are targets for further study with a view to increasing the proportion of the covalently bounds antioxidants in fruit.

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# Meristem activation in potato: impact on tuber formation, development and dormancy

R. Viola, M.A. Taylor & K.J. Oparka

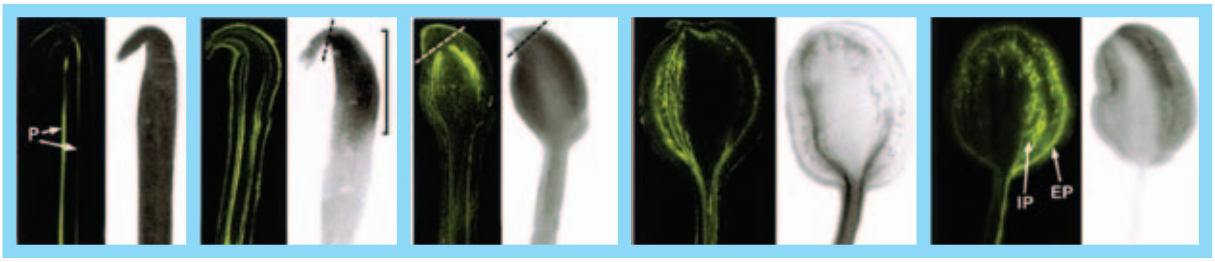


Figure 1 Unloading of CF (colour plates) and $^{14}C$ (autoradiographs) in potato stolons and tubers following treatment of leaves with CFDA or $^{14}CO_2$.

**The life cycle of the potato tuber** The life cycle of the potato tuber includes organogenesis, tuber development, dormancy and sprouting. This developmental sequence requires the co-ordinated control of a complex set of interlocked physiological processes and metabolic pathways and impacts directly on many qualitative and quantitative traits of the potato crop. Meristem activity plays a key role in this process. In elongating stolons and growing sprouts, the apical meristem acts as a sink for nutrients, which are sourced from the photosynthetic apparatus and tuber storage parenchyma respectively. Inactivation of the apical meristem, as a result of tuber induction, impacts greatly on the physiology of the entire plant, as the developing tubers subsequently become the largest sinks present. Increased cell division and expansion in the developing tuber is followed rapidly by a massive deposition of starch and storage protein. Following tuber maturation and during the rest phase, several tuber tissues undergo a functional and metabolic sink-source transition. The completion of these transitions coincides with the re-activation of meristem functionality at dormancy break.

**Meristem activity and tuber formation** We have adopted a multidisciplinary approach to investigate developmental changes occurring in the tuber (cv. Désiree) during its life cycle, focusing on the apical meristem[1]. Firstly we examined the physiological and metabolic changes accompanying tuber induction. Carboxyfluoroscein diacetate (CFDA) or $^{14}CO_2$ were applied to mature source leaves of potato plants to image changes in phloem unloading to the various regions during the tuber induction phase. Confocal images (colour plates) and autoradiographs (B&W plates) of stolons and tubers show unloading of CF and $^{14}C$ respectively (Fig. 1). In non-swelling stolon tips, the CF signal is restricted to the phloem (P) in both the stolon axis and hook regions, whilst radioactive assimilates appear distributed evenly across the stolon axis. Following tuber initiation, substantial unloading of CF and $^{14}C$ occurs from both internal (IP) and external (EP) phloem networks, suggesting the activation of cell-to-cell (symplastic) phloem unloading. In elongating stolons, substantial unloading of nutrients is observed in the apical region. However, irrespective of the developmental stage of swelling tubers, CF or $^{14}C$ were not found at the apex of the tuber within the dormant apical bud. Closer examination of the apical buds in growing tubers showed that although phloem connections were present immediately below, and leading into, the apical bud, no unloading of CF occurred in this region (Fig. 2).

These findings indicate that tuber formation is characterised by a switch from apoplastic to symplastic

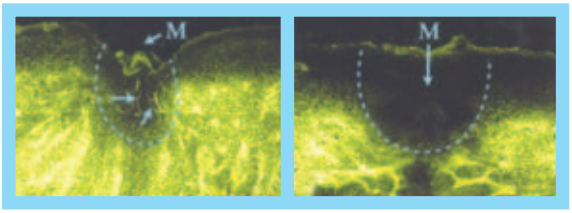


Figure 2 Close-up of CF unloading in the apical part of developing tubers. In the region containing the apical meristem (M), dye is apparent in the phloem (arrows) but does not unload. Abundant unloading is observed in the subtending swelling region.

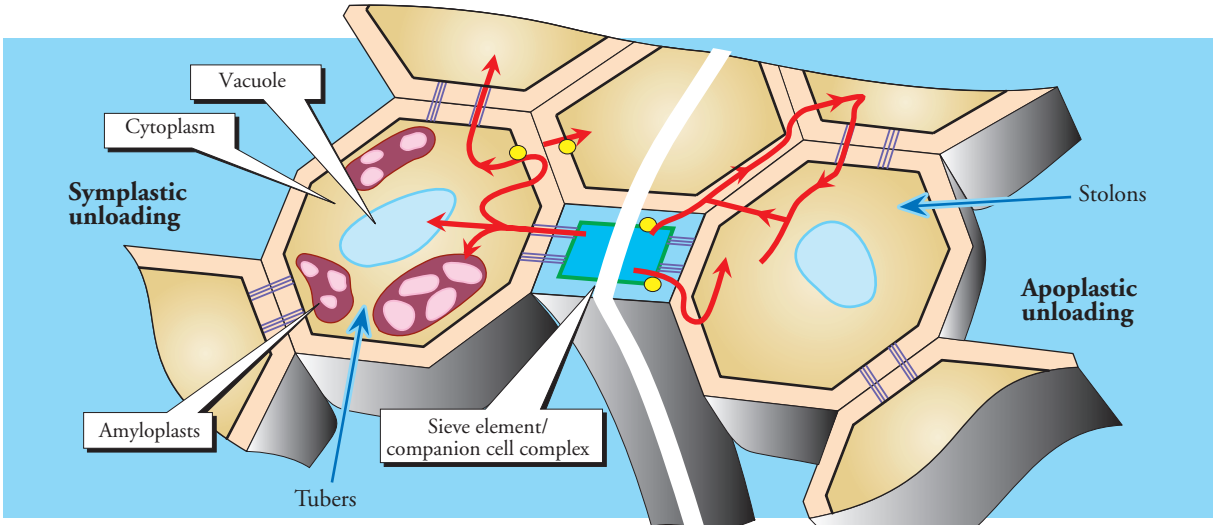


**Figure 3** Schematic representation of phloem unloading in stolons and tubers.

unloading of assimilates in the subapical stolon region (Fig. 3). In elongating stolons, sucrose and other nutrients are unloaded from the phloem transport system into the apoplast. In the subapical region, sucrose is retrieved by parenchyma cells via sucrose transporters. In swelling stolons, this step is bypassed as a result of the establishment of symplasmic communication between the phloem system and the parenchyma cells, resulting in enhanced sink potential within stolon tips (Fig. 4) and a marked increase in the compartmentalisation of unloaded sucrose (Fig. 5). The latter may be directly responsible for the up-regulation of gene expression (starch and protein deposition) observed during tuber development. The induction of symplastic phloem unloading in tuberizing stolons appears spatially confined to the subapical stolon region and the apical, meristematic region becomes symplastically isolated from the rest of the tuber. Here, sucrose hydrolysis may occur in the free space before retrieval of hexoses, as suggested by the localization of cell wall

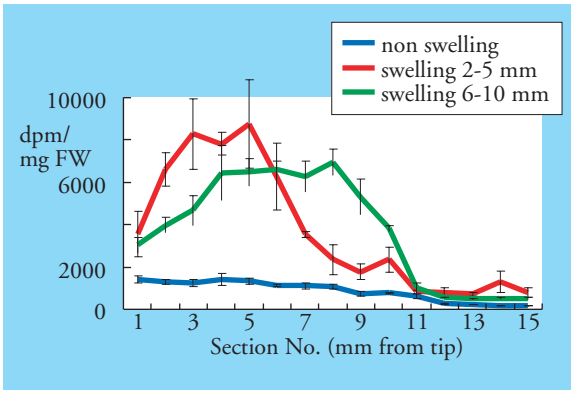


**Figure 4** Concentration of radioactivity in 1-mm sections excised along the axis of non-swelling or tuberising stolon tips. Data collected 4h after labelling of foliage with $^{14}CO_2$.

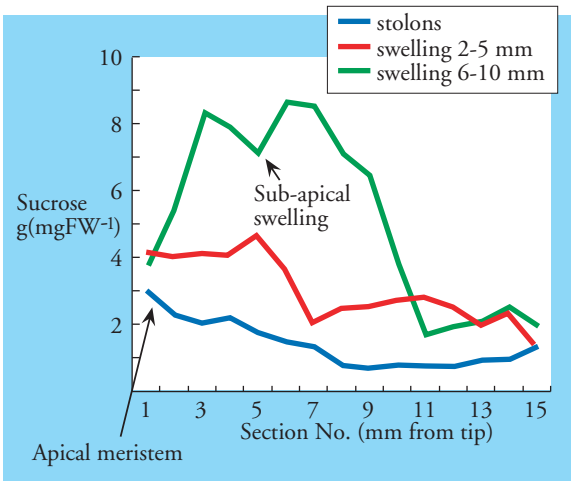


**Figure 5** Distribution of sucrose along the axis of non-swelling and tuberising stolon tips.

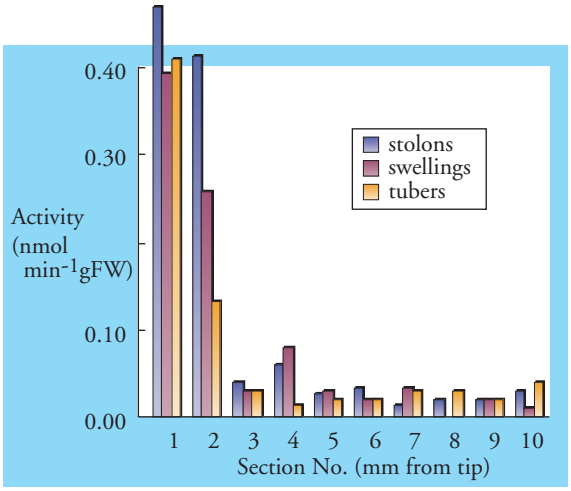


**Figure 6** Distribution of cell wall-bound acid invertase activity along the axis of stolon and tuberising tips.

invertase activity (Fig. 6). This hypothesis is also supported by the highly localized expression from the *invGE* promoter in the apical region of stolons and tubers (Fig. 7). The symplastic isolation of the apical meristem from the rest of the tuber coincides with the cessation of cell division in the apical meristem and the induction of dormancy.

Figure 7 Expression of an apoplastic invertase (*invGE*) in stolons and tubers revealed by GUS staining.

**Meristem activity in mature tubers** Following maturation of potato tubers, there is an obligate period of rest, e.g. lack of bud growth. It is not known whether the repression of bud growth in this phase is controlled by factors within the buds themselves or by factors within the tuber. In preliminary experiments, we observed that dormant tubers appear to have the competence to produce substrates for bud growth (e.g. sucrose) and to transfer these substrates from the storage parenchyma into the transport system. We also found evidence that the apical bud of dormant tubers is metabolically competent to metabolise exogenously labelled substrates (not shown). Compositional analyses of apical buds during storage of mature tubers revealed a marked increase in carbohydrates (Fig. 8) in advance of visible bud growth. The pattern of CF

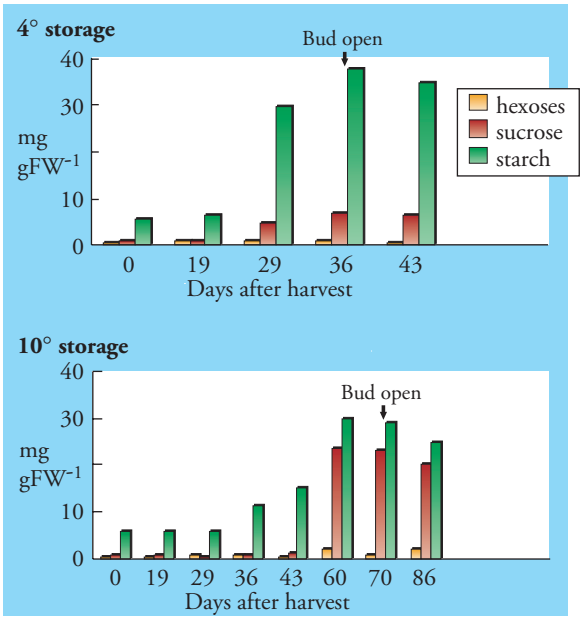


**Figure 8** Change in carbohydrate content of apical buds during tuber storage.

unloading in the bud at various developmental stages (Fig. 9) shows that dormancy release is correlated with the activation of phloem unloading. Thus it appears

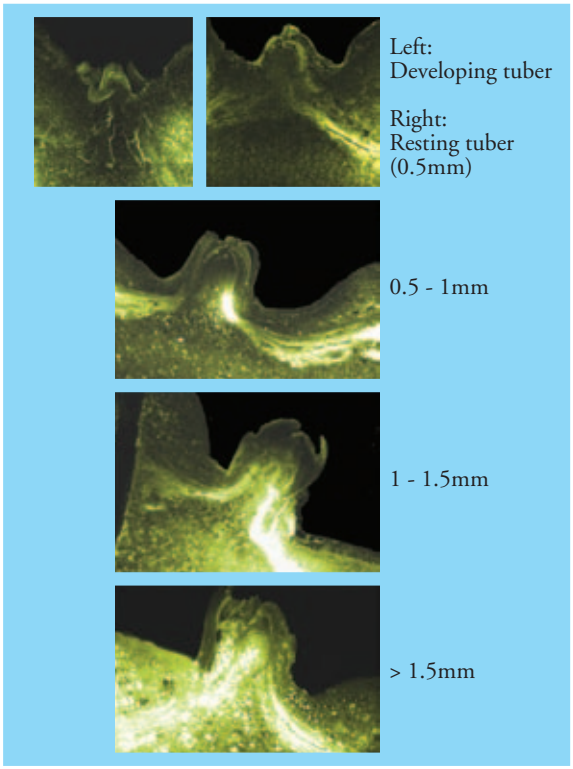


Figure 9 Unloading of CF in the apical buds at different developmental stages after application of CFDA through a well at the stolon-end of the tuber.

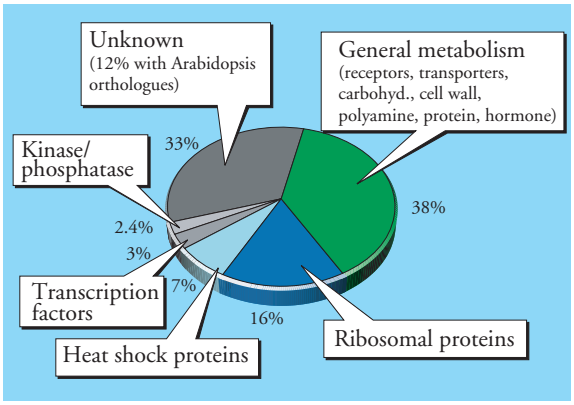


**Figure 10** Genes up-regulated in the very early stages of dormancy release isolated by subtractive hybridisation methodology.

that the re-activation of metabolic processes and of mitotic activity occurring in the apical meristem upon release from dormancy is a direct result of activation of phloem unloading and ensuing carbohydrates accumulation in the tissue.

The large carbohydrate accumulation observed in apical tuber buds prior to any visible signs of bud growth, has proved a useful tool to identify buds in the very early stages of dormancy release. Using a suppression subtractive hybridisation approach, a library of cDNA clones, enriched for genes up-regulated at the very early stages of dormancy release, has been produced. Approximately 400 different cDNA sequences (ESTs) have been obtained (Fig. 10). *In situ* hybridisation methodology has confirmed tissue-specific and developmental stage-specific expression of some of the genes isolated via the SSH approach (Fig. 11). Further functional characterisation of these genes through DNA arrays and transgenic approaches is in progress.

**Conclusion** We have established the crucial role of meristem activity in the life cycle of the potato tuber. Inactivation of the apical meristem is accompanied by symplastic isolation during tuberisation of stolons and this status is maintained throughout tuber development, maturation and rest. The resumption of cell-to-cell communication between the apical bud and the rest of the tuber coincides with dormancy release and results in carbohydrate accumulation in the bud. Specific events at the interface between the meristem

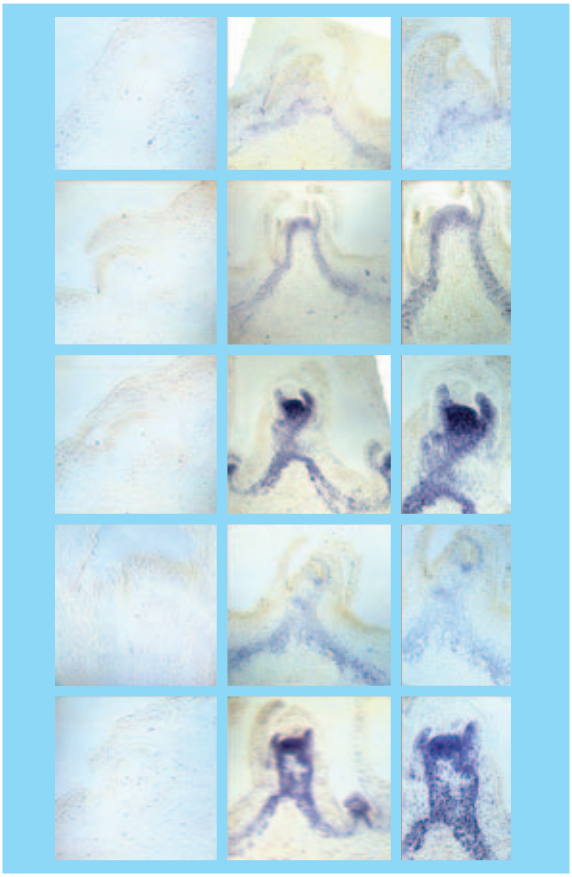

Figure 11 Tissue-specific expression patterns of selected genes isolated by the SSH approach.

and the plant transport system have been identified as control points for meristem activation and deactivation. In addition, biochemical markers and genes specifically expressed at these stages have been discovered making possible, for the first time, the dissection of the mechanisms responsible for meristem activity at the cellular and molecular level. These mechanisms may well be widespread and form the basis for improved understanding of phenomena such as bud dormancy and apical dominance in plants.

Acknowledgements

Many other colleagues contributed to this work, in particular Alison Roberts and Sophie Haupt (Unit of Cell Biology) and Rob Hancock (Unit of Plant Biochemistry). Their input has been greatly appreciated. Support for this work has been provided by EU (Framework IV) and by SEERAD (SCR/550/00).

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# Gene shuffling improves the function of *Tobacco mosaic virus* movement protein

R.L. Toth, S. Chapman & G. Pogue

In a major collaborative venture between SCRI and Large Scale Biology Corporation (LSBC), a Californian-based biotechnology company, we are exploring the use of viral vectors for the expression of foreign proteins in plants. Vectors based on plant viruses offer an alternative to stable transformation for the production of biopharmaceuticals in plants and much research has focused on their use for vaccine production, through the expression either of short pathogen-derived peptides as fusions to viral coat proteins or of complete proteins[1]. The speed and ease with which high levels of foreign gene expression can be achieved has also led to their extensive use as research tools for virological and cell biological studies, and for *in planta* analysis of protein functions[2]. Virus expression vectors based on the *Tobacco mosaic virus* (TMV) genome have proved to be powerful tools for expression of pharmaceutically-relevant proteins and have speeded plant functional genomics through either over-expression of plant genes or gene silencing. Despite the utility of the present vector systems, the inclusion of additional genetic load, in the form of foreign genes, reduces the replication and movement efficiencies of the vectors when compared with their wild-type counterparts and these reductions can negatively effect host range and expression levels. Previous improvements in levels of foreign protein expression, genetic stability and infection phenotypes of TMV-based vectors have been made through the inclusion of extra promoter elements to drive foreign gene expression and the creation of hybrid tobamovirus genomes[2]. These approaches have suffered from their reliance on the available genetic diversity of virus isolates and did not seek to evolve optimal viral components to compensate for deficiencies present in the expression vectors.


We are using various techniques to improve TMV as a vector for expression of useful foreign proteins. TMV has a positive-sense, single-stranded, RNA genome of 6396 nucleotides and encodes four protein products. One of these, a 30kDa protein (30K or movement protein), is essential for cell to cell movement of the virus. It has been shown to associate with plasmodesmata, intercellular cytoplasmic channels, to bind single-stranded nucleic acids *in vitro* and to interact with plant cytoskeletal elements. Further to its coding function, the 30K gene contains an RNA structure, from which assembly of viral RNA and coat protein into virus particles is initiated, and a subgenomic promoter sequence that in the natural virus directs the synthesis of the mRNA encoding the coat protein. The multifunctional nature of this viral gene, and its requirement for viral movement, makes it an ideal target for improvement through mutagenesis and DNA shuffling.

The technique of DNA shuffling[3] is a PCR-based method for fragmentation and reassembly of mutated copies of a gene of interest to produce a population of recombined gene sequences. This *in vitro* method mimics, in an accelerated fashion, the natural evolutionary process of genetic recombination. Many of the shuffled gene sequences produced will be nonfunctional or debilitated in function, but a proportion will have an improved function, which can be identified using an appropriate screen. The technique has proved to be extremely successful in evolving enzymes with novel specificities or enhanced activities and more recently in altering the cell tropisms and processing characteristics of retroviruses[4-6]. We have performed DNA shuffling to evolve the 30K gene and its product to compensate for losses in activity

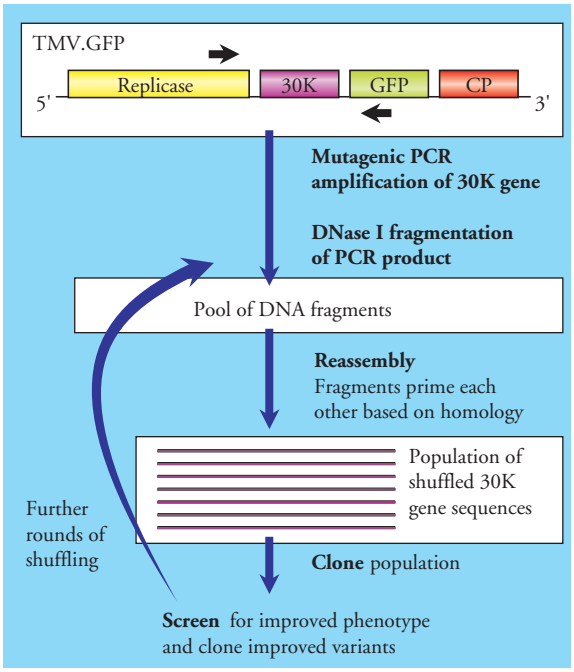


**Figure 1** Shuffling of the TMV 30K gene.

incurred by foreign gene insertion. A previously described TMV-based vector that carries the gene for green fluorescent protein (TMV.GFP, Fig. 1) was used to permit an easy visual screen for improvements in cell to cell movement.

## Shuffling of the movement protein gene

A portion of the TMV genome encompassing the subgenomic promoter for the 30K gene and the 30K gene itself was subjected to DNA shuffling (Fig. 1). The population of shuffled fragments was cloned into TMV.GFP and inoculated to leaves of the highly susceptible experimental host *Nicotiana benthamiana* as reassembled virus particles. The shuffled population produced about 20% of the number of fluorescent infection foci produced by unshuffled virus and the size of the infection foci, generally smaller, was more disparate for the population than the control, indicating that the majority of the population was disfunctional or disabled (Fig. 2A). Visual screening of approximately 5000 fluorescent infection foci identified 70 lesions that appeared larger than the control lesions, indicating faster cell to cell movement. To confirm the improved phenotypes, homogenates prepared from the variant lesions were inoculated to half-leaves of the more restrictive host *N. tabacum* cv. Xanthi nn with controls on the opposing half-leaf (Fig. 2B). A total of 53 variants showed faster cell to cell movement and the shuffled portions of their genomes were cloned back into the progenitor plasmid through RT-PCR.

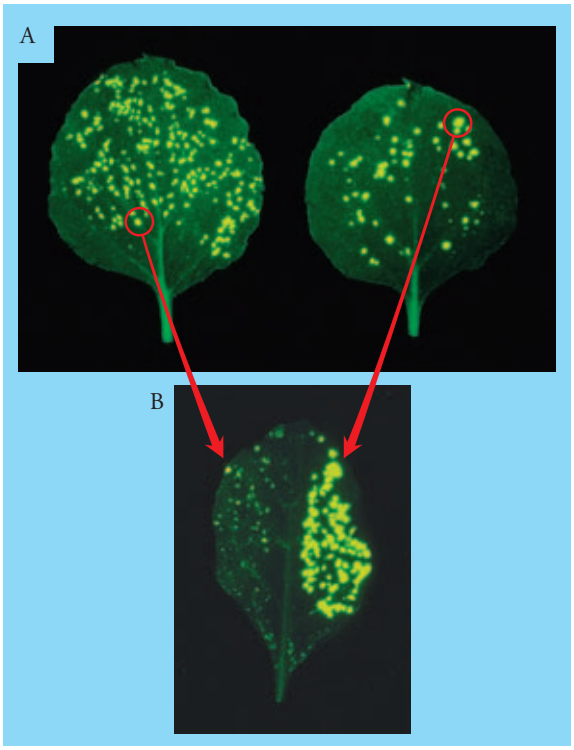


**Figure 2** Screening shuffled populations for improved movement phenotype. A; *N. benthamiana* leaves inoculated with unshuffled control virus (on the left) or 30K shuffled population (on the right). B; Individual lesions passaged to *N. tabacum* cv. Xanthi. Unshuffled control on left side of leaf; improved variant on right side of leaf.

Clones produced from the 53 variants were used in a second round of shuffling to generate recombinants with new patterns of mutations and phenotypes. The population obtained was screened on *N. benthamiana* and *N. tabacum* cv. Xanthi nn as before. Variants showing faster cell to cell movement than the best clone from the first round were selected and 37 clones obtained. These clones were input into a third round of shuffling in the same way. The population produced was screened on the restrictive production host *N. tabacum* cv. MD609 to facilitate discrimination of differences in cell to cell movement rate. After secondary screening, three clones showing faster cell to cell movement than the best clone from the second round were obtained.

## Shuffled clones are improved for cell to cell and systemic movement

The cell to cell and long-distance movement rates of the progenitor, unshuffled, clone (*unshuff*) and the best clone from each of the three rounds of shuffling (*shuff1*, *shuff2* and *shuff3*) were assessed to evaluate

| Inoculum | Mean area of fluorescent infection foci (mm²) | |
|---|---|---|
| | *N. tabacum* cv. MD609 | *N. benthamiana* |
| *unshuff* | 0.219 | 0.172 |
| *shuff1* | 0.309 | 0.554 |
| *shuff2* | 0.816 | 0.660 |
| *shuff3* | 0.967 | 0.913 |

**Table 1** Fluorescent infection foci area measurement. The areas of fluorescent foci on inoculated leaves of *N. tabacum* cv. MD609 and *N. benthamiana* were measured at 5 dpi or 3 dpi, respectively. Analysis of variance gave values of 0.0599 and 0.0683 for the least significant difference at the 5% level for measurements of foci on *N. tabacum* cv. MD609 and *N. benthamiana*, respectively.

more critically the visually discriminated improvements. Rates of cell to cell movement were investigated through area measurements of multicellular, fluorescent, infection foci produced by the four clones on *N. benthamiana* and *N. tabacum* cv. MD609 (Table 1). Statistical analysis of the data showed that, on both hosts, *shuff1* produced significantly larger lesions than the progenitor construct and that the two clones from subsequent rounds of shuffling produced larger lesions than the best clone from the previous round of shuffling.

To test whether the mutations that improved cell to cell movement also affected the speed at which uninoculated leaves became infected, the time at which systemic fluorescence developed was monitored for the four clones on the two different hosts. All the shuffling progeny produced systemic fluorescence at the same time on the experimental host *N. benthamiana*, one day faster than the progenitor. On the production host *N. tabacum* cv. MD609, there were more marked differences in the timing of systemic GFP expression (Fig. 3). The shuffling progeny produced systemic fluorescence at least 13 days in advance of the progenitor and the clones *shuff2* and *shuff3* produced systemic infections one to two days in advance of *shuff1*. By 20 dpi, when *shuff1* had only systemically infected 2-4 leaves, *shuff2* and *shuff3* had systemically infected 4-5 or 5-6 leaves respectively. Furthermore, a higher proportion of the surfaces of systemically infected leaves displayed green fluorescence in *shuff2* and *shuff3* infections than in *shuff1* infections. Although the 30K protein has been proposed to have a role in systemic movement, it seems more probable that the enhanced long-distance movement is a consequence of the faster cell to cell move-

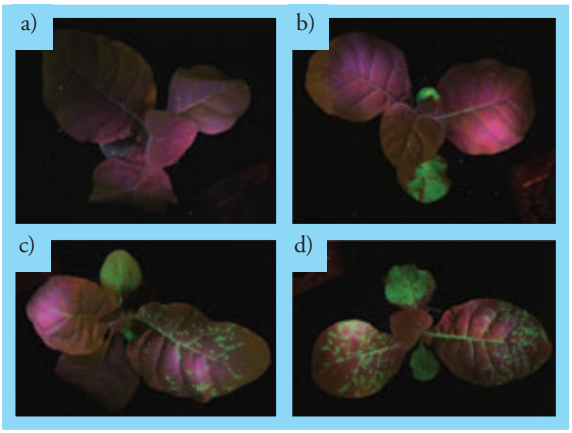


Figure 3 Systemic infections of *N. tabacum* cv. MD609 plants 10 days post-inoculation with *unshuff* (a), *shuff1* (b), *shuff2* (c) and *shuff3* (d). Plants were inoculated with reassembled virus and maintained at 28°C prior to observation of green fluorescence under UV illumination.

ment, possibly allowing the virus to evade host responses more effectively.

Detection of 30K in protein extracts prepared from protoplasts inoculated with transcripts of *shuff1*, *shuff2* and *shuff3*, showed that they accumulated higher levels of 30K protein than *unshuff*, indicating that they contained mutations that stabilised the movement protein. Whether the mutations affect other aspects of 30K protein functionality is the topic of current studies in our laboratory. Whatever the mechanisms, the shuffling process has produced mutations that can compensate for the reduced levels of 30K expression that result from the introduction of a foreign gene. In addition, the mutations not only improve movement on permissive hosts but also broaden the host range to previously restrictive hosts, such as *N. tabacum* cv. MD609.

### Effects of mutations on wild-type virus

Given the high mutation rate reported for TMV and susceptibility of RNA viruses to recombination, it seemed unlikely that if the changes found in the improved vectors could confer a selective advantage to unmodified wild-type virus that they would not have evolved naturally. To test this hypothesis and to address possible environmental concerns of the escape from contained facilities or future field use of such improved vectors, the mutated regions from the *shuff2* and *shuff3* clones and the equivalent region from the progenitor GFP-expressing vector were engineered back into a wild-type TMV cDNA clone. The cell to cell movement rates of virus produced from these clones was assessed by measuring the areas of necrotic

lesions induced on *N. tabacum* cv. Xanthi NN, which has the hypersensitivity resistance gene N. The mean lesion areas at 2 dpi for the *unshuff*, *shuff2* and *shuff3* derivatives were 0.566, 0.581 and 0.573 $mm^2$, respectively. Analysis of variance gave a value of 0.0571 for the least significant difference at the 5% level, indicating no statistical difference in the rates of cell to cell movement. Further, all the viruses produced systemic symptoms on the tobacco cultivar MD609 at 4 dpi and accumulated to similar levels in systemically infected tissue (data not shown). Thus, the 30K mutations found in the improved shuffled clones are only beneficial in a virus vector background, and compensate for losses in movement brought about by the insertion of a foreign gene. The ineffectivenes of the mutated sequences in a wild-type background is in accord with previous experiments that have shown that the level of 30K expression does not limit the rate of cell to cell movement of wild-type TMV infections.

## Conclusions

The purpose of this work was to evolve the TMV 30K gene and its product to compensate for losses in activity induced by foreign gene inclusion. The sequences of plant RNA viruses, such as TMV, do not simply encode proteins, but also have roles in replication, subgenomic mRNA transcription, directing assembly and as translational enhancers. Their base composition may be constrained further by packaging requirements and effects on RNA stability. The successful application of shuffling to improvement of the multifunctional 30K gene encourages us to believe that, with the development of appropriate screens, shuffling other portions of the genome should make improvement of desirable vector traits, such as genetic stability and high levels of foreign gene expression, easier than through rational design. The improved vectors produced in this study will be of utility for *in planta* production of therapeutics due to the increased biomass of infected material and will provide more robust tools for basic plant research.

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# A DNA-shuffled movement protein enhances virus transport by evasion of a host-mediated degradation pathway

T. Gillespie, R. Toth, S. Haupt, P. Boevink, A.G. Roberts, S. Chapman & K.J. Oparka

In the preceding report, we described a viral movement protein (MP) that was improved in its transport functions through the technique of DNA shuffling. *Tobacco mosaic virus* (TMV) vectors expressing the shuffled MP *(shuff3*MP*),* moved from cell to cell at rates that were substantially greater than the same vectors expressing a wild-type MP *(wt*MP*).* We were curious to determine the nature of the improved performance of *shuff3*MP clones, and designed a series of experiments to examine the subcellular basis of improved MP function. This work was carried out in collaboration with Biosource Genetics Corporation as part of an ongoing programme aimed at understanding and improving the functions of plant viral vectors.

## The TMV movement process

TMV is a single-stranded RNA virus capable of local and long-distance movement. Local movement occurs through plasmodesmata, which become transiently enlarged ('gated') by the 30 kDa viral MP to allow the intercellular passage of the viral genome[1]. The TMV movement complex most likely exists in the form of an elongated ribonucleoprotein complex, the size and structure of which is compatible with the gated plasmodesmal pore. In order to reach the next cell, the MP-viral RNA complex must be transported from the subcellular site of replication to, and through, plasmodesmata. This movement is thought to occur on microtubules (MT), as MP shows a conspicuous association with MT during the viral replication cycle[2]. Once the viral genome has passed into new cells, the replication and movement cycles are repeated. Unlike other viral proteins, the MP of TMV is expressed transiently in infected cells and undergoes a cycle of synthesis and degradation at the leading edge of an infection site[3].

To study the subcellular behaviour of the TMV MP, we made translational fusions of the green fluorescent protein (GFP; from *Aequorea)* or the red fluorescent protein (DsRed; from *Discosoma)* to *wt*MP and *shuff3*MP. We then inoculated plants with viral vectors expressing these fluorescent proteins onto plants and studied their subcellular localisation using confocal laser scanning microscopy (CLSM). To aid in the identification of subcellular organelles, we inoculated viral vectors expressing DsRed fusions onto transgenic *Nicotiana benthamiana* plants expressing GFP either in the endoplasmic reticulum *(er*GFP*;* ex. Baulcombe) or fused to the cytoskeletal protein α-tubulin *(tua*GFP*;* constructed at SCRI by S. Mitchell).

## *wt* MP is transferred from ER to MT during infection

During early infection of cells with vectors expressing *wt*MP-DsRed, we noticed that the *wt*MP became associated with punctate cortical bodies at the leading edge of expanding infection sites (Fig. l). On *er*GFP plants, the *wt*MP-DsRed fusion was seen to accumulate at the cisternal vertices of the cortical ER network (Fig, 1A). In cells immediately behind the infection

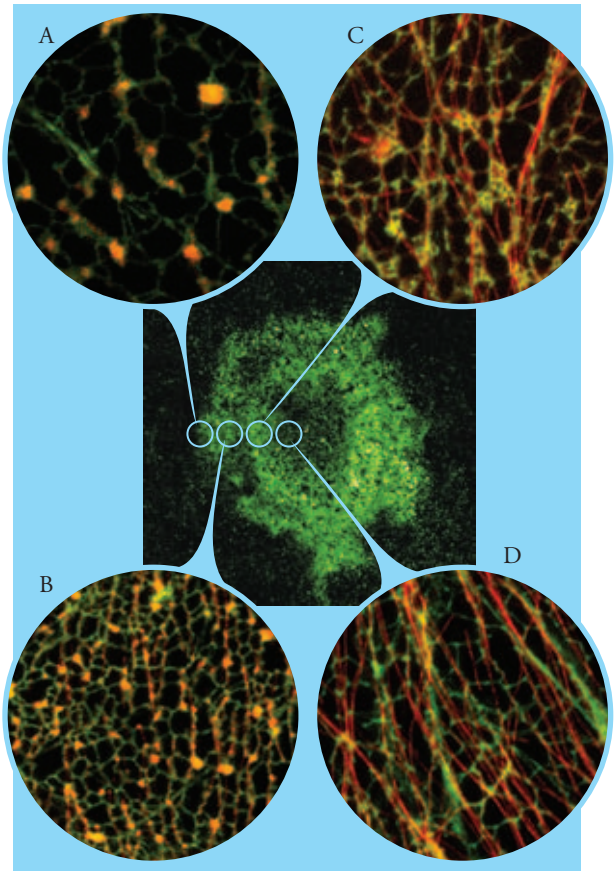


**Figure 1** A *wt*MP infection site has a characteristic ring-like structure due to a cycle of MP synthesis and degradation (centre). At the leading edge of infection (A) *wt*MP (red) is localised to the vertices of the cortical ER network (green). Behind the infection front (B), the MP causes a distortion of the ER network. Subsequently, the *wt*MP is transferred onto microtubules (MT; red in C). After transfer, the ER resumes its polygonal shape (D).

front, these cortical bodies began to enlarge, and in older, infected cells the *wt*MP induced a transient deformation in the cortical ER network, causing ER tubules to become elongated in places, reminiscent of a fishing net being stretched between specific points (Fig. 1B). Subsequently, the fluorescent *wt*MP was passed onto underlying MT, causing these structures to fluoresce intensely (Fig. 1C). Confirmation that the underlying structures were MT was obtained by inoculating *tua*GFP plants with viral vectors expressing *wt*MP-DsRed (data not shown). In yet older infected cells (i.e., those towards the centre of an infection site), most of the *wt*MP was transferred from ER onto underlying cortical MT and, in these cells, the ER had once again resumed its normal, polygonal appearance (Fig. ID). In cells at the very centre of an infection site, most of the *wt*MP was degraded, giving the infec-

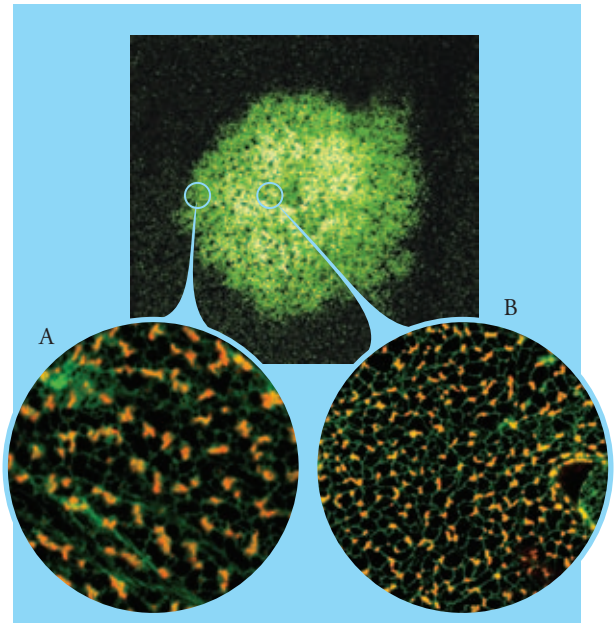


**Figure 2** *shuff*MP infection sites are uniformly fluorescent throughout due to a lack of MP degradation (centre). At the infection front (A), *shuff*MP (red) localises to cortical ER (green). At the centre of infection (B), *shuff3*MP remains on the ER, without transfer to MT.

tion sites a 'ring'-like appearance (centre image, Fig.1). These observations suggest that during infection with TMV, *wt*MP is transferred from cortical ER to MT as part of the ongoing viral infection process.

### *shuff*3MP fails to transfer from ER to MT

Next, we examined the behaviour of *shuff*MP-DsRed expressed from an identical TMV vector. The initial stages of infection were similar to those observed with *wt*MP, i.e., *shuff3*MP became attached to the vertices of the cortical ER network (Fig. 2A). However, in marked contrast to the *wt*MP, *shuff3*MP aggregates continued to enlarge at these sites without transfer to MT (Fig. 2B). Furthermore, the ER network did not undergo the distortion associated with *wt*M*P* expression, and retained its polygonal appearance throughout infection At the very centre of the infection site, *shuff3*MP was not degraded and remained associated with the cortical ER. These data suggest that successive rounds of DNA shuffling had induced changes in the *shuff3*MP that prevented the transfer of MP from ER to MT.

### A single amino acid change prevents the transfer of *shuff*3MP from ER to MT

As the *shuff3*MP contains a number of coding (stuctural) and non-coding (silent) nucleotide changes that confer improved stability (see preceding report), we

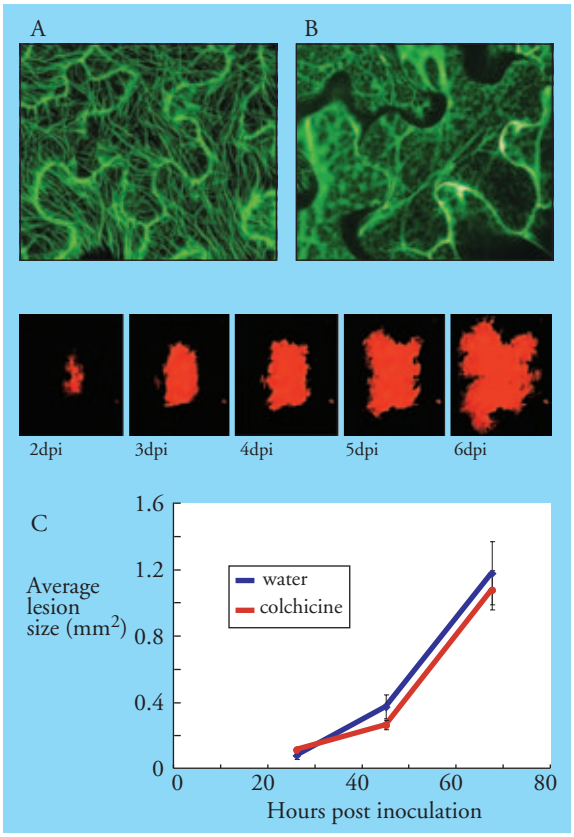


**Figure 3** *Tua*-GFP transgenic plants before (A) and after (B) treatment with the MT-depolymerising drug, colchicine. In the presence of colchicine, infection sites continued to expand at the same rate as water-infiltrated controls (C). Inset shows growth of a single infection site in colchicine).

made fusions of GFP to clones containing the individual mutations. Clones expressing only the silent changes showed a MT-localisation phenotype identical to the *wt*MP (data not shown). However, the single amino acid coding change L-72-V was found to be responsible for the lack of MT association characteristic of the *shuff3*MP. This amino acid change, and its effects on the stability and function of the MP, are currently the subject of further studies in our laboratory.

### MT are dispensable for TMV movement

MT have been suggested to play a central role in the TMV cell-cell movement process[2]. We were surprised that, given its ability to improve vector movement, *shuff3*MP did not strongly associate with MT at the leading edge of the infection. We therefore questioned whether MT are an absolute requirement for cell-cell transport of TMV. Leaves of transgenic plants expressing *tua*GFP (Fig.3A) were exposed to colchicine, a MT -depolymerising drug, to determine concentrations that would depolymerise the MT cytoskeleton, without affecting other cell functions. At O.5mM colchicine, the MT cytoskeleton was completely depolymerised while cytoplasmic streaming was unaffected (Fig. 3B). When viral vectors expressing either *wt*- or *shuff3*MP fusion proteins were inoculated onto colchicine-treated tissues, these vectors moved unimpeded from cell to cell (Fig. 3C), demonstrating that an intact MT cytoskelton is not required for normal TMV movement functions.

### Accumulation of *shuff3*MP improves viral transport functions

The failure of *shuff3*MP to transfer from cortical ER to MT suggests that MT might function as part of a degradation pathway for viral MP. As inhibition of the ER-MT step led to accumulation of *shuff*MP on the ER, we examined the relative levels of MP accumulation in protoplasts transfected with either *wt*- or *shuff3*MP clones. As expected, increased levels of stable MP were detected in *shuff3*MP clones (Fig.4). Furthermore, the same amino acid change (L-72-V) responsible for inhibiting ER-MT transfer, was shown to be responsible for the increased accumulation of *shuff3*MP in protoplasts. These data suggest that blockage of part of the host degradation pathway for viral MP leads to accumulation of stable MP in infected cells.

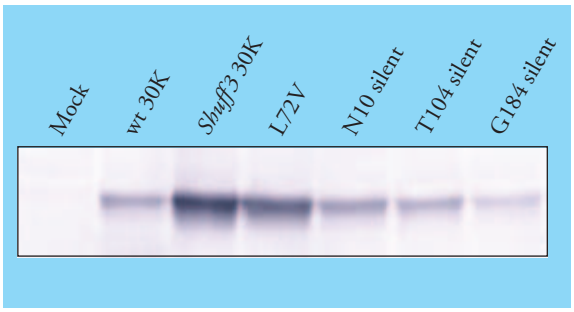


**Figure 4** The L-72-V coding change, characteristic of all *shuff*MP clones, causes MP to accumulate in transfected protoplasts. Silent changes have no effect on MP accumulation.

We next examined whether two known functions of viral MPs, namely plasmodesmal gating and viral RNA trafficking, were improved in the *shuff3*MP clones. To examine plasmodesmal gating, we biolistically bombarded plasmids expressing GFP ($M_r$ 27 kDa) under control of the 35S promoter into single leaf epidermal cells with and without plasmids expressing *wt*- or *shuff3*MP-DsRed. These data are shown in Figure 5. In control leaves (no MP expres-

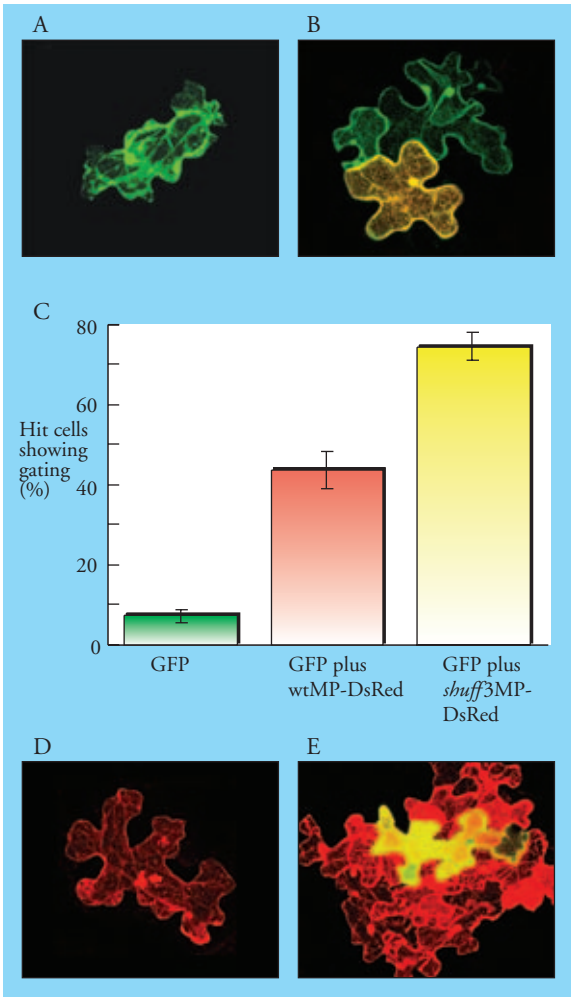


**Figure 5** *shuff*MP improves both plasmodesmal gating (A-C) and viral RNA trafficking (D,E) compared to controls. To assess gating, MP was co-bombarded into single cells along with free GFP (27kDA; A-C)). To assess viral RNA trafficking, RNA transcript of a MP-defective virus was bombarded into single epidermal cells in the absence (D) or presence (E) of viral MP.

sion), GFP showed restricted trafficking to neighbouring cells (Fig. 5A), a result confirming the low size exclusion limit of source-leaf plasmodesmata (*Annual Report, 1999, 76-79*[4]). In the presence of *wt*MP, plasmodesmal gating was increased significantly above controls, as seen by the number of bombarded cells showing movement of GFP (Fig. 5C). However, values for plasmodesmal gating induced by *shuff3*MP were approximately double those of the *wt*MP, indicating substantial improvement in this viral MP function (Fig. 5B,C). To examine the capacity of *wt*- and *shuff3*MP to traffic viral RNA, we constructed a movement-defective viral vector expressing DsRed as a cytosolic protein (TMV. *FS*MP.DsRed). We then co-bombarded this vector into single cells with or without plasmids expressing *wt*- or *shuff3*MP*s* (expressed as GFP fusions). As expected, TMV.*FS*MP.DsRed failed to move from cell to cell (Fig. 5D). In the presence of the *wt*MP, viral RNA trafficking was rescued, and replicating virus was detected in epidermal cells outside the bombarded cell (data not shown). However, *shuff3*MP rescued viral RNA trafficking from a greater number of bombarded cells, and over a greater number of cell boundaries, than the *wt*MP (Fig. 5E).

### Does *shuff3*MP evade a host degradation pathway?

The improved viral MP functions described above were consistent with the hypothesis that levels of functional *shuff3*MP were enhanced by evasion of a MP degradation pathway. The L-72-V coding change that leads to loss of MT association, and subsequent accumulation of *shuff3*MP, does not occur in regions of the MP known to be involved in plasmodesmal gating and viral RNA trafficking, suggesting that evasion of MP degradation led directly to increased MP levels, and indirectly to improved MP functions. Recently, it was shown that the TMV MP becomes ubiquinated prior to degradation by the 26S proteasome pathway[5]. The proteasome is a subcellular protein complex in animals and plants that is involved in the degradation of abnormal proteins, allowing amino acids to be recycled by the cell. Ubiquitin tagging is a prerequisite for this pathway, allowing the aberrant protein to be recognised and processed by the 26S proteasome. Our data are consistent with a model in which the *wt*MP is mobilised from the viral replication 'factories' established on the cortical ER prior to degradation. Instead of playing a role in cell-cell transport of the viral genome through plasmodesmata, we suggest that MT function as part of the host degradation pathway, perhaps in redistributing excess MP when the proteasome is saturated.

### Epilogue

Three rounds of DNA shuffling led to the improvement of viral movement functions of TMV -based vectors. Intuitively, we expected the altered coding changes that conferred improved cell-cell movement to have occurred in regions of the viral MP known to have roles in viral transport, such as plasmodesmal gating and viral RNA trafficking. However, the most significant coding change induced by random DNA shuffling was associated with a stage of the MP 'life cycle' characterised by degradation rather than movement, an area of viral infection that has received

almost no attention. Our data emphasise that the viral replication cycle involves a complex interplay between viral and host factors, the balance of which determines the success of viral spread. The fact that genetically modified vectors based on TMV, but not *wt*TMV itself, are improved in their movement functions (see preceding report) suggests that *wt*TMV, in an evolutionary sense, is extremely 'fine tuned' to its host in terms of cell-cell movement. However, the burden of an increased genetic load on the viral vector leads to reduced stability and movement, facets of the infection process that apparently can be enhanced. We are therefore encouraged that viral vectors might be improved further in desirable traits through random DNA shuffling rather than by rational design.

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# Pathology

James Duncan, Hugh Barker & Peter Palukaitis

*In the year of the completion of the human and Arabidopsis genomes, great strides have being made in characterising the genomes of plant pathogens and pests and the interactions of plants with their pathogens and pests. Characterisation of the genomes of bacteria, fungi, and nematodes at SCRI has yielded some exciting results, with more to follow. With viruses, where the genomes are much smaller and were characterised some years ago, research has already moved into the area of functional genomics. With the new Institute focus on (functional) genomics, proteomics, bioinformatics, and metabolic profiling, Pathology has found a new niche, allowing new interactions with other research groups at the SCRI. This means we need to reduce our efforts in some more traditional aspects of agriculture over the next few years. However, at present, we are still heavily involved in a number of projects under the broad headings of Aetiology & Epidemiology, Microbial Variation & Diagnostics, and Development of Disease Control Measures. In the last year, we have made substantial progress in all these areas but, for this Annual Report, we will highlight our achievements in the areas of Microbial Genomics and Plant-Pathogen/Pest Interactions. The struggle against plant disease continues on several fronts.*

## Microbial Genomics

A physical map of the genome of *Erwinia carotovora* subspecies *atroseptica* (*Eca*) is 90% completed, with over 130 genes mapped. Good progress has been made in mapping the genome of *Erwinia carotovora* subspecies *carotovora* (*Ecc*). A number of these genes are novel and may have an important role in the pathogenicity or host range of this organism.

A comparison of the *Eca* and *Ecc* physical maps shows that the genomic organisation of the two subspecies is very similar, although the positions of some genes differ between the genomes, which may explain differences in pathogenicity and host range between these pathogens.

Sample sequencing of two *Eca* bacterial artificial chromosome (BAC) clones has demonstrated major alterations in the ordering of conserved genes in the *Eca* genome vis-à-vis *E. coli*.

Sample sequencing of two *Eca* BAC clones has identified a number of novel genes that may help *Eca* survive in the soil or *in planta*. These include sequences similar to opine catabolism genes from *Rhizobacteria*, regulatory genes from *E. chrysanthemi* and sequences similar to genes from an operon required for the attachment of *Agrobacterium* to host cells.

End sequencing of *Eca* BAC clones has identified sequences that may have a role in pathogenesis. These include sequences similar to toxin genes in

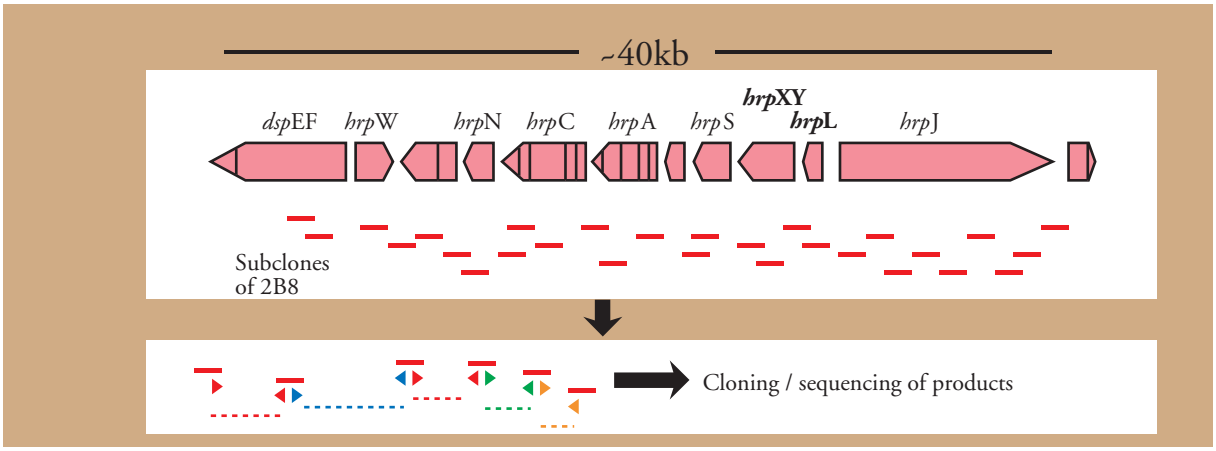


**Figure 1** A number of novel pathogenicity- and host range-related genes have been identified in *Erwinia carotovora* subsp. *atroseptica (Eca)*. A Bacterial Artificial Chromosome library has been used to locate the position of an entire *hrp* gene cluster (approx. 40 kb). The cluster, thought to be responsible for the export of avirulence genes, has been sequenced using a combination of random sequencing and PCR. Phenotypic tests suggest that the cluster is functional in *Eca*.

*Pseudomonas syringae* and genes involved in iron scavenging in *P. aeruginosa*. Important genetic determinants of pathogenesis in *Eca*, some with analogues in animal pathogens, have been identified and characterised. An intact Type III secretion system (*hrp* gene cluster) has been fully sequenced (Fig. 1); it is very similar to the *hrp* gene cluster in *E. amylovora*, and its functionality has been confirmed by the export of the *avrB* gene in transformed *P. syringae*. Two more *E. amylovora* disease-specific genes adjoin it. This finding is surprising, as pathogenesis in these two species is very different. The *hrp* gene cluster may have some hitherto unforeseen role in the early stages of infection by *Eca*.

Sequences also have been identified in *Eca* that are homologous to haemolysins in various animal pathogens. Although some are similar to the *hecAB* genes from *E. chrysanthemi,* others have no similarity to any known *Erwinia* genes. Haemolysin homologues are present in various other plant pathogens and have a role in attachment of *P. putida* to plant seeds.

A number of important proteins and/or genes encoding such proteins have been identified associated with the potato cyst nematode (PCN). These include the following:

A gene for pectate lyase, encoding an enzyme that probably aids in the penetration of the host by the nematode. This is the first report of a pectolytic enzyme in any animal.

A peroxiredoxin, a major hydrogen peroxide removal enzyme, is located on the surface of PCN. Similar peroxidases in nematode parasites of animals are involved in cuticle cross-linking and in protecting the nematode from superoxides generated by the host's defence mechanisms.

A family of chitin synthase genes. One gene product is involved in the synthesis of eggshells and another in part of the nematode feeding apparatus. Also identified was a subgroup of collagen genes responsible for fuelling the cuticle growth of adult nematodes.

A novel family of nematode secreted proteins has been characterised and the proteins have been shown to be expressed in different nematode secretory tissues, implying different functional roles in the development of PCN.

In viral genomics and functional genomics, there also have been a number of advancements.

*Raspberry bushy dwarf virus* (RBDV, genus *Idaeovirus*) has a bipartite genome that consists of a *c.* 5.4kb RNA-1 and a *c.* 2.2kb RNA-2. RNA-1 encodes the proteins that are involved in virus replication. RNA transcripts from full-length cDNA clones of each of the genome RNAs of RBDV were shown to be infective, opening up the possibility of reverse genetics, to determine the nature of resistance breakage by the RB isolate.

The cell-to-cell movement protein (MP) of *Groundnut rosette virus* (GRV), encoded by ORF4, was expressed from *E. coli* and affinity purified. Gel retardation analysis demonstrated that in contrast to many other viral MPs, including the 3a MP of *Cucumber mosaic virus* (CMV), the ORF4-encoded protein bound to viral single stranded (ss) RNA non-

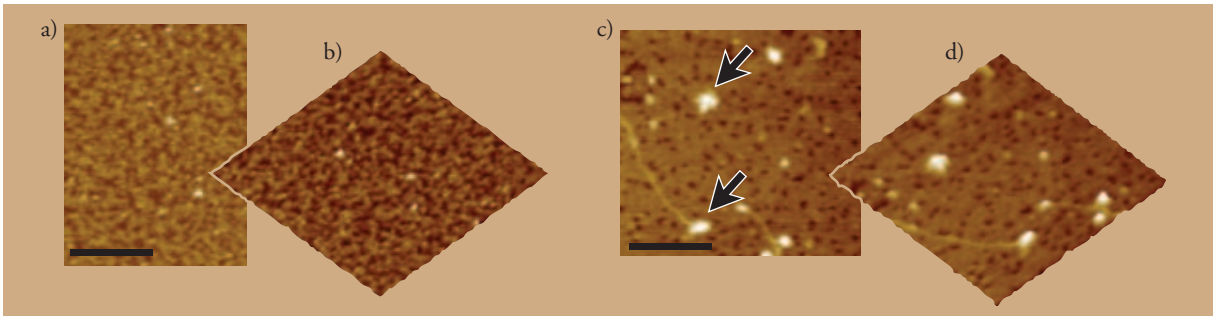


**Figure 2** Atomic force microscopy (AFM) allows three-dimensional imaging and measuring of individual biomolecules in the nanometre range under ambient and physiological conditions. AFM was used to characterise the architecture of complexes formed *in vitro* by viral RNA and movement protein (MP) encoded by two different plant viruses, *Cucumber mosaic virus* (CMV) and *Groundnut rosette virus* GRV). Analysis of CMV MP-RNA complexes revealed chains of CMV MP molecules, presumably bound to RNA (a, b). By contrast, GRV MP formed unexpected structures containing chains of ribonucleoprotein extending from large globules (arrows) (c,d). Bars = 300 nm.

cooperatively and formed complexes of low protein:RNA ratio. UV crosslinking and nitrocellulose membrane retention assays confirmed that the both GRV MP and CMV MP formed complexes with ssRNA and the complexes were of similar stability. Probing MP-RNA complexes with atomic force microscopy demonstrated that the ORF4-encoded protein bound to limited regions of viral RNA while the CMV 3a protein formed highly packed complexes (Fig. 2).

The GRV ORF4-encoded protein was also shown by immunofluorescence microscopy to generate tubular structures protruding from the protoplast surface. Such tubules are also produced by the CMV MP.

Nucleotide sequences of the cylindrical inclusion (*CI*) gene of 15 isolates of *Potato virus Y* (PVY) showed there to be greater diversity between strain sub-groups than was previously supposed. There seem to be two classes of CI sequence that are characteristic of the two main PVY sub-groups, but there is also evidence that recombination has occurred between viruses in different sub-groups.

Preparations of *Tobacco rattle virus* (TRV) of sufficient concentration and purity for measurement of Raman optical activity were made. Studies on the TRV preparation (collaboration with L. Barron, University of Glasgow) provided experimental support for a previous theoretical model of TRV particle structure, but with some additional details.

A self-interaction was demonstrated in the yeast two-hybrid system by protein p51 encoded by RNA-2 of *Potato mop-top virus* (PMTV). Furthermore, the protein was shown to bind ssRNA. This work indicates that p51 functions to transport RNA from cell to cell during the virus movement process.

The PMTV RNA-2 proteins p21 and p13 were expressed as fusion proteins with GFP from the TMV viral vector. Localisation of p21-GFP by confocal laser scanning microscopy indicated that it accumulated at or near plasmodesmata, whereas p13-GFP appeared as small aggregates in the cytoplasm. No interaction was found between p13 and p21 in the yeast two-hybrid system. This work will lead to the development of a model for virus protein function in virus movement.

### Plant-Pathogen/Pest Interactions

Many genes that are important in early cell signalling, plant defence and response to stress, have been isolated from potato after challenge with pathogenic bacteria, fungi and nematodes.

Using RT-PCR (TaqMan), initial experiments have been carried out quantifying expression analyses of potential signalling and response genes, including a protein phosphatase (PP2A) regulatory subunit, a TATA-binding associated factor, a receptor-like kinase, and a ubiquitin-specific protease. Expressed sequence tags (ESTs) for these genes had been obtained from a suppression subtractive hybridisation (SSH)-derived library of potato genes up-regulated 1 hour after inoculation of potato leaves with *Eca*.

A potato gene, *erg-1* (Erwinia-response gene), is rapidly induced by *Eca*, *Phytophthora infestans*, ethylene and salicylic acid. This gene is a member of a family of related stress-responsive genes of unknown function.

A potato gene encoding a WRKY-like transcription factor was shown to be induced in interactions with *Eca* and *P. infestans* and to be co-regulated with a class 1 endochitinase. Sequences of the 5' untranslated region (UTR) for St-WRKY-1 and the endochitinase

were determined and found to contain a number of potential binding sites for cis-regulatory elements. The 5'UTR of the chitinase contained potential W-box WRKY-binding regions.

SSH has identified genes expressed in tomato roots infected with *Meloidogyne chitwoodi*. Genes previously associated with stress, infection and apoptosis were found, including the *Le Mir* gene, proteases, pathogen related proteins, a nodulin homologue and the resistance gene *Mi-1*. A similar approach with compatible and incompatible plant PCN interactions has generated an EST library, unexpectedly rich (30 - 50%) in nematode sequences. cDNA-AFLP of plant-PCN interactions has also identified a large number of plant genes putatively involved in the host response, including three NBS-LRR genes not currently in public databases.

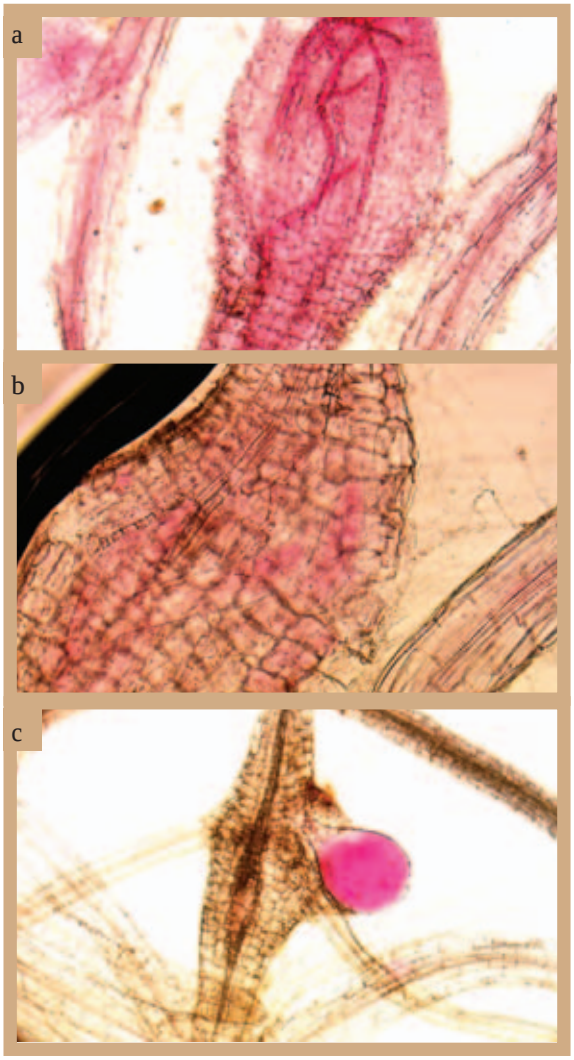


Figure 3 The time course of *Meloidogyne chitwoodi* parasitising the resistant potato, *S. bulbocastanum*. (A) At 3 days post infection, second stage juveniles (J2s) invading the roots are seen moving intercellularly and orientating themselves in the root tips before migrating to the vascular cylinder. (B) At 10 days post infection, the nematode, having established a feeding site, is seen enlarging and moulting. Oesophageal gland secretions have altered the expression of host genes and multinucleate 'giant cells' form the established feeding site. Characteristic swelling of the host plant cortical cells has occurred (cf. adjacent, unthickened, normal root). (C) At 21 days post infection, after moulting three times, the adult, female nematode is seen breaking through the cortex of the root.

A histological examination of nematode development in Pentland Ivory (susceptible to PCN (*Globodera pallida*) and potato clone 12601ab1 (with PCN resistance derived from *Solanum tuberosum* spp. *andigena* CPC2802) has been made. Nematode development is retarded in 12601ab1 relative to Pentland Ivory, which is consistent with the typical delayed resistant response for cyst nematodes. A histological examination of *Meloidogyne chitwoodi* (Virulent line V6) successfully parasitising the resistant potato *S. bulbocastanum* is shown in Figure 3.

SSH was used to discover genes involved in compatible (virulent) and incompatible (avirulent) challenges by *Rhynchosporium secalis* in barley. Several novel and resistance-related genes were identified and their regulation is being characterised.

The *Hero* gene is a major wide-spectrum disease resistance gene of tomato that confers resistance to PCN. It has been isolated by a map-based cloning method in a close collaboration with a German research group. PCN-susceptible tomato plants, became resistant to PCN after transformation with the *Hero* gene. This provides an excellent opportunity to dissect the molecular mechanisms of PCN resistance in plants and to exploit this useful gene to develop PCN resistant potato cultivars.

Virus resistance responses have been characterised in several host-derived and pathogen-derived (transgenic) systems.

Simultaneous expression in tobacco of transgenes encoding sense and antisense sequences of the *CI* gene obtained from a $PVY^{O}$ sub-group isolate, gave very strong resistance to other isolates of $PVY^{O}$ but not to isolates from the $PVY^{N}$ subgroup.

Plants of the *S. tuberosum* clone G8107(1) were found to be immune to infection with *Potato leafroll virus* (PLRV) following inoculation with very large numbers of viruliferous aphids, although the plants were readily infected by grafting. Results of sensitive detec-

tion tests on inoculated plants suggest that virus delivered by aphid inoculation is unable to exit the inoculated leaves. This type of resistance has not been identified in other lines of *S. tuberosum*.

Resistance to CMV in CMV RNA-1 transgenic plants was found to be sequence specific but not to be caused by post-transcriptional gene silencing. Resistance to both long-distance movement of CMV and replication of CMV RNA-1 could be overcome by graft inoculation, suggesting the presence of a component of the resistance mechanism that could be saturated.

Specific topics not covered above are given in more detail in the following articles.

# A novel *N* gene-associated resistance to the movement of TMV vectors neutralised by a CMV RNA 1 transgene

T. Canto & P. Palukaitis

The *N* gene is the best characterised resistance gene to a plant virus, viz., *Tobacco mosaic virus* (TMV). This resistance gene was introgressed into tobacco from *Nicotiana glutinosa* in the 1930's and is still in widespread use in field tobacco plants. In the early 1990's, the *N* gene was the first plant virus resistance gene cloned and its resistance mechanism has been analysed extensively[1-3]. Infection of tobacco containing the *N* gene by TMV results in a hypersensitive response (HR) and the confinement of TMV to cells surrounding the initial site of infection. This response also leads to a systemic acquired resistance (SAR) mediated by salicylic acid (SA). At temperatures above 28°C, the HR and the restriction response associated with the *N* gene are inactive, and so TMV spreads throughout the plant. Lowering the temperature of incubation to below 28°C allows activation of the *N* gene resulting in necrosis in all tissues containing TMV. Physiological and cellular events that take place during the induction of the HR were described recently by Wright and Santa Cruz (Ann. Rep. 99/2000, 136-139).

During an analysis of transgenic tobacco expressing RNA 1 of *Cucumber mosaic virus* (CMV) and showing resistance to CMV, it was observed that *N* gene-mediated resistance to TMV was retained in these plants at temperatures below 28°C.

However, at higher temperatures, when the *N* gene was inactive, TMV appeared to show greater spread into leaves and an increase in accumulation of TMV in the CMV RNA 1 transgenic plants. To determine whether this increase was due to increased replication, movement or both, non-transgenic tobacco plants as well as CMV RNA 1 transgenic tobacco plants were infected with TMV expressing the gene encoding the green fluorescent protein (GFP). These experiments showed that an additional mechanism of resistance (associated with the *N* gene), limiting TMV movement was still active at higher temperatures. This mechanism could be overcome by expression of CMV RNA 1.

Figure 1 Infection by TMV-GFP at 25 °C and at 4 days post inoculation resulted in visible necrotic lesions in inoculated leaves of tobacco transgenic for CMV RNA 1 (right leaf), that were absent in inoculated leaves of non-transformed tobacco (left leaf).

**CMV RNA 1 enhances cell-to-cell movement of TMV-GFP in *N* gene tobacco**

Infection of tobacco (cv. Samsun NN) expressing CMV RNA 1, by TMV containing the gene encoding the GFP (TMV-GFP), at 25°C, resulted in the appearance of visible necrotic local lesions in the inoculated leaf (Fig. 1, right leaf). Surprisingly, such macroscopic lesions were not visible in tobacco (cv. Samsun NN) that was not transgenic (Fig. 1, left leaf). Since

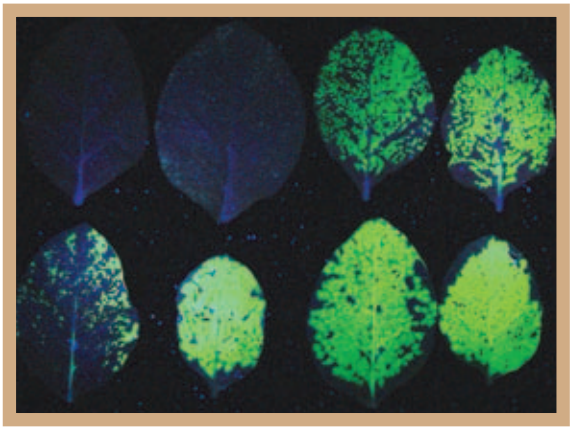

Figure 2 GFP fluorescence (under a UV lamp) in tobacco leaves 4 days after their inoculation with TMV-GFP, kept at the *N* gene-active temperature of 25 °C (upper row) or at the *N* gene-inactive temperature of 33 °C (lower row). From left to right: inoculated leaves of non-transformed tobacco NN, CMV RNA 1-transgenic tobacco NN, non-transformed tobacco nn, and CMV RNA 1-transgenic tobacco nn (indicated as NN, NN-TG, nn and nn-TG, respectively).

wildtype TMV gave necrotic lesions of comparable sizes in the two types of plants, this retarded local movement appears to be a consequence of slower movement of some TMV vectors in *N* gene-containing tobacco. The enhanced effect by the CMV RNA 1 transgene on the cell-to-cell movement of TMV-GFP in tobacco containing the *N* gene, also occurred at 33°C, where the ability of the *N* gene to induce an HR and restrict cell-to-cell movement of TMV is known to be inactive (Fig. 2, compare lower left two leaves).

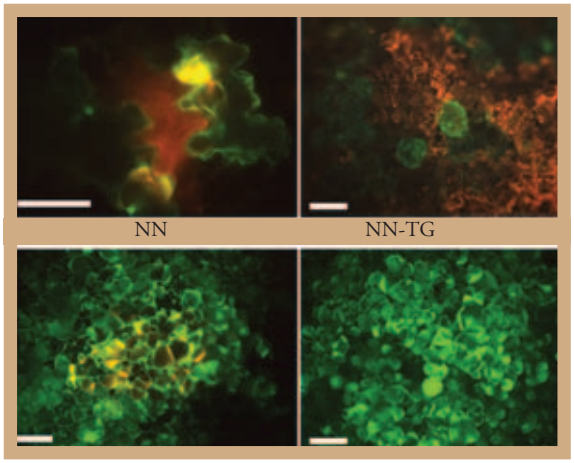


Figure 3 GFP fluorescence (by UV microscopy) in tobacco leaves 4 days after their inoculation with TMV-GFP, kept at the *N* gene-active temperature of 25 °C (upper row), or at the *N* gene-inactive temperature of 33 °C (lower row). From left to right: infection loci in non-transgenic tobacco NN, and CMV RNA 1-transgenic tobacco NN, (indicated as NN and NN-TG, respectively). An FITC/Rhodamine filter was used in which GFP fluorescence is green, while autofluorescence from dead tissue is orange. Healthy tissue appears as a dark background. Bars represent 100 µm.

**TMV-GFP shows very limited cell-to-cell movement in *N* gene tobacco** The accumulation of TMV or TMV-based vectors was found to be similar in isolated tobacco mesophyll cells (data not shown). Therefore, the differences observed here are due to effects on cell-to-cell movement rather than on intracellular accumulation of viral RNA *per se*. This was confirmed by examining the cell-to-cell movement of TMV-GFP using fluorescence microscopy (Fig. 3). In tobacco cv. Samsun NN at 25°C, movement of TMV-GFP was limited to a few cells, resulting in a microscopic lesion (Fig. 3, upper left panel). By contrast, in CMV RNA 1 transgenic Samsun NN tobacco, TMV-GFP spread to a larger number of cells, but further movement was restricted by activation of the *N* gene (Fig. 3, upper right panel). At 33°C, TMV-GFP showed cell-to-cell movement to numerous cells in both types of plants (Fig. 3, lower panels), indicating that the severely restricted movement of TMV-GFP at 25°C was due to effects associated with the *N* gene.

**CMV RNA 1 enhances long-distance movement of TMV-GFP in *N* gene tobacco** At 33°C, TMV can systemically infect both non-transgenic as well as CMV RNA 1 transgenic plants (not shown). However, at this temperature, movement of TMV-GFP was mostly restricted to within the inoculated leaves, and to the main veins of one or a few leaves above the inoculated leaves in tobacco cv. Samsun NN (Fig. 4, right panel, top row). By contrast, in Samsun NN tobacco plants transgenic for CMV RNA 1, systemic infection of leaves above the inoculated leaf occurred with TMV-GFP spreading throughout the invaded leaves (Fig. 4, right panel, second row). Thus, there appears to be some factor in tobacco plants restricting the movement of TMV-GFP even at temperatures when other aspects of *N* gene-mediated resistance are inactive.

**CMV RNA 1-mediated cell-to-cell movement of TMV-GFP is *N* gene associated** To ascertain whether the *N* gene was associated with the restriction of TMV-GFP in non-transgenic tobacco, plants of the tobacco cultivar Samsun nn, containing the *n* allele, were tested for their ability to restrict the cell-to-cell movement of TMV-GFP. Such plants were also made transgenic for CMV RNA 1, to compare the

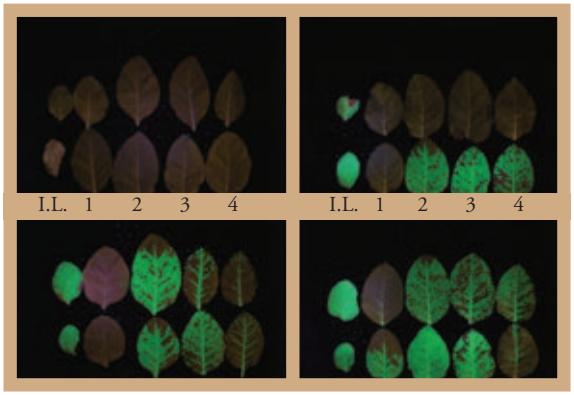


Figure 4 GFP fluorescence (under a UV lamp) in tobacco plants 14 days after their inoculation with TMV-GFP, kept at the *N* gene-active temperature of 25°C (left panel), or at the *N* gene-inactive temperature of 33°C (right panel). From top to bottom: non-transformed tobacco NN, CMV RNA 1-transgenic tobacco NN, non-transformed tobacco nn, and CMV RNA1-transgenic tobacco nn (indicated as NN, NN-TG, nn and nn-TG, respectively). Each row of leaves shows the detached inoculated leaf (indicated as I.L.) followed by the detached, ascending, four consecutive leaves (numbered 1 to 4).

effects of the CMV RNA 1 transgene on TMV-GFP movement in the two lines (Samsun NN vs. Samsun nn). In fact, in the absence of the *N* gene, TMV-GFP moved efficiently from cell to cell in the presence or absence of the CMV RNA 1 transgene (Fig. 2 upper right two leaves). This was true at 25°C and at 33°C (Fig. 2, upper right two leaves vs. lower right two leaves). Thus, the restriction on cell-to-cell movement exhibited in Samsun NN plants at 25°C and 33°C (Fig. 1, and Fig. 2, left leaves), was associated with the presence of the *N* gene.

**CMV RNA 1-mediated long-distance movement of TMVGFP is *N* gene associated** The above Samsun nn lines were also assessed at both 25°C and 33°C to determine whether the inhibition of long-distance movement of TMV-GFP was associated with the *N* gene, or with a gene(s) not linked to the *N* gene. Interestingly, both non-transgenic and CMV RNA 1-transgenic Samsun nn tobacco supported the long-distance movement of TMV-GFP, and at 25°C as well as at 33°C (Fig. 4, both panels, lower two rows). Thus, the restrictions to both cell-to-cell and long-distance movement of TMV-GFP are associated with the presence of the *N* gene.

It is possible that the temperature-independent resistance observed against TMV movement might not be due to an effect of the *N* gene itself, but rather to one of the genes closely linked to the *N* gene that were co-introgressed from *N. glutinosa*. To test this possibility, tobacco plants from an nn cultivar made transgenic for the *N* gene alone[1] have been tested for their resistance to the cell-to cell movement of TMV-GFP. These plants showed the same restrictions of movement observed in tobacco cv. Samsun NN (cf. Fig. 1). Thus, this resistance phenotype is associated with the *N* gene and not specifically to genes linked to the *N* gene.

**Resistance to TMV-GFP movement occurs independent of the SAR pathway** The effects of the CMV RNA 1 transgene could be due to an interaction with either the *N* gene itself or some factor downstream of the pathway activated by the *N* gene. SAR, induced by TMV infection, is activated by an SA-dependent pathway. To determine whether the restriction to the movement of TMV-GFP was associated with this pathway, transgenic Samsun NN tobacco plants expressing the bacterial *nahG* gene, which results in SA degradation, were infected with TMV-GFP. When such plants are infected with wildtype TMV, they develop necrotic lesions, but the SA-mediated restriction response is not activated, and so TMV continues to move slowly through the plant, inducing necrosis in infected tissues. In the Samsun NN, *NahG*-expressing tobacco, TMV-GFP was confined to a micro-lesion, as was observed in non-transgenic Samsun NN tobacco (cf. Fig. 3). Thus, it appears that the resistance associated with the *N* gene is triggered by a pathway different from that associated with SA production. It would be interesting to determine whether similar domains of the *N* gene are associated with the SA-mediated resistance response and the novel, CMV RNA 1-suppressible resistance response. Testing transgenic plants expressing various *N* gene mutants[2] as well as an alternatively spliced *N* gene transcript[3] could lead to a clearer understanding of the nature of the elictor of the two distinct resistance responses. This may also provide more information on how CMV RNA 1, or the encoded 1a protein, is able to suppress a resistance mechanism that has a limited effect on the movement of wildtype TMV, but a strong effect on the movement of a TMV vector.

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# Cross-kingdom activity of plant virus-encoded silencing suppressors

B. Reavy & S.A. MacFarlane

Plants have evolved several ways of resisting infection by pathogens including viruses. Systemic acquired resistance (SAR) is a general resistance mechanism that exists in tobacco and other plants. Typically, challenge to the lower leaves of a plant with a virus such as tobacco mosaic virus (TMV), induces partial resistance to both TMV and to other unrelated pathogens in upper, uninoculated leaves. More specific resistance to viruses results from the presence of resistance genes in the plant. For example, potato plants carrying the Ry gene are resistant to strains of *Potato virus Y* but are not resistant to other, related viruses. Recently, it has been discovered that plants may also combat virus infection by targeting the virus RNA for sequence-specific degradation by a mechanism known as post transcriptional gene silencing (PTGS). PTGS in plants was first identified as the cause of cosuppression, in which transformation of plants with an additional copy of a host gene could abolish expression of both the new transgene and the host homologue by inducing degradation of cytoplasmic mRNA. This sometimes results in a visible phenotype depending on the gene involved (Fig. 1). This system was shown also to act on viruses; firstly by the demonstration that transformation of plants with non-translatable viral sequences often resulted in either extreme resistance to the same virus, or in a 'recovery' phenotype where plants developed resistance following an initial, virus-susceptible phase. Subsequently, it was shown that PTGS can be induced in plants by virus infection without the need for plant transformation. Some plant viruses have developed suppressor genes that interrupt PTGS in order to protect themselves from inactivation by the plant PTGS system. For example, *Tobacco etch virus* (TEV) and *Cucumber mosaic virus* (CMV) contain genes (HC-Pro and 2b, respectively) that suppress PTGS. These genes appear to operate in different ways so that HC-Pro reverses silencing in all tissues including those in which it is already established, whereas the 2b gene prevents initiation of silencing in newly emerging tissues but has no effect on already established silencing.

Figure 1 Silencing of the endogenous phytoene desaturase gene in *Nicotiana clevelandii* leads to photobleaching of the leaves.

PTGS is not unique to plants and similar mechanisms, called RNA interference or RNA silencing, have been observed in a variety of organisms, including fungi, mice, nematodes, zebrafish and the fruit fly *Drosophila melanogaster*. Recently, it was shown that silencing could be induced in cultured *Drosophila* cells by treatment with double-stranded (ds) RNA, and biochemical studies of this system have revealed details of some of the enzymatic activities involved in this process. The *Drosophila* system is ideally suited to allow the identification of cellular components that interact with and regulate the activities of the biochemical components of the silencing process. Because dsRNA-mediated silencing occurs in a wide variety of organisms, we hypothesised that components of the process might be common between the different organisms. In particular, we were interested to determine if suppressors of silencing from plant viruses could also act in *Drosophila* cells. Double-stranded RNA and DNA plasmids encoding different viral suppressors and the enzyme β-galactosidase were introduced into the cells by a process known as transfection. This leads to a short term or 'transient' expression of the introduced genes, and the induction of PTGS by the dsRNA. The *Drosophila* system mimics some but not all of the events occurring during PTGS in plants, as there is no movement of proteins or RNA molecules between the insect cells and no events similar to the systemic spread of silencing in plants. Nevertheless, it is a very useful system for studying parts of the PTGS mechanism that may be common to a number of organisms.

**Suppression of silencing in *Drosophila* cells by the HC-Pro protein** As a first test of the activity of plant virus silencing suppressors in *Drosophila* cells, we looked at the effect of expression of the HC-Pro gene on silencing of the *lacZ* gene. The *lacZ* gene codes for the enzyme β-galactosidase and the activity of this enzyme can be detected by staining cells with a substrate that produces a blue colour in the presence of the enzyme. Introduction of this gene alone into *Drosophila* cells produces a blue colour in the cells that have taken up and expressed the DNA. Co-introduction of dsRNA derived from the *lacZ* gene is expected to silence expression of the *lacZ* gene, reducing the number of cells that stain blue. The function of plant virus silencing suppressors can be examined by adding plasmids encoding the suppressor genes to *Drosophila* cells together with the *lacZ* gene and dsRNA, and assaying the number of cells that subsequently stain blue. In these experiments, transfection of the *lacZ* gene alone into *Drosophila* cells resulted in ~80% of the cells staining blue (Fig. 2). The number of cells staining blue was reduced to only ~10% when the *lacZ* expression gene and dsRNA corresponding to the first ~500nt of the *lacZ* gene were introduced into the cells. By contrast, introduction of the *lacZ* gene and *lacZ*-specific dsRNA, together with the TEV HC-Pro gene, resulted in staining of ~50% of the *Drosophila* cells, indicating that the silencing was partially suppressed by expression of the virus suppressor protein.

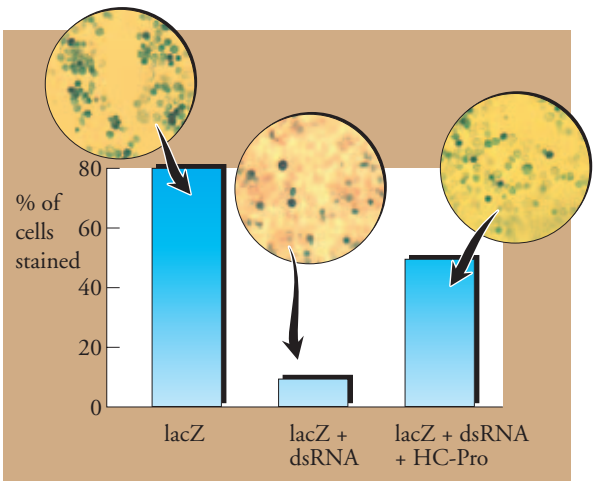


**Figure 2** Suppression of gene silencing in *Drosophila* cells by TEV HC-Pro. Cells were transfected with the *lacZ* gene alone (+ lacZ) or with the *lacZ* gene and dsRNA to induce silencing (+ lacZ + dsRNA) or with the *lacZ* gene, dsRNA and the HC-Pro gene to assess suppression of silencing (+ lacZ + dsRNA + HC-Pro). Cells expressing the *lacZ* gene stain blue. The number of cells stained with each treatment is shown in the chart.

This assay is dependent upon simultaneous introduction of three molecules into the cells and the transient expression of the genes involved. Thus, complete suppression of silencing is very difficult to achieve. The efficiency of the silencing suppression assay was increased by production of a stable cell line (DS2-HC-Pro) expressing the HC-Pro protein constitutively. An unrelated cell line (DS2-VCL) expressing a recombinant antibody was used as a control in order to eliminate the possibility that the process of stable transformation of the cells could in some way interfere with the gene silencing mechanism. Silencing was strongly induced in the DS2-VCL control cells when the *lacZ* gene and dsRNA were introduced together, and only 5% of the cells stained for β-galactosidase activity compared to 42% when the *lacZ* gene was introduced alone (Fig. 3). However, ~33% of cells expressed β-galactosidase when the *lacZ* gene and dsRNA were introduced into the DS2-HC-Pro cells, compared to 42% when the *lacZ* gene was introduced alone. This indicates that silencing was significantly suppressed in the cell line expressing the TEV HC-Pro silencing suppressor protein.

**Protection of *lacZ* RNA in cells producing the HC-Pro protein** A feature of suppression of silencing in

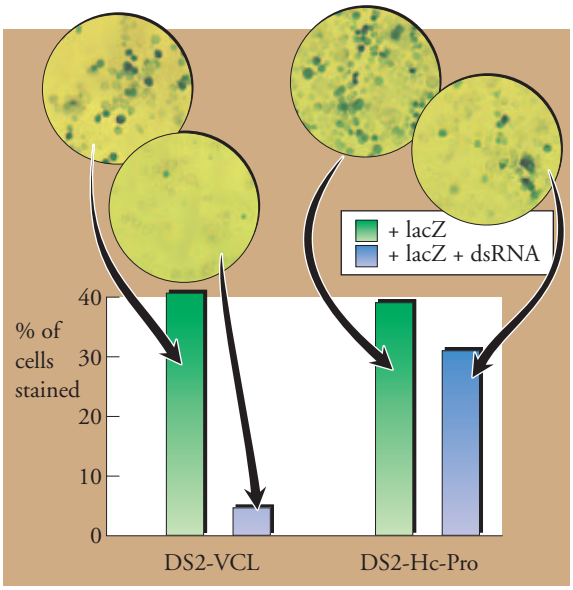


**Figure 3** Suppression of gene silencing in a stably-transformed cell line expressing TEV HC-Pro. Control cells (DS2-VCL) on the left, or cells expressing TEV HC-Pro (DS2-HC-Pro) on the right, were transfected with either the *lacZ* gene alone (+ lacZ) or with the *lacZ* gene and dsRNA (+ lacz + dsRNA). The number of cells from each line that stained with either treatment is shown in the chart.

plant cells is that the RNA species targeted by the silencing process is protected from degradation. The RNA present in *Drosophila* cells after transfection with different combinations of the *lacZ* and HC-Pro genes with dsRNA, was examined by northern blotting to determine if the HC-Pro protein was effective in preventing *lacZ* mRNA degradation. No RNA with the expected size of the *lacZ* transcript RNA could be detected in extracts of *Drosophila* cells transfected with the *lacZ* gene and dsRNA (Fig. 4). In contrast, intact *lacZ* RNA was present in *Drosophila* cells when the *lacZ* gene and dsRNA were introduced along with the HC-Pro gene. Similarly, the *lacZ* RNA was intact in DS2-HC-Pro cells after transfection with the *lacZ* gene and dsRNA.

**Suppression of gene silencing by the *Tobacco rattle virus* 16K gene** Previous studies have indicated that many plant viruses are able to overcome PTGS but often the particular genes involved in this activity have not been identified precisely. We were interested to determine if the *Drosophila* cell system could be used to ascribe silencing suppression activity to other uncharacterised plant virus proteins. *Tobacco rattle virus* (TRV) has been shown to suppress transgene silencing but the specific viral protein responsible for this activity has not been identified. TRV has two genomic RNA species that have been fully sequenced. The larger RNA (RNA1) codes for 134K and 194K proteins that form the viral replicase, a 29K cell-to-cell movement protein, and a 16K cysteine-rich protein. The smaller RNA (RNA2) codes for the coat protein (CP) and may encode other (2b and 2c) proteins involved in virus transmission by nematodes. A characteristic of tobraviruses is that RNA1 can infect plants systemically in the absence of RNA2, i.e. without CP expression and virion formation. This type of infection, referred to as NM-infection, occurs frequently in vegetatively propagated crop plants such as potato and bulbous ornamentals, and is often associated with increased symptom severity. RNA1, therefore, contains all the functions necessary for virus multiplication including, possibly, suppression of gene silencing. The 16K protein is the only protein encod-

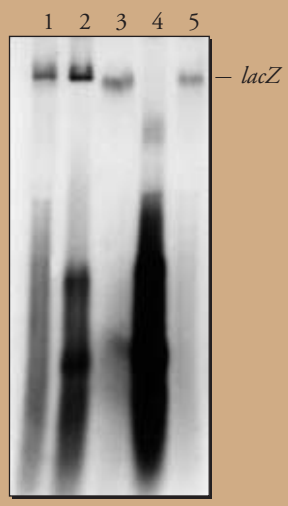


**Figure 4** Northern blot analysis of *lacZ*-gene specific RNA in control cells (lanes 3-5) or in cells expressing TEV HC-Pro (DS2-HC-Pro, lanes 1,2). Cells were transfected with the *lacZ* gene alone (lanes 1,3), or with the *lacZ* gene and dsRNA (lanes 2,4), or with both the *lacZ* and HC-Pro genes and dsRNA (lane 5).

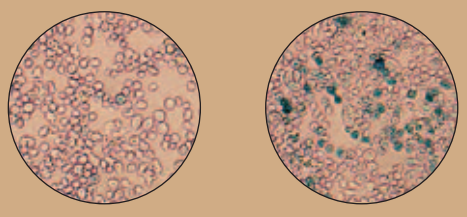


**Figure 5** Suppression of gene silencing by the TRV 16K gene. *Drosophila* cells were transfected with the *lacZ* gene and dsRNA (+ lacZ + dsRNA) to induce silencing, or with the *lacZ* gene and dsRNA along with the TRV 16K (+ lacZ + dsRNA + 16K) gene to assess suppression of silencing. Cells expressing the *lacZ* gene stain blue.

ed by RNA1 without an assigned function and we decided to test this gene for silencing suppression activity in *Drosophila* cells.

As described earlier, introduction of the *lacZ* gene and dsRNA resulted in only ~6% of cells staining blue with β-galactosidase activity compared to ~80% staining when the *lacZ* gene alone was introduced. Inclusion of the 16K gene along the *lacZ* gene and dsRNA increased the number of cells staining blue seven-fold when compared to treatments lacking the 16K gene. Expression of the TRV 16K protein in *Drosophila* cells thus prevented dsRNA-mediated silencing of the *lacZ* gene confirming our hypothesis that this protein can function as a silencing suppressor. Currently, we are using this system to screen proteins from a wide variety of plant viruses for silencing suppressor activity.

**Conclusions and prospects** Our demonstration of suppression of PTGS in *Drosophila* cells by plant virus proteins indicates that at least part of the pathway of PTGS is conserved between plants and *Drosophila.* The TEV HC-Pro and CMV 2b proteins are thought to target different components of the silencing system, as the CMV 2b protein prevents initiation of silencing only in newly emerging tissues, whereas the potyvirus HC-Pro protein suppresses silencing in all tissues. We have been unable to observe any suppression of silencing in *Drosophila* cells by the CMV 2b protein. This could be because the 2b protein is not functional as a suppressor in this cell system, or perhaps the early stages of dsRNA-induced silencing in *Drosophila* differ from the initiation of silencing in plant tissues, possibly by-passing the step at which the 2b gene functions. The different types of silencing suppressor proteins found in plant viruses may be useful in dissecting the biochemical pathway of silencing in *Drosophila* and possibly in other organisms. In addition, it is becoming apparent that silencing may play a role in other processes as well as defence against foreign RNAs. Perturbation of the silencing process has been found to affect development and fertility in plants and germ-line development in nematodes. Intervention with silencing suppressor proteins from plant viruses may therefore have significant utility in determining the involvement of silencing in development and differentiation in both plants and animals.

# Oxidative processes involved in soft rots caused by *Botrytis cinerea*

I. Muckenschnabel, G.D. Lyon, N. Deighton, B.A. Goodman, D. Stewart & B. Williamson

*Botrytis cinerea* causes grey mould disease and associated soft rots in a wide range of horticultural crops world-wide. The fungus is a necrotroph, whereby it invades plant tissues by killing cells in advance of mycelial penetration and is subsequently able to grow on dead tissues. In contrast, biotrophs, such as rusts and mildew, are only able to grow in living plant cells. There are therefore some fundamental differences in the type and speed of some of the molecular events associated with the infection process between biotrophs and necrotrophs. Previously, emphasis has been placed on investigating the role of cell wall degrading enzymes and toxin production during infection by *B. cinerea*. As partners in an EU-funded project named 'Oxidative attack by necrotrophic pathogens-new approaches for an innovative and non-biocidal control of plant diseases' (AOS-PLANT), we have investigated the oxidative burst during infection with an emphasis on quantifying redox-related changes through electron paramagnetic resonance (EPR) spectroscopy[1], quantification of compounds influencing the redox state of cells, and chemical markers for consequential lipid peroxidation.

Increasing evidence suggests that oxidative processes involving highly reactive free radicals (chemical species with unpaired electrons), metal ion species and toxic products of the peroxidation of lipids present in cell membranes are involved in the disease processes in plants in a manner analogous to processes in animal cells. We have studied some of these oxidative processes in fruits of sweet pepper (*Capsicum annuum*) and leaves of French bean (*Phaseolus vulgaris*) and *Arabidopsis thaliania* during necrotrophic infection by *B. cinerea*. Comparison of cellular processes using tissues from these three plant families (Solanaceae, Leguminoseae and Brassicaeae, respectively) has enabled us to identify responses common to all three plant species and also to note some important differences.

It has been reported that *B. cinerea* produces hydrogen peroxide that is free to pass across lipid membranes in much the same way as water molecules. Hydrogen peroxide would be expected to oxidise Fe(II) to Fe(III) with the formation of the hydroxyl radical (HO•) through the Fenton reaction: $Fe(II) + H_2O_2 \rightarrow Fe(III) + HO^- + HO\bullet$. Fe(II), however, is relatively rare in plant tissues, with Fe being transported predominantly as Fe(III) citrate and stored as ferritin, where the Fe occurs as Fe(III) oxyhydroxide polymers. Solubilisation of Fe from ferritin is accomplished readily by oxalate, a product of the metabolism of ascorbic acid by *B. cinerea*, and reaction of this Fe(III) with reductases or antioxidant molecules such as ascorbic acid yields the Fe(II) for the Fenton reaction. EPR spectroscopy was used to follow the changes in the oxidation states of transition metal ions such as Mn (II) and Fe(III) that can be readily detected in plant tissues and quantified through changes in signal intensity. Being transition metals, manganese and iron can exist in both EPR-detectable [e.g. Mn(II) and Fe(III)] and EPR-silent forms [e.g. Mn(III) and Fe (II)]. EPR therefore has the potential to provide information on the redox status of tissue samples and can also directly detect and characterise free radicals associated with complex molecules. We obtained evidence for effects of *B. cinerea* infection in pepper not previously recognised[2] by the detection of an unidentified, single-peak free radical

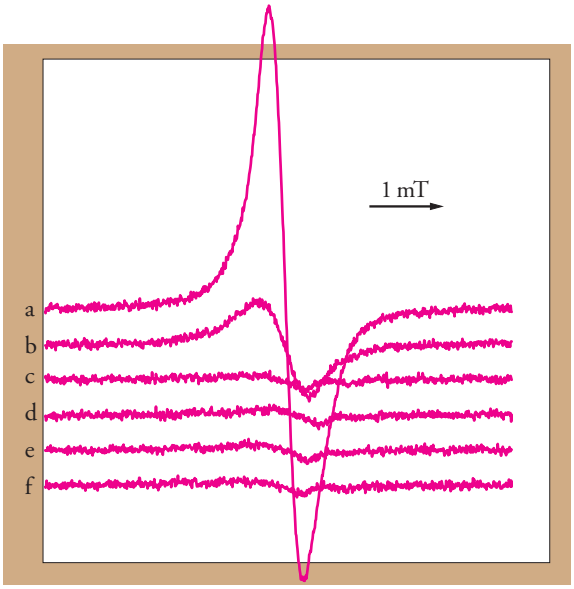


**Figure 1** Typical EPR spectra (free radical region) of *B. cinerea*-inoculated pepper fruit. Samples were taken from increasing distances from the centre of the lesion: (a) centre of lesion, (b) edge of lesion, (c) 7mm from edge of lesion, (d) 15mm from edge of lesion, (e) 22mm from edge of lesion, (f) 30mm from edge of lesion.

(Fig. 1) and elevated levels of Fe(III) at g=4.3, especially within necrotic vascular traces that extend beyond the rotting lesion (Fig. 2). We obtained similar results in infected young leaves of French bean[3] and *A. thaliana*. There is therefore an indication that the plant deploys antioxidant systems in an attempt to redress the imbalance created by the pathogen. The levels of ascorbic acid decline appreciably in diseased tissues (Fig 3). When the spin trap POBN (α-(4-pyridyl-1-oxide)-N-*t*-butylnitrone) was introduced into samples from bean leaves, evidence was found also for an unstable free radical being involved in the disease process beyond the edge of the lesion (Fig. 4)

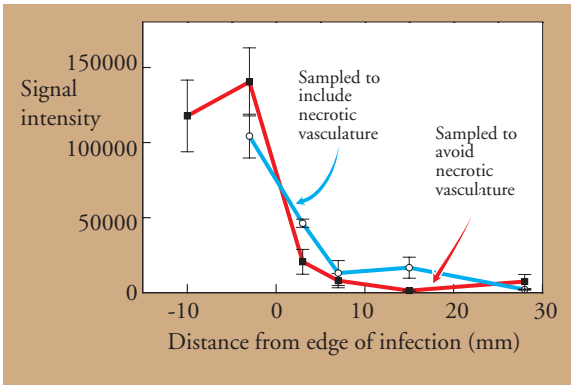


**Figure 2** Intensity of EPR-detectable Fe(III) signal associated with specific lesions in *B. cinerea* infected pepper fruit.

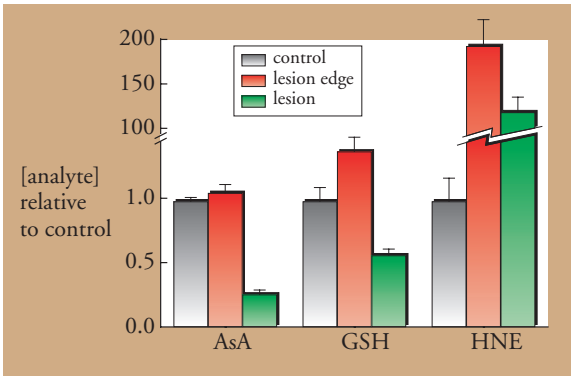


**Figure 3** Ascorbic acid and glutathione depletion and accumulation of lipid peroxidation products from *B. cinerea* infection in *P. vulgaris*. AsA - ascorbic acid, GSH - reduced glutathione, HNE - 4-hydroxynonenal.

at the same time as a destabilisation of the ascorbate radical (Asc•) occurred. Similar results were found in pepper fruits[3].

Oxidative stress induced during the onset of plant disease results in degradation of cellular membranes by lipoxygenases and the peroxidation of lipids by peroxygenase. Linolenic acid from cell membranes is broken down to malondialdehyde (MDA) and 4-hydroxyhexenal (4-HHE) and linoleic acid to *n*-hexanal and 4-hydroxynonenal (HNE). These four aldehydes were quantified as their 2,4-dinitrophenylhydrazone derivatives by liquid chromatography-mass spectroscopy (LC-MS) to provide a measure of the lipid peroxidation that occurred in plant tissues as a result of infection.

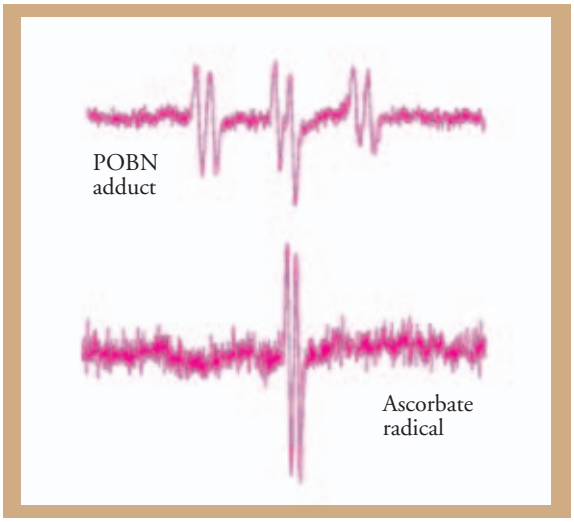


**Figure 4** EPR detection of an unstable free radical (using the spin trap POBN) and the ascorbate radical in *B. cinerea* infected *P. vulgaris* leaves.

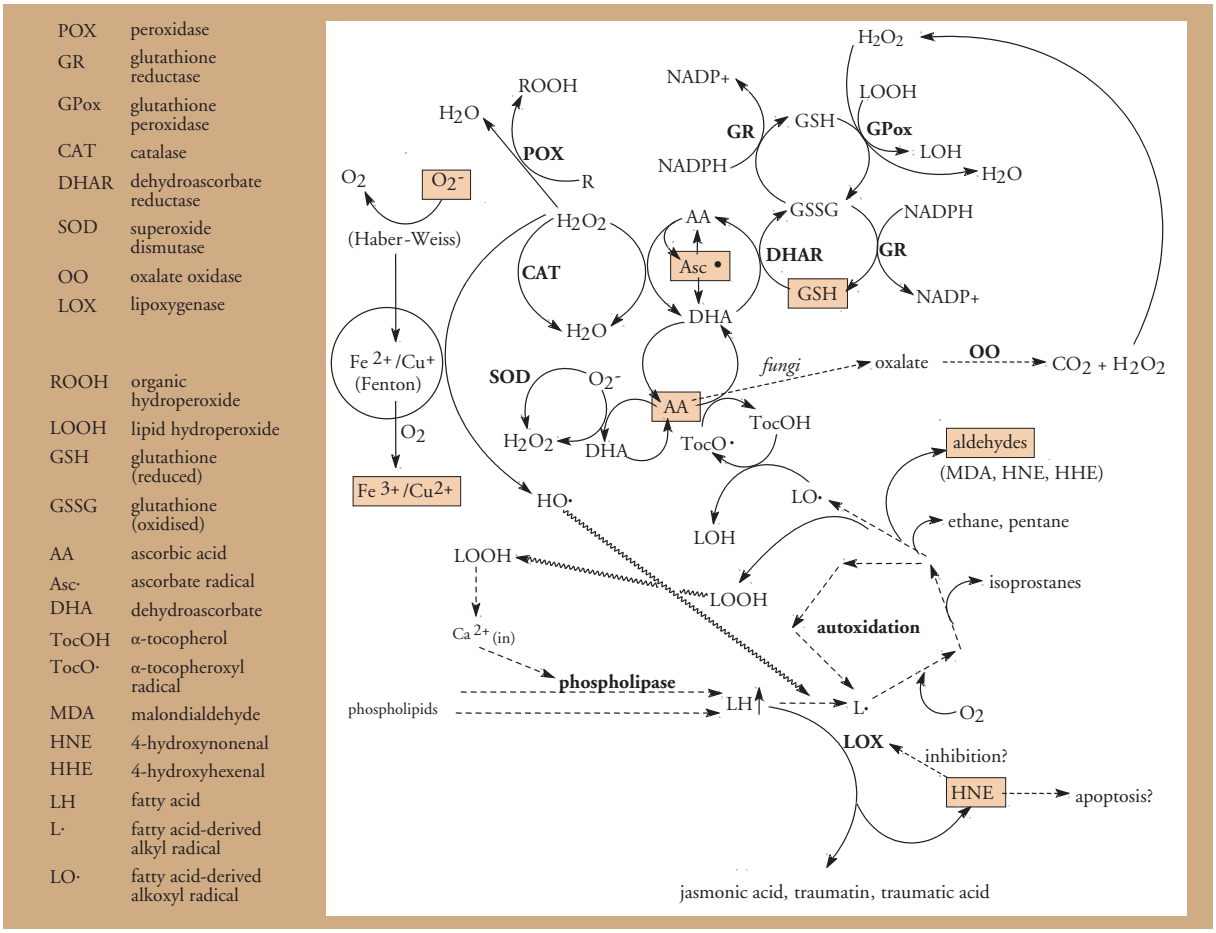


**Figure 5** Relationship between oxidative processes associated with the infection process.

In sweet pepper fruits, elevated levels of HHE, HNE, MDA and *n*-hexanal were detected within the lesion and at the lesion margin. There was also some evidence of their extension into apparently healthy tissues[2]. The high levels of HHE and HNE found in diseased plant tissues is interesting because, in animal systems, they are known to be cytotoxic and genotoxic, reacting with amino and thiol groups in proteins and also with deoxyguanosine to form cyclic compounds at pH 7.4.

In leaves of four genotypes of *P. vulgaris* inoculated with *B. cinerea*, large increases in HNE and MDA were found adjacent to rotted tissue again indicating the powerful influence of this necrotroph on the host[4]. However, the expected accompanying increases in the levels of these aldehydic products of lipid peroxidation were not observed in *A. thaliana* inoculated under similar conditions, indicating that this species responds differently to infection compared with tissues from other plant families studied earlier. Thus, whilst *Arabidopsis* is an excellent model plant to study aspects of gene function and transcriptional regulation, there are limitations to the extent that information derived from *Arabidopsis* can automatically be related to plants in other families.

Massive depletion of ascorbic acid pools ahead of visible infection and generation of the EPR signals associated with the plant-pathogen interaction in representative species from three plant families, all indicate damage to the antioxidant mechanisms as an early event in the infection process (Fig. 5). It seems that once there is imbalance in oxidative processes, driven by the invading necrotrophic fungus, the latter is favoured and disease is the outcome.

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# Use of the *C. elegans* model system to understand parasite gene function

J. Jones, L. Gray, P. Veronico[1], C. De Giorgi[1] & P. Bazzicalupo[2]

**Introduction** In the 1960s and 70s, a small group of scientists chose a previously unheralded organism, the free living nematode *Caenorhabditis elegans*, as a model system for the study of genetics, behaviour and developmental biology. Their reasons for doing so were that it had many of the features desirable for an experimental system: it had a rapid (3.5 day) life cycle, could be easily cultivated on plates of bacteria, it was small (1.5mm in the adult stage) and composed of sufficiently few cells (1000) that determination of the lineage was feasible. Furthermore, the nematode was translucent (allowing simple microscopical observations of internal morphology) and had a mode of reproduction that was convenient for genetic analysis. As more workers began to appreciate the benefits of *C. elegans,* other techniques were developed which further enhanced its utility as a model system. Systems for genetic transformation, gene knockouts, synchronous culture and large-scale culture were developed. By the end of 1998, the entire 100 million base pair genome of this organism had been sequenced. This resource, coupled with the other achievements of the *C. elegans* project which included the determination of the entire cell lineage, a full understanding of the anatomy (including the nervous system and all its synaptic connections) at the electron microscope level, a library of over 1600 mapped mutations in genes and an integrated physical and genetic map, meant that *C. elegans*, a small soil dwelling nematode, was the most completely understood metazoan on the face of the planet.

Contrast this with the situation faced by scientists working with parasitic nematodes. No *in vitro* culture systems for these organisms exist and, as a consequence, many developmental stages occur within a host and are inaccessible for study. Obtaining sufficient material for analysis is almost always difficult and most parasites are intractable to standard genetic analysis. Since many of these parasites have a devastating medical or economic impact on the activities of man, it is important that the information gained about nematode biology and the techniques developed during the *C. elegans* project are exploited by scientists studying nematode parasites. This article sets out to explain how *C. elegans* has been used in our laboratory in order to enable us to understand the function of genes identified from plant parasitic nematodes far more fully than would otherwise have been possible.

**Chitin synthase** Chitin metabolism is considered an important target for control strategies, particularly in fungi: chitin synthases are targets for several important groups of fungicides and insecticides and some evidence suggests that chitin synthase inhibitors have potential for control of plant parasitic nematodes. Little is known about the functional role of chitin in nematodes, although this polymer has been found in eggshells of many nematodes and other, less thorough, studies have suggested that chitin may be present in the feeding apparatus or body wall of some nematodes. Collaborative studies with a group in Italy led to the cloning of a gene encoding a potential chitin synthase from a plant parasitic nematode *Meloidogyne artiellia*. Database similarity searches led to the discovery that two homologues exist in *C. elegans*. Given the problems of examining gene function in plant parasitic nematodes, we used *C. elegans* for functional studies of the chitin synthase gene.

A full-length genomic sequence was obtained for the plant parasite chitin synthase gene and very basic experiments examining gene expression were possible. These showed that the gene is expressed in adult females – the nematode stage containing developing eggs. These results are consistent with a role for the chitin synthase protein in synthesising the chitin of the eggshells. Considerably more detailed experiments were possible with the *C. elegans* genes. RT-PCR, using cDNA extracted from synchronous cultures sampled at every 2 hours throughout the life cycle, was used to investigate temporal expression patterns of the genes. Constructs, in which the promoters of each the *C. elegans* genes were fused to GUS/GFP reporter proteins, were used to generate transgenic animals. Examination of the localisation of the reporter proteins was used to determine the spatial expression patterns of the chitin synthase genes.

The two *C. elegans* genes showed different temporal expression patterns as indicated by RT-PCR. One gene (T25G3.2) was expressed in adult hermaphro-

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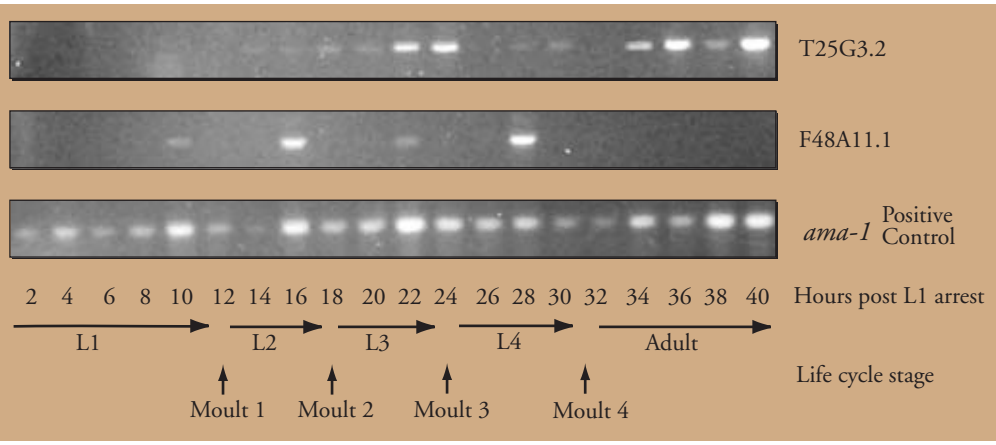


**Figure 1** RT-PCR analysis of the temporal expression patterns of the *C. elegans* chitin synthases. Each lane shows the results of an RT-PCR experiment conducted using cDNA extracted from a synchronous *C. elegans* culture. The presence of a band indicates that the gene is expressed at this time in the life cycle. One gene (T25G3.2) is expressed in later larval stages and the adult hermaphrodite. The other gene (F48A11.1) is expressed only in a short period before each moult with no evidence for expression in the adult hermaphrodite.

dites and later larval stages of the nematode (Fig. 1). Chitin is known to be present in eggshells of many nematodes and since the T25G3.2 gene seems to be expressed at a time when eggshells are being synthesised, it seems reasonable to suggest that the product of this gene is responsible for synthesis of chitin in the eggshell. The RT-PCR data from the other gene (F48A11.1) suggested an entirely different role for this protein. This gene was expressed specifically in the period immediately preceding each moult (Fig 1). Eggshells are not being synthesised at this point of the life cycle, so what is the chitin being made by this protein used for? Transgenic animals containing the construct in which the 5' region of the F48A11.1 gene was cloned upstream of the reporter had GFP present in the cells of the pharynx (Fig. 2) – the structure which forms the feeding apparatus.

The feeding apparatus of many nematodes, including *C. elegans,* is replaced during each moult. The spatial and temporal expression patterns of the F48A11.1 gene therefore suggest that the chitin that it synthesises is used in the feeding apparatus. One part of the pharynx of *C. elegans* (the terminal bulb) bears a thick, ridged cuticle on its inner surface. This grinder is responsible for physically breaking bacteria ingested by the nematode prior to digestion and absorption of nutrients. It is possible that this grinder contains chitin to provide structural rigidity. Our data suggests that this is indeed the case, with the product of the F48A11.1 gene responsible for the synthesis of this chitin.

The use of *C. elegans* as a model to study gene function has therefore allowed us to discover that chitin

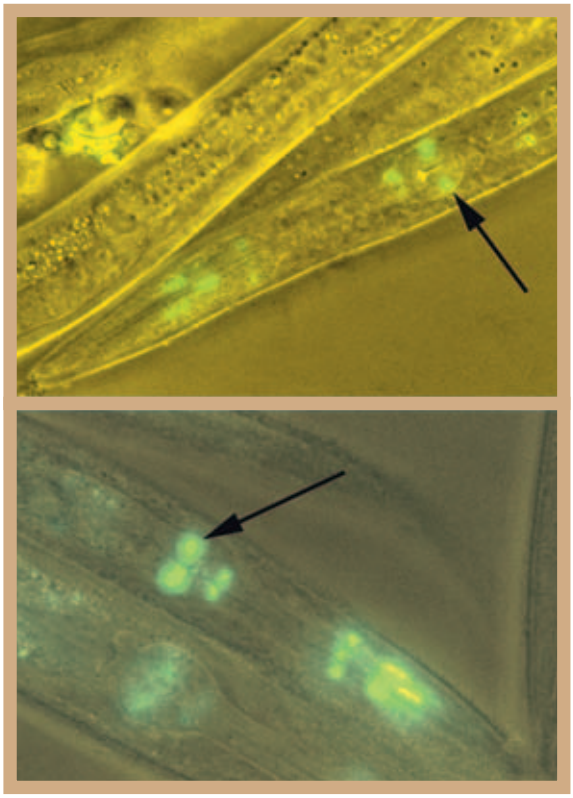

**Figure 2** Spatial expression pattern of the F48A11.1 gene. The image shows transgenic animals carrying a plasmid in which the 5' upstream region (promoter) of the F48A11.1 gene is fused to a GFP reporter with a nuclear localisation signal. The presence of GFP in a cell indicates that the gene is normally expressed in that cell. GFP is observed solely in the epithelial and glandular cells of the pharynx.

synthase proteins have two roles in *C. elegans*. Having uncovered a second function for this protein, which our studies on the plant parasite did not reveal, it is now possible to examine the plant parasite in more detail in order to determine whether this second function also operates in this nematode.

These studies show that chitin plays an important role in several aspects of nematode biology. Since chitin is not present in vertebrates or plants, chitin biosynthesis may provide an excellent target for novel control methods against a variety of parasitic nematodes. The use of *C. elegans* has allowed functional studies on this protein to progress quickly and will also allow assessment of the suitability of these proteins as control targets.

**Collagens** All nematodes are covered by an outer layer or cuticle. The cuticle provides a flexible exoskeleton which allows directed movement while forming a barrier between the organism and its external environment preventing damage, desiccation and, in parasitic species, attack from host defences. The predominant protein constituent of the cuticle is collagen.

The sequencing of the *C. elegans* genome has allowed the identification of the entire complement of genes encoding cuticular collagens. A total of 154 collagen genes were identified which have been subdivided into families on the basis of the number and position of conserved cysteine and tyrosine residues which are thought to be involved in forming bonds between individual collagen polypeptide chains. Other species of nematode, including the animal parasites *Ascaris suum* and *Brugia malayi* and the plant parasites *Globodera pallida* and *M. incognita,* contain collagen genes which share the same basic structure as *C. elegans* collagens. The collagen families in these species have not been described in such detail but they appear to be of a similar size to those observed in *C. elegans*. Genes that are closely related to each of the six families defined in *C. elegans* can be identified. Thus, the characterisation of collagen gene function in *C. elegans* may have wider implications in predicting the function of gene homologues throughout the Phylum Nematoda.

Previous studies have shown that the most abundant collagen protein in adult plant parasitic nematodes falls into the group 1a family – of which almost nothing is known in *C. elegans*. Therefore, we characterised this family of genes both in PCN and *C. elegans*. The *C. elegans* group 1a collagen family contains 12 gene members. These have some unusual properties in that they are clustered in the genome – four family members are arranged as repeats and a further three are grouped in a cluster of three. We used RT-PCR to examine the expression patterns of some of the group 1a family members (Fig. 3). Like other

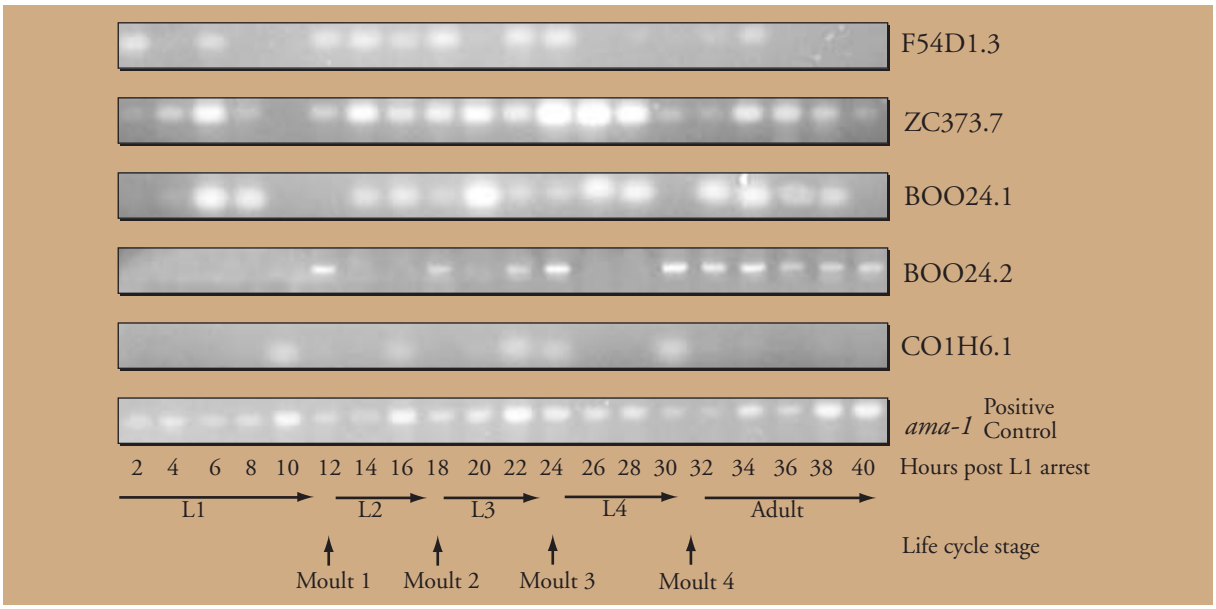


**Figure 3** RT-PCR analysis of expression of some of the *C. elegans* group 1a collagen genes during development from L1 larvae to adulthood. Each lane shows the results of an RT-PCR experiment conducted using cDNA extracted from a synchronous *C. elegans* culture. The presence of a band indicates that the gene is expressed at this time in the life cycle. Expression of some genes is linked to the moulting cycle. Several of the genes are expressed in the adult stages.

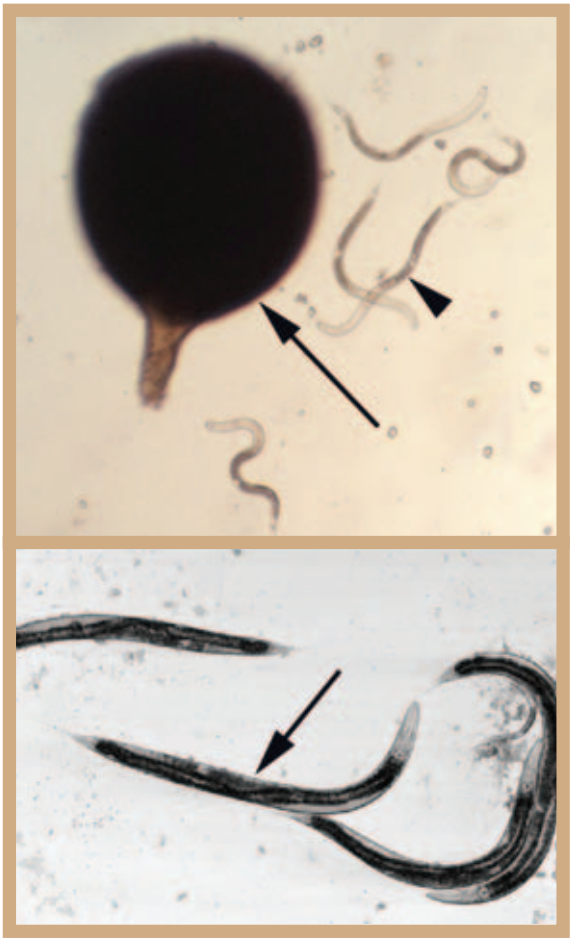

Figure 4 Comparison of the adult stages (arrows) of *Globodera pallida* (A) and *Caenorhabditis elegans* (B). The adult female of *G. pallida* is spherical and opaque, whereas the adult of *C. elegans* retains the worm-like morphology and is translucent. Note also the difference between the *G. pallida* female (arrow) and juvenile stage (arrowhead).

collagen genes in *C. elegans,* the expression of several of the group 1a genes is linked to the moulting cycle. Two of the genes (C01H6.1 and B0024.2) are expressed at, or immediately before each of the four moults that occur from L1 to adulthood while expression of another two (B0024.1 and ZC373.1) looks to be increased around the middle of each larval stage (Fig. 3). The most notable feature of these experiments was the finding that several of the *C. elegans* group 1a collagen genes (ZC373,7, BOO24.1, BOO24.2 and F54D1.3) are expressed in the adult nematode (Fig. 3). Previous observations suggest that a restricted set of collagens is expressed in the adult animal and it is therefore possible that one of the roles of the group 1a family of collagens is to provide material for the adult cuticle.

If the adult stages of *C. elegans* and plant parasites are compared (Fig. 4), the differences in morphology are striking. The *C. elegans* adult retains the transparent, elongated, cylindrical form seen in its earlier life-cycle stages, whereas the *G. pallida* adult female is roughly spherical and opaque. Is it possible that the function of the group 1a collagens is conserved in such diverged nematodes? Our studies suggest that this is indeed the case. Although it was not possible to perform analysis with PCN in the same detail as for *C. elegans*, RT-PCR experiments suggested that the PCN

group 1a genes are also expressed in the adult stages (Fig. 5). Database searches also suggest that similar genes are present in adult stages of other nematodes, including animal parasitic forms. Therefore, it seems that this collagen subfamily has a conserved functional role, in formation of the adult cuticle, throughout the Nematoda.

Although parasitic nematodes have evolved specializations in order to acquire a successful parasitic behaviour, these adaptations have been built on a framework of basic nematode anatomy and physiology. The availability of the information from the *C. elegans* project therefore represents a remarkable resource for understanding the biology of other nematodes. Our own studies, summarised here, show that in spite of the vast differences between *C. elegans* and plant parasitic nematodes in terms of their morphology and life cycles, plant nematology can benefit enormously from the *C. elegans* research effort.

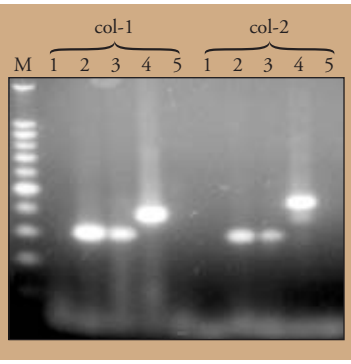


**Figure 5** RT-PCR analysis of expression of two of the *G. pallida* group 1a collagen genes in J2s (1); virgin females (2) and gravid females (3) using primers specific for each gene. A positive control using a clone of each collagen gene (4) was performed. A negative control (5) in which water replaced template DNA was carried out for each set of primers. The presence of a band indicates that the gene is expressed at this time in the life cycle. The *G. pallida* genes are both expressed in the adult female stages.

# *Phytophthora infestans* genomics: positional cloning of avirulence genes

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*Phytophthora infestans* is the causal agent of late blight of potato (Fig.1) and tomato, and thus responsible for significant losses in these crops worldwide[1]. This is particularly the case for the cultivated potato, where it is regarded as a threat to global food security. *P. infestans* belongs to the Oomycetes, a class of organisms that includes many important plant pathogens. The Oomycetes exhibit a mycelial growth habit but are distinct from the Fungi[1]. *P. infestans* exhibits a gene-for-gene interaction with potato, in which avirulence gene (*Avr*) products from the pathogen are recognised by the host plant expressing the cognate resistance gene[2,3] (Fig. 1). This leads to the hypersensitive response (HR), a localised programmed cell death in the plant that inhibits further spread of the pathogen.

Control of late blight has been achieved mostly by the use of fungicide applications, and the deployment of resistance (either major resistance genes or field resistance). Wild species of potato, principally *Solanum demissum*, have been used as sources of resistance in breeding programs. Presently, at least 11 major genes for resistance (R1 to R11) have been identified and incorporated into differential lines for identifying races of *P. infestans*. Where R genes have been used to control late blight, they are quickly overcome by new races.

Figure 1 Gene-for-gene interaction between potato and *P. infestans*. Potato cultivars (L - R) Stirling, Bintje, 1512C, and Pentland Ace uninoculated (top row), and inoculated with different *P. infestans* races (middle and lower rows).

While the genetic basis of resistance to late blight in potato has been well characterised, the genetic basis of virulence/avirulence in *P. infestans* is less well defined. Only relatively recently has the genetic basis of avirulence been determined for more than a few avirulence genes matching R genes. Most recently, a genetic cross between two *P. infestans* races was extensively analysed by the group of Francine Govers at Wageningen University, the Netherlands[3,4]. Six dominant avirulence genes at single loci were shown to segregate in this cross. Three of the *Avr* genes, *Avr*3, *Avr*10, and *Avr*11 were tightly linked to each other. The other three *Avr* genes, *Avr*1, *Avr*2 and *Avr*4, all segregated independently.

At the molecular level, *P. infestans* is becoming a model Oomycete for the study of pathogenicity mechanisms and avirulence. Central to these studies are resources such as a molecular-genetic linkage map[4], an expressed sequence tag (EST) database[5], as well as techniques for the analysis of gene function such as transformation[6], gene silencing[7], *in planta* reporter systems[8] and heterologous expression of genes from other *Phytophthora* species[9]. DNA libraries, particularly large insert genomic libraries, are a critical resource for structural genomics and, by definition, for positional cloning.

The system most amenable to manipulating large cloned DNA inserts is the *Escherichia coli* bacterial artificial chromosome (BAC)[10]. These cloning vectors, based on a single copy F factor plasmid, are capable of stable maintenance of insert DNA up to 300 kb. Insert sizes above 100 kb are preferred for positional cloning applications, since fewer clones are required for chromosome walking or chromosome landing across linkage map intervals containing genes of interest. This is of particular consideration if the genome size is large, or if the gene of interest lies in a genomic region where the frequency of recombination is low. The genomes of many

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fungal and Oomycete plant pathogens are relatively small compared to other organisms where positional cloning has been undertaken[11]. However, the genome size of *P. infestans* has been estimated to be 250 Mb[12], which is relatively large for an Oomycete plant pathogen, and larger than the genome of the model plant *Arabidopsis thaliana*.

When producing a BAC library that is primarily for the positional cloning of multiple target genes, it is more efficient to use a heterozygous individual from a selected $F_1$ mapping population as a source of high molecular weight DNA. An $F_1$ from a mapping population that possesses all required markers and phenotypic traits can be selected then if all the target genes segregate in the population. Recently, using a segregating population, the positions of six dominant *Avr* genes have been located on the molecular genetic linkage map of *P. infestans*. All have tightly linked AFLP markers[3]. Using one $F_1$ from this population, which contains all six *Avr* alleles from the genetic cross[3,4], we have produced a BAC library comprising ten-fold genome representation and an average insert size of 100 kb[13].

To convert from the genetic map to a physical map, BAC clones were pooled in an ordered manner to allow the library coordinates of *Avr*-linked BAC clones to be determined. The pools of BAC clones were screened for the AFLP markers determined to show close linkage to the six *Avr* genes. Positive clones were then fingerprinted individually and positioned relative to each other, the AFLP markers, and the *Avr* genes. For *Avr*1, which had only two linked markers 8 cM from the *Avr* locus, six BAC clones were identified. In this instance, both markers are one side of the gene, and it remains to be determined how far towards *Avr*1 the BAC contig extends (Fig. 2). This will determine how many further chromosome walking steps need to be taken to span this locus, and clone the gene.

The *Avr*2 locus is flanked by two AFLP markers. One marker is 1 cM away and the other is 5 cM distant. BAC clones containing the AFLP markers were identified and aligned. By fingerprinting the BAC clones and by testing hybridisation to each other, it was shown that the two small BAC contigs do not overlap (Fig. 2). To close the gap between the BAC contigs, chromosome walking has been initiated from the ends of the BACs. Thus, four steps from the ends of the two BAC contigs have been taken. Again, the newly identified BACs do not overlap, and so further steps are required to close the gap. However, if the ends of the extended BAC contig closest to *Avr*2 can be mapped beyond *Avr*2, then no further chromosome walking is required to clone this locus.

Physical mapping surrounding the *Avr* gene cluster was considerably simpler than for *Avr*1 or *Avr*2 due to the greater density of AFLP markers linked to these genes. In total, 11 AFLP markers are located in a 5 cM region containing *Avr*3, 10, and 11. A BAC contig was constructed which contains 11 BAC clones, spanning approximately 300 kb of genomic DNA. This contig incorporates ten AFLP markers and should also contain the *Avr*11 locus (Fig. 2). *Avr*3 and *Avr*10 are distal to the last AFLP marker, and that end of the BAC contig must be mapped in order to ascertain the position of the *Avr* genes relative to the BAC contig.

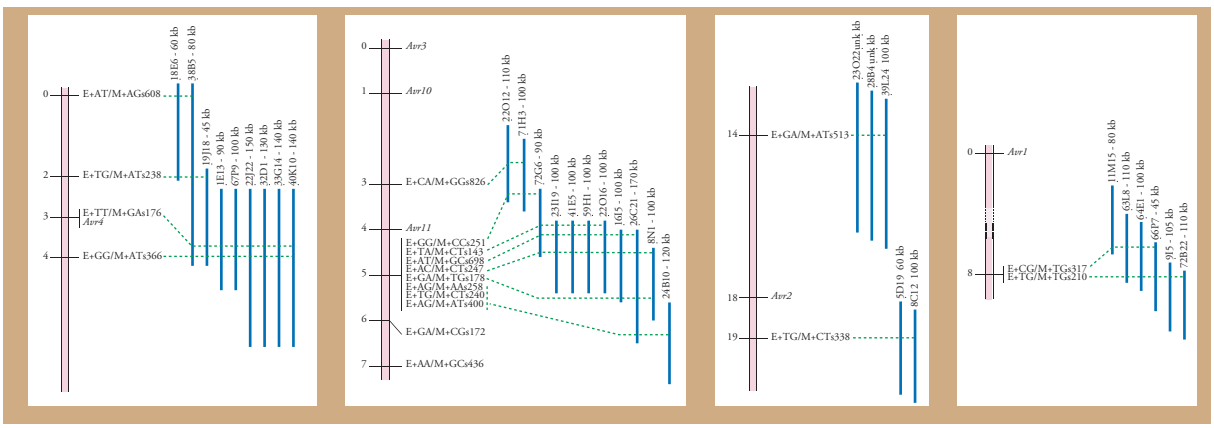


**Figure 2** Section of *P. infestans* linkage groups (adapted from van der Lee et al. 2001). Indicated are the map positions of AFLP markers used in screening for identifying and verifying BAC clones spanning these genomic regions. Contigged BAC clones (not to scale) are shown as solid vertical lines to the right of the linkage group. Their respective insert sizes are indicated at the top of the solid vertical lines. The relative positions of AFLP markers in the BAC contigs are shown by dotted lines.


Similar to the *Avr* gene cluster, the genomic region surrounding *Avr*4 has several linked AFLP markers. A BAC contig across this 4 cM region has been constructed and contains 9 BAC clones spanning approximately 200 kb of genomic DNA. One BAC clone of 80 kb spans the entire 4 cM region containing the *Avr*4 locus (Fig. 2). The relatively small physical size of this region therefore makes *Avr*4 an attractive target for cloning.

To identify the gene from the non-coding DNA present on the BAC clone, we are first determining the DNA sequence of the entire 80 kb containing *Avr*4. From this, we will predict open reading frames (ORFs) for further analysis. Despite *P. infestans* being a eukaryote, introns are relatively rare in gene sequences analysed to date and so should not greatly interfere with gene prediction. Predicted ORFs can be tested for avirulence gene function in at least two approaches. Firstly, a virulent isolate can be genetically transformed with the candidate sequence. If the sequence is the *Avr* gene, then a shift to avirulence will be observed. Transformation of *P. infestans* has been used by numerous research groups for over ten years, and is being refined continually[6,14]. In the past, this has been a complicated procedure, but newer methods taking advantage of biolistic transformation are now available[15].

The second approach to proving *Avr* gene function relies on the assumption that the host plant recognises the *Avr* gene product and triggers the hypersensitive response to effect resistance. By transiently expressing the candidate *Avr* gene in a host expressing the R gene, tissue necrosis will be indicative of the HR, and therefore the interaction of host R gene with the pathogen *Avr* gene. Transient expression systems used to date have involved *Agrobacterium* delivery of the DNA expression cassette, or viral vectors such as potato virus X (PVX)[16].

**Conclusions and future prospects** Crop plant disease has a significant impact on the productivity and profitability of UK and overseas agriculture. Current approaches to disease control often rely on the application of expensive, highly toxic chemicals that pose serious health risks to humans and animals, and contribute to pollution of land and water supply. Understanding the molecular basis of plant resistance to pathogen challenge will allow us to identify key genes that integrate the plant defence-signalling networks, and will provide the groundwork to obtain broad-spectrum, durable resistance to crop pathogens using novel strategies that are both cost-effective and benign in the wider environment.

This project is providing the genetic and physical resources for isolation of avirulence genes (recognition of the products of which triggers resistance in the host) from *P. infestans*, the model Oomycete pathogen of potato. As yet, no avirulence genes have been isolated from Oomycetes, although considerable efforts have been directed towards obtaining the host *R* genes with which they interact. Isolation of the avirulence genes will allow the plant-pathogen gene-for-gene interaction to be studied in detail, and will provide genes that may be directly deployed to develop durable disease resistance in the plant. It is only

through a thorough understanding of the biology of *P. infestans*, the nature of its interaction with the host plant (potato), and the identification of genes involved in these processes, that we will be able to develop novel control strategies to protect potato production in Scotland.

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# *Phytophthora* and gummosis of pistachio in Iran

J.M. Duncan, D.E.L. Cooke, N.A. Williams & M. Mirabolfathy[1]

For most people in the West, pistachios are a delicious snack normally consumed at parties - green kernels locked temptingly, and sometimes frustratingly, in a slightly opened thin shell. Elsewhere in the world, especially Iran, the cultivated pistachio (*Pistacia vera*) is an important staple crop, a source of food and cooking oil. Iran has about 250,000 ha of pistachio orchards producing 210,000 tonnes of nuts per annum and worth *c.* US$ 400M. Nearly 90% of Iranian production is concentrated in Kherman province, where wild *P. vera* also grows; members of the genus occur principally in an area stretching from the Mediterranean to W. China, but there are also native species in Mexico and USA.

Gummosis is one of the most important diseases of pistachio trees. It is characterised by a gummy or tarry exudate from the crown and lower trunk, whilst the underlying tissues are usually stained brown or dark brown and rotted. This, and severe root rotting, often kills the tree with average tree mortality in Kherman *c.* 10-12% or higher (Mirabolfathy, 1988 and unpublished observations).

*Phytophthora* species cause gummoses of a number of crop plants and several species have been isolated from affected pistachio trees: *P. citrophthora*, *P. cryptogea,* and two described originally as *P. megasperma* and *P. drechsleri*. All are pathogenic to pistachio seedlings and scions. In particular, the last two species above represent more than 90% of all *Phytophthora* isolates recovered from pistachio and both produced typical symptoms of gummosis and decline when re-inoculated onto seedlings and young trees[1,2].

*P. megasperma* and *P. drechsleri* are poorly resolved species and many *Phytophthora* isolates have been assigned wrongly to them. This is a common problem in *Phytophthora* taxonomy as there is a dearth of reliable morphological and physiological characters for identification. For example, one of the main characters used to identify *P. drechsleri* is the ability to grow at 35°C, a character not confined to this species.

**ITS identification of *Phytophthora* isolates from pistachio** SCRI has been at the forefront of developing rapid and reliable molecular methods for identifying *Phytophthora* species[3,4,5]. Principal among these has been a PCR-based system based on the sequence and restriction digests of the ITS region of the genomic ribosomal RNA gene repeat (rDNA)[5]. Using this system, we were able to compare the pistachio isolates of *P. megasperma* and *P. drechsleri* with isolates from international mycological collections that had been well characterised by classical and molecular criteria, including ITS. DNA fingerprints, generated by amplified fragment length polymorphism (AFLP), provided further comparisons among isolates.

**ITS identification of *Phytophthora*** Obtaining PCR products containing the ITS sequence of the *Phytophthora* isolates from pistachio was straightforward[3,4,5]. Small fragments of hyphae, picked off from cultures with sterile toothpicks, were added to the PCR mixture with primer ITS6, a version of the universal primer ITS5, which gives good amplification of Oomycetes, and ITS4. Thereafter, the amplicon was either sequenced or digested with restriction enzymes to yield ITS-RFLPs. Sequences were aligned against the database containing the ITS sequences of many isolates of nearly all described species of *Phytophthora*[3]. Likewise ITS-RFLP patterns could be compared with the patterns in a similar database[4,5]. A phylogram (Fig. 1) based on a sequence alignment shows the relationship of the pistachio isolates to the rest of the genus.

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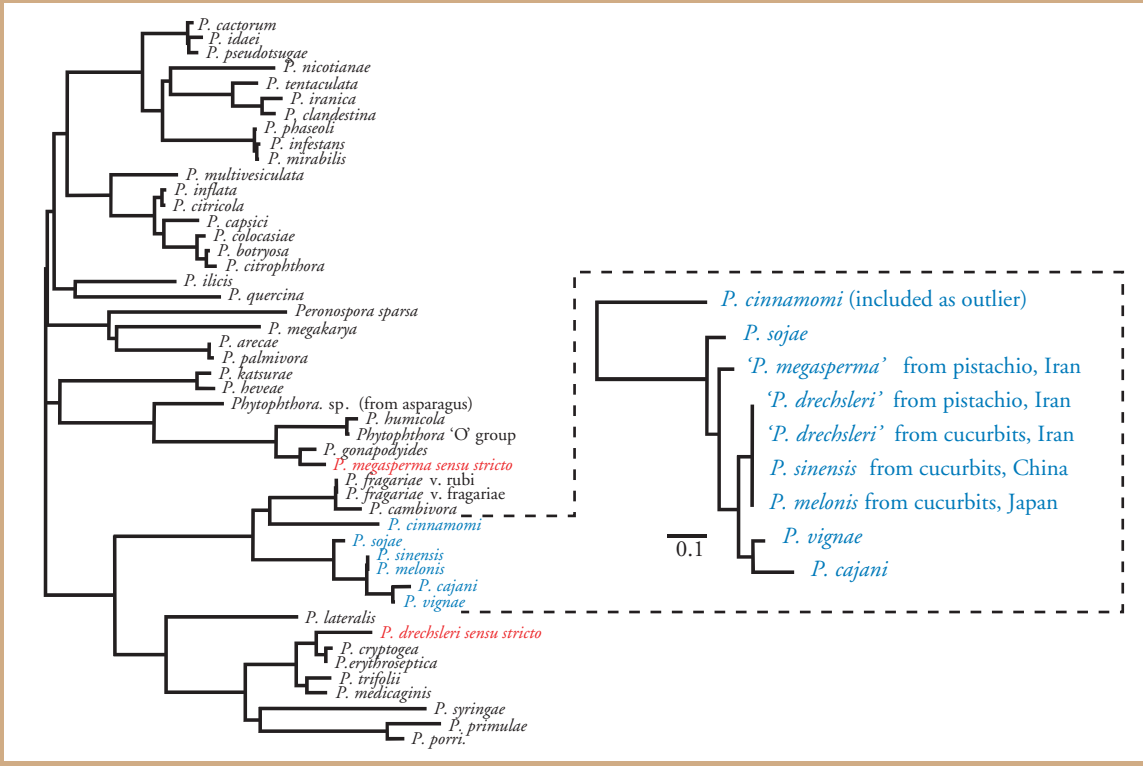


**Figure 1** Neighbour-joining DNA-distance phylogenetic tree based on the ITS sequences of *Phytophthora.* Part of the tree has been expanded to include the *Phytophthora* species from pistachio trees and isolates, supposedly of *P. drechsleri,* from cucurbits in Iran. *P. cinnamomi,* as one of the most closely related species not within the clade, has been included as an outlier.

The pistachio isolates that had been assigned previously to *P. megasperma* and *P. drechsleri* belonged to neither of these species. Instead they aligned within a closely related group of non-papillate species that comprised *P. cajani, P. melonis, P. sinensis, P. sojae,* and *P. vignae* (Fig. 1). The *'P. megasperma'* isolates obviously share a common ancestor with *P. sojae,* a species that causes a severe root rot of soya world-wide and one that was also once designated as *P. megasperma*[6]. In fact, neither is closely related to *P. megasperma sensu stricto* (Fig. 1). All the sequences or restriction digests of the ITS region of a large collection of *'P. megasperma'* pistachio isolates were identical. Their AFLP patterns were also very similar (Fig. 2), indicating that they all belonged to the same species. AFLP is time-consuming and expensive but it gives reproducible and detailed DNA 'fingerprints' and, because they are well dispersed throughout the genome, is an ideal counterweight to a single molecular marker like ITS.

Further support for the isolates belonging to a single species was their similarity in appearance on a range of agars and in other classical characteristics in culture. All molecular, morphological and physiological evidence marked them out as different from all other described *Phytophthora* species and, therefore, the name of the species *P. pistaciae* sp. nov. was erected for the *'P. megasperma'* isolates causing gummosis of pistachio[1].

Similarly, isolates of *'P. drechsleri'* from pistachio were not related to *P. drechsleri sensu stricto* but had identical ITS sequences (Fig. 1) and RFLPs and very similar AFLP fingerprints (Fig. 2) to *P. melonis* and *P. sinensis*, species that attack cucurbits in Japan and China respectively. Pathologists had long thought that *P. melonis* and *P. sinensis* might be the same species[7] and the present study confirmed it, while extending the host and geographical ranges of what should now be called *P. melonis,* the name with priority.

This finding promoted a re-examination of other so-called *P. drechsleri* isolates from cucurbits from Iran. These also belonged to *P. melonis,* a result that has important implications for control of pistachio gummosis in Iran, where cucurbits are widely grown, often in close proximity to pistachio trees. *Phytophthora* root

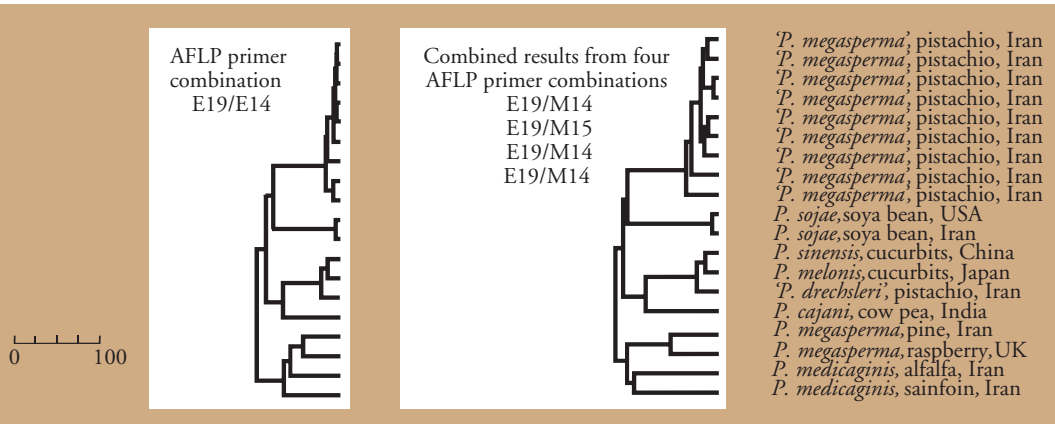


**Figure 2** AFLP-based dendrograms and respective images of banding patterns from which they were derived indicating the level of relatedness of the *P. megasperma*-like and *P. drechsleri*-like isolates from pistachio to other *Phytophthora* taxa. The top phylogenetic tree has been constructed form the results of a single AFLP primer combination (E19/M14); the bottom from combined results of four primers combinations (E19/M14, E19/M15, E19/M14, E19/M14). The scale bar indicates percentage similarity.

rot of cucurbits is common in Iran, as it is elsewhere in the region. Again the findings were confirmed by AFLP but, interestingly, the similarity among isolates in classical morphology and physiology seen in *P. pistaciae* was not seen in *P. melonis*. There were marked difference in these characters, although all isolates from whatever host and origin grew at temperatures >35°C.

So far, all described species belonging to the clade containing *P. pistaciae* and *P. melonis* attack hosts of Old World origin[8]: cowpea *(P. cajani)* in Asia; pigeon pea *(P. vignae)* in Africa and Asia; cucurbits *(P. melonis)* in Asia and world-wide; and pistachio *(P. pistaciae* and *P. melonis)* in Asia. Thus, it may be that this clade of *Phytophthora* is also of Old World origin, although an isolate that clearly belongs to the same group has recently been isolated from cassava in S. America (Alvarez, Lokke, Williams and Duncan, unpublished results). Whatever their origins, it is clear that *P. melonis*, at least, may be a much more common and more economically important pathogen than previously suspected.

### A PCR diagnostic for *P. pistaciae* and *P. melonis*

One aim of the project was to develop a diagnostic that could be used to identify and detect the main *Phytophthora* spp. causing pistachio gummosis and possibly detect them in tree nurseries on rootstocks. Many, if not most *Phytophthora* diseases, are spread through planting infected material and, where grafts are involved, it is logical to test the rootstocks for disease. In apples for instance, a survey within the USA showed that hardly any apple rootstocks were free of infection by *P. cactorum*, and infection by other species, such as *P. cambivora*, was also very common[9].

SCRI has developed molecular diagnostics for a number of *Phytophthora* diseases, most notably for red core (*P. fragariae*) of strawberries[10]. The approach taken has been a generic one based on a two-round nested PCR. In the first round, which is the same for all Phytophthoras, DNA extracted from plants (also soil and water) is amplified with primers DC6 and ITS4. These primers amplify only DNA of the Peronosporales of the Oomycetes (all *Phytophthora* spp. and their allies such as *Pythium*, the downy mildews and white blister rusts – *Albugo*). The product of the first round of PCR is then used as a starting point for a second, in which a forward primer located in ITS1 and a reverse primer in ITS2 amplify the DNA of a particular *Phytophthora* species, or a small group of closely related species.

In the case of the pistachio *Phytophthora*, all the species in the clade had very similar ITS sequences, making difficult the design of a set of unique primers for each species. A different approach, combining PCR and ITS-RFLP, was therefore adopted. A set of second-round primers was developed that amplified all the species in the clade but none from any other clade. Individual species then could be identified by digesting the resultant PCR product with restriction enzymes, which exploited small, single base-pair differences between the species of interest. The restriction enzymes that differentiated all the species in the clade from one another are given in Table 1. Note that *P. pistaciae* was differentiated from all others by digestion with restriction enzyme *Bsl*I (Fig. 3).

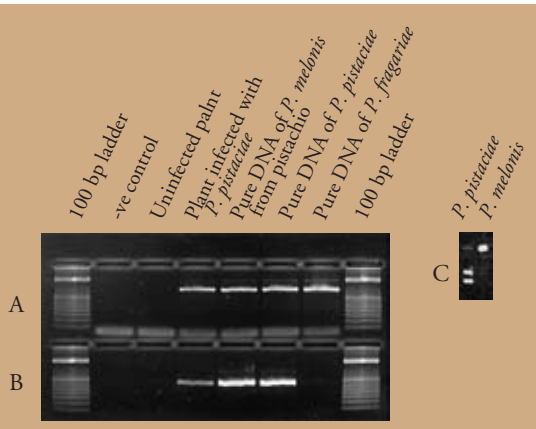


**Figure 3** *Right:* pistachio seedlings nearly 3 weeks after inoculation with a zoospore suspension of an isolate of *P. pistaciae* (formerly *'P. megasperma'*). On the right of this picture is a pot of un-inoculated seedlings (control). The inoculated seedlings which have collapsed (left) had badly rotted roots. Roots from inoculated and un-inoculated seedlings were tested by nested PCR.
*Left:* Nested PCR on infected and healthy roots from inoculated pistachio seedlings
A. - First round nested PCR with primers DC6 & ITS. B. - Second round nested PCR with primers PISfwd1 and PISrev1.
*Centre:* C. - Digestion of PCR amplicon generated by primers with restriction enzyme BslI.

Although the new diagnostic could not be tested on naturally infected pistachio trees at SCRI, it was tested on artificially inoculated pistachio seedlings in growth cabinets (Fig. 3). The test worked well in this environment and there is no obvious reason why it should not work under more practical conditions in Iran and elsewhere where gummosis of pistachio is a problem. It should also be applicable to any other disease caused by a *Phytophthora* species belonging to the same clade as *P. pistaciae*. Some of the diseases caused by other species in the clade are very serious: root rot caused by *P. sojae* is of world-wide economic importance and cowpea blight (*P. cajani*) and root rot of pigeon pea are locally important in India and Australia. The work therefore could have much wider application than originally intended, an unforeseen but welcome bonus!

| Restriction Enzyme | Phytophthora species | | | | |
|---|---|---|---|---|---|
| | *P. melonis* | *P. vignae* | *P. cajani* | *P. pistaciae* | *P. sojae* |
| AatII | | | + | | |
| AgeI | | + | + | | |
| AluI | | | | + | + |
| BsaW1 | | + | + | | |
| BslI | | | | + | |
| BsiE1 | | | | | + |
| DrdI | + | | | + | + |

**Table 1** Digestion by restriction enzymes of the PCR product obtained with primers ITS6 (forward sense) and ITS4 (reverse sense) from *Phytophthora* species within the same clade as *P. pistaciae*.

Acknowledgements

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# Plants, soils and environment

Geoff Squire, Karl Ritz & Donald Mackerron

*Not far into* Naturalists Voyage Round the World[1], *the traveller asked at a house whether robbers were about, and got the enigmatic reply 'the thistles are not up yet'. To appreciate this reply, the reader is told about the simple three-way interaction between thistles, travellers and robbers. The thistles grew very large at a certain time of the year, and in great beds, affording hiding places for robbers to attack the travellers. The nature of the vegetation determined the safety or otherwise of the people. The modern reader might indeed be fanciful. Would not the locals want to remove the thistles, or at least prevent them growing in such dense thickets? They would need to know how far to diminish them so they no longer provided sufficient space to hide robbers. They could sow a competitive species in among the thistles, or encourage a pest to spoil them, or sow a field nearby with a sexually compatible dwarfing form; or despatch them by an ingenious chemical. In time, by a variety of means, the thistles would have retracted and disappeared. But would not the people begin to miss them, to recall that the beds looked quite attractive in a great mass on a clear evening, catching the low sun, and to realise their roots prevented the soil being washed away in the wet season. Even robbers had become more sophisticated and no longer needed thistle beds to hide in. Some of the people wanted the thistles back, at least as a presence. Fanciful, yes, but these not-quite-so-simple matters of tripartite interactions are what the Division is attempting to understand, if not in every detail, then in enough to be able to suggest how people might coexist better among soil, bugs and vegetation.*

## Loss of diversity – loss of function?

Among the more newsworthy aspects of plant biology in 2001 was the matter of halting and reversing the loss of biodiversity from agricultural habitats. Farmland that supports a wide range of plant and animal species is the concerted aim of government advisory bodies such as Scottish Natural Heritage and English Nature, popular organisations such as the RSPB and the Scottish Wildlife Trust, and a great range of more local interests, as well as many farmers and scientists. The loss of weeds is becoming a test case of the effect of humans on their environment, and a focal point for ethics and art – witness a recent

commission for the 2001 season of the BBC Promenade Concerts[2]. A high level of biodiversity in arable fields and their margins will become a necessary output of farming. Safeguarding arable diversity will be a prerequisite for the development of high value products from crop biotechnology, including GM varieties. Getting profitable arable farming and biodiversity is not straightforward however. A definition is needed of a suitable level of biodiversity, and then a means found to achieve it. The Division's science is contributing to both of these aims.

On a practical scale, research groups are working to define criteria for the diversity and health of an ecosystem. The tests of soil resilience, described in the previous Annual Report and published in the journal *Oikos,* were later profiled as an 'editor's choice' in *Science*[4]. Our studies into soil biophysics (one of the research profiles following this introduction) and the molecular profiling of soil microorganisms will also contribute to a suite of methods for assessing the state of soil. Research on the arable seedbank has defined simple community-scale features that enable a comparison to be made of UK seedbanks at various times since 1915. Modelling of plant communities will now seek to define the crop genotypes and agronomy that will move seedbanks towards the desired states. The seedbank and the weed vegetation are themselves important sources of food and habitat for insects and mites. Some detailed studies of ecological interactions at these higher orders of complexity are given later in the second of the research profiles.

Our innovative theoretical approach, which defines biodiversity as a distribution of individuals across physiological 'trait space', gained further acceptance through publication in the journal *Nature*[3]. Further progress was achieved during the year through measuring and modelling the life history trajectories of plants. In order to relate vegetation to insects and higher organisms, the two-dimensional distributions of arable plants have to be translated to three-dimensional root and canopy structures. The modelling of this transition needs an appreciation of the way groups of individuals 'self-organise' and thereby distribute carbon and nitrogen among the plates and platforms of the plant canopy.

### Geneflow and ecological safety

Research on geneflow and persistence of genes and plants in the environment continued with new funding from SEERAD. The unambiguous pollen sources of the GM Farm Scale Evaluations in Scotland are allowing us to refine estimates of gene movement

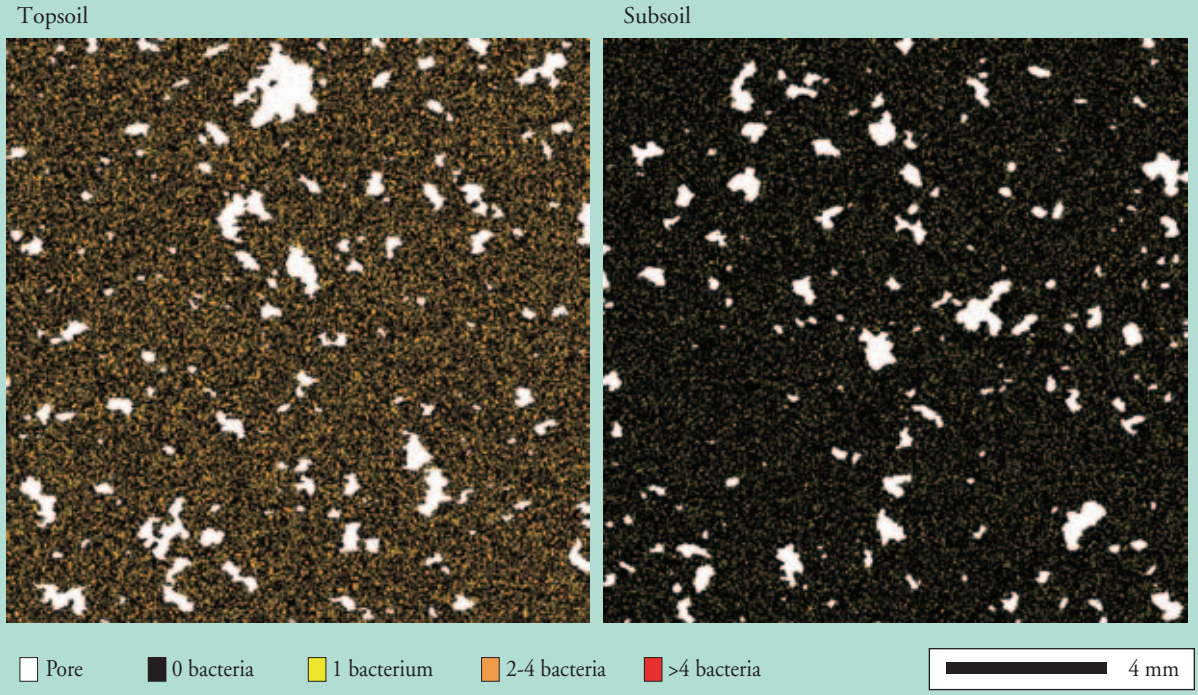


**Figure 1** Simulation of bacterial distribution and associated pore networks in mineral soils using Monte-Carlo method Markov-chain model, based on measurement of indigenous cells in an arable soil. The basic mapping unit (i.e. pixel size) here is 20 μm.

from such crops and to define the roles of different insects. (This work is joint with the Unit of Applied Genetics). Staff from SCRI have been asked to give evidence on and to shape policy in this complex area, both in the UK[5] and for the European Commission[6]. These are instances of impartial scientific investigation being translated into sound advice to members of Scottish, UK and European parliaments. The examination of farmland biodiversity in the Farm Scale Evaluations of GM crops has proceeded to schedule. Around 20 visits for data collection were made to each of the farm sites this year. The first results will be available near the end of 2002.

### The measurement and role of heterogeneity

To understand such ecological processes needs knowledge of how spatial and temporal heterogeneity in the physico-chemical environment affects the biota. The Division is now able to study heterogeneity over scales from soil pores to regions. For instance, using a combination of high-resolution multiple-scale mapping and innovative modelling, the first-ever detailed simulation maps of bacterial distribution at scales up to 5 cm in soil have been attained (Fig. 1). At scales of several millimetres, bacteria appear to be everywhere, but at smaller scales the cells are heterogeneously distributed and relatively large regions of soil are devoid of microbes. This distribution is important to a wide range of processes in soils, including those by which soil bacteria make certain toxic substances harmless. Flows of solutes through soil are very non-uniform, often following tortuous paths, which once established may persist. Transformation of the toxic material will only occur when compounds are in contact with microbes capable of mediating them, but the main pathways of flow might miss many of the microbes. Typically, only 1-10% of the microbial community can degrade toxins, i.e. most of the cells mapped in Figure 1 will be unable to degrade them. Mathematical models of bacterial locations, solute flows and degradation potential are being extended to three-dimensional space, and should indicate how soil and soil organisms might be managed in a way that brings the solute closer to the bacteria.

Another example is the process of nitrification (conversion of ammonium to nitrate), which is a very important part of the nitrogen cycle in arable soils. Nitrate is highly mobile and can therefore be delivered to plant roots with the mass flow of water, but can also be easily leached from the rooting profile into groundwater. Nitrous oxide is also produced during this process, leading to further N losses from soils, generating a potent greenhouse gas. Previous studies over many growing seasons at SCRI have shown temporal variation in potential nitrification rate (PNR) in arable soils. A new project aimed at detailed mapping of PNR in a study field has shown extensive spatial variation in the process at any one time (Fig. 2), and a unique data set that will enable modelling of the major soil N-cycling processes through the agricultural year. Further work is underway to understand the cause of this variation through molecular analyses of the bacterial communities (e.g. the eubacterial and ammonia-oxidiser bacteria) that are responsible for nitrogen transformations.

### Resilience on a still larger scale

Manipulating genotype and resource *together* remains the principal means by which we can continue to feed off the earth's resources without destroying them. The

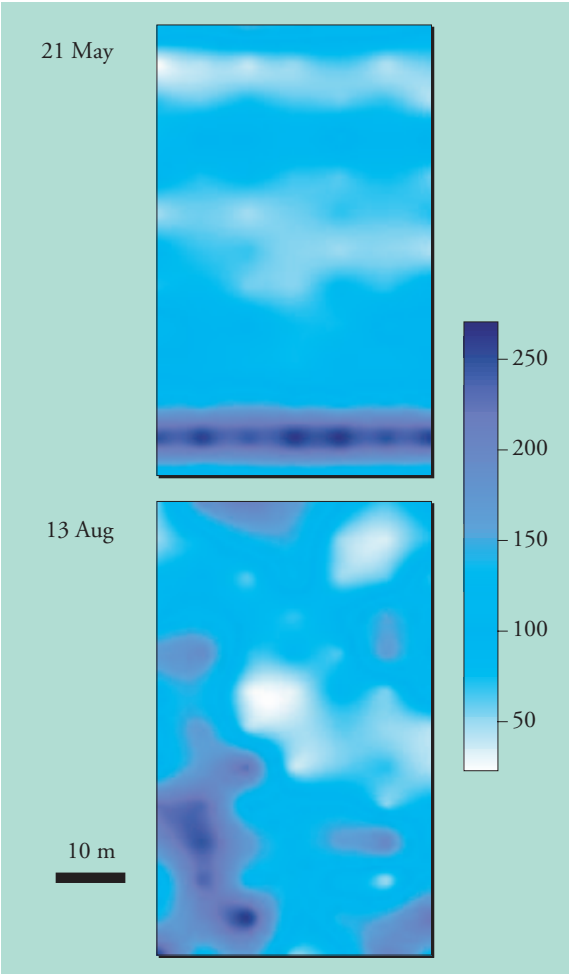


**Figure 2** Maps showing spatio-temporal variation in potential nitrification rate (PNR) in soils under spring barley in Bullionfield 2001. Scale shows PNR as μg $NO_2$-N/g/h.

enormous growth in actual and potential agricultural production over the past 200 years has encouraged the development of society whenever good agricultural principles have been applied. Conway[7], in his influential book on world-wide agriculture, has stressed that being able to continue producing food will require application of the soundest ecological principles with the latest biotechnology. The model ecological system suggested by Conway – the tropical, tiered 'garden' – has justifiably been criticised[8], but the general message remains true. Science, together with the wider interests of society, needs first to define the kinds of arable ecosystems that will satisfy the different ends of cropping, wildlife and greatly reduced pollution. Given this, crop genotypes and agronomy can be devised to meet those ends. Research should be proactive – designing safe and resilient production systems – rather than just reacting to assess the safety of the latest innovation.

The Division has as much to contribute to these global matters of food and environmental security as to the more esoteric arts of fine soil structure and population dynamics. Central to our role is defining and quantifying both the ecosystems and the crop genotypes that fit within them. Research in the Division has therefore seen further movement into barley as the central plant and barley-based arable rotation as the main system of study. This aligns us better with much of the genetical research at SCRI and with the arable systems in the nearby regions and in many other parts of the globe. Research has begun on genetic differences in root architecture and root function in barley, a programme with the potential to link gene expression in roots to rhizodeposition, microbial community function and soil biophysical properties. The ultimate prize is to understand how loss of root material to the soil through root skeletons, sloughing off of root cells, excretion of mucilage and exudation of a variety of chemicals, many at signal strength and of little use as food for soil microorganisms, might regulate the complex behaviour of soil microorganisms and hence the integrity of soil structure. Above ground, research teams have continued to investigate the three-dimensional features of barley canopies, as they affect pathogenesis and the coexisting arthropods. The potential for this science is a predictive understanding of how small changes in genetic information in crop varieties or wild populations propagate through phenotypes to determine features at and ultimately beyond the patch and field.

Finally, we give continued thanks to SEERAD, DEFRA, the research councils and other funders for our growing and diversifying research portfolio, and to those farmers and members of the public with whom we have interacted.

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# Evolution and adaptation of insects and mites to vegetation systems

B. Fenton, G. Malloch, R. Brennan & A.N.E. Birch

Plant living insects and mites provide biologists with excellent material to study the dependence of herbivores on the plants they consume. There are two strategies used by these organisms. The first is to exploit as wide a range of plants as possible (polyphagy). An insect such as the peach potato aphid (*Myzus persicae*) is a good example of this strategy[1]. It can colonise hundreds of different types of plants from many families and species. This path leads to competition from other insects, but has the advantage of offering plentiful food sources throughout the year. The second strategy is to become a specialist. This occurs by an organism becoming more and more adapted to the vegetation system i.e. the seasonal, physiological and ecological conditions of a host plant and its environment. In this second scenario, increased specialisation over evolutionary time is expected to lead to ever increasing selection pressure on the herbivore. It is forced to adapt to any changes in the plant, further restricting its ability to make use of alternative food sources, thus reinforcing the specialisation.

In the last decade, work at SCRI has started to use the highly sensitive techniques of molecular biology to examine the molecular ecology and evolution of groups of arthropods in vegetation systems. Where these are pests, it is important to understand how they have become so, whether they are generalists or specialists, how related they are, what their virus transmitting capabilites are and how readily they will be able to adapt to control measures, particularly plant resistance genes transferred between plant species.

This article describes work on two groups of arthropod pest, one insect and one mite, which has provided new insights to their evolution. The insect species belong to the byturid beetles. This is an interesting group that contains the important raspberry pests; *Byturus tomentosus* and *B. unicolor*. The mite species belong to the genus *Cecidophyopsis,* amongst which the most important pest species is the blackcurrant gall mite, *C. ribis*. However, other species of both groups are found on many other crop and non-crop plants. The host range of individual species in both groups is believed to be restricted to one or two related host plant species, but it was not clear how many species there are, or their exact host ranges. In addition to their use in applied research, the methodologies also contribute information to help identify and conserve endangered organisms, such as the byturids in tropical vegetation systems.

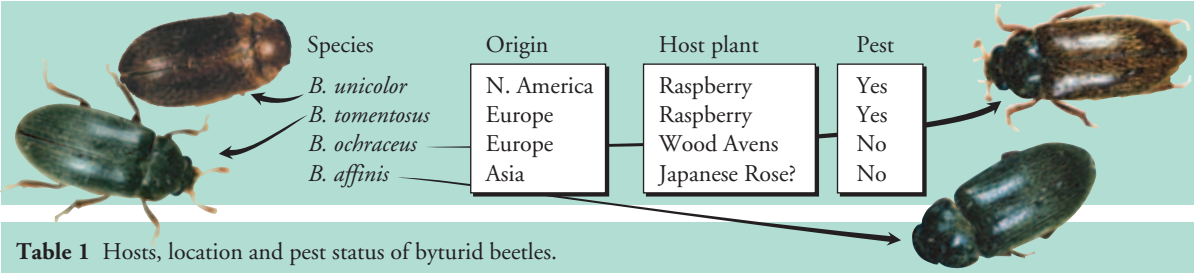


**Table 1** Hosts, location and pest status of byturid beetles.

The byturid beetles that have been included in this study are shown in Table 1. There are more species, but these are very rare in nature. In Figure 1, the life cycle of the European raspberry beetle is represented. The larvae of this beetle are only associated with two plant species, *Rubus idaeus* (raspberry) or *R. fruticosus* (bramble). There are a large number of physiological 'locks and keys' that are required for this beetle to complete its life-cycle. Firstly, it must emerge at the right time of year and find flowers on which to feed and develop its ovaries/testes (hawthorn), and then find flowers of wild or cultivated raspberry or blackberry on which to mate and lay its eggs. With only one generation a year, the adults get no feedback as to the success of their offspring, as they only live for a few weeks. Therefore, the attraction to plant chemicals must be 'hard wired', i.e. innate. In addition to finding host plants, the beetles must find mates of the same species, another critical step requiring recognition processes. Knowledge of these recognition processes can be particularly useful for developing control strategies[2]. For the beetles, their life cycle is aided by the relatively stable vegetation system with which they are associated. Specifically, their wild hosts are perennial, propagate through their roots and are associated with climax vegetation, remaining in the same area for a long time. Other byturids, where the life cycle is known, have a restricted number of plants on which the larvae can develop (Table 1). *B. ochraceus* has the narrowest range of all, as the larvae only develop on *Geum urbanum* - the wood avens. From these observations, it is clear that any biochemcial and evolutionary modifications made by their host plant, including speciation, would need to be matched by the insect. Thus, over extended periods of time, beetle evolution would be driven by their host plant's evolution and, where different species are found on two closely related plants, they should reflect the relationships of the plant, i.e. be two closely related species.

A similar situation is encountered with *Cecidophyopsis* mites. These tiny creatures can form galls on plants, hence the common name - gall mites. However, not all species form galls (Table 2). These mites are also believed to be highly host specific and, like the byturids, this specialism is likely to have been positively reinforced. One major difference between the mites and the beetles is in their sexual reproduction. The mites are haplo-diploid, with males developing from unfertilised (haploid) eggs, like bees and wasps, and the female is in control of their production. This

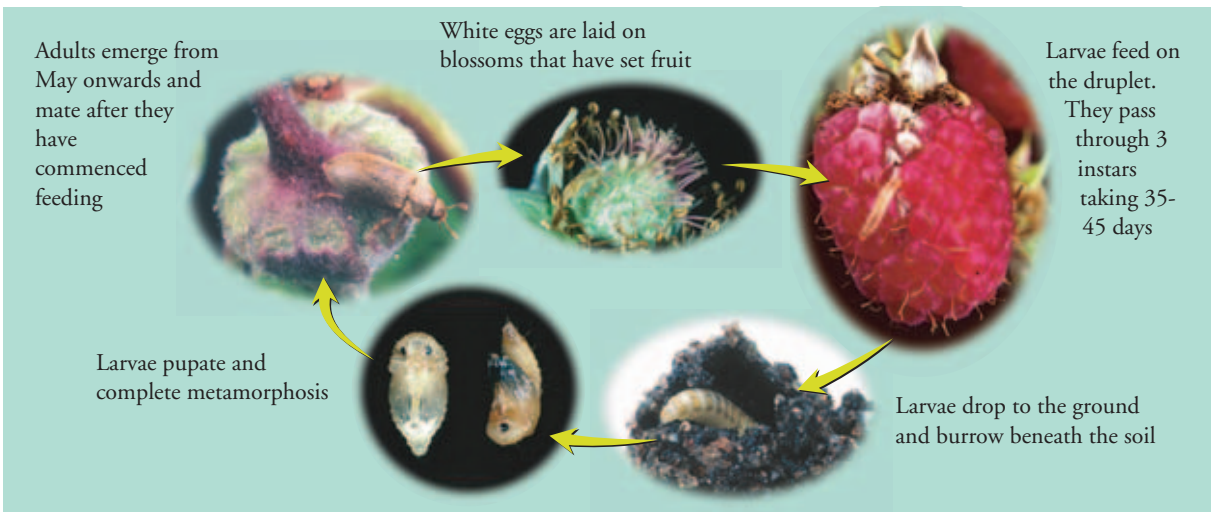


**Figure 1** Life cycle of raspberry beetle.

| Species | Host | Molecular ID | Morphological ID | Damage |
|---|---|---|---|---|
| *C. aureum* | Golden currant | Yes | Amrine | Galling |
| *C. alpinum* | Alpine currant | Yes | Amrine | Galling |
| *C. grossulariae* | Gooseberry / Blackcurrant | Yes | Collinge | Leaf browning |
| *C. ribis* | Blackcurrant | Yes | Westwood | Galling |
| *C. selachodon* | Red currant | Yes | Eyndhoven | Galling |
| *C.* WC (new species) | Red currant | Yes | No | Leaf surface |
| *C. spicata* | Red / Blackcurrant | Yes | No | Galling |

**Table 2** Hosts, descriptions and damage caused by the known *Ribes* infesting *Cecidophyopsis* species.

means that she can alter the sex ratio in her offspring, laying unfertilised eggs that develop into males when the timing is right. Therefore, unlike a byturid beetle, a *Cecidophyopsis* mite does not have to search for a partner. The single founder of a colony will generate both males and females. However, the other processes of adaptation to the vegetative system, i.e. seasonal movement, host finding and recognition, selection and biochemical adaptation, will be present alongside, in some cases, an ability to alter plant growth to form protective structures.

Classical study has long considered that host-specialised organisms will have co-evolved with their vegetative system. It is now possible to examine objectively the extent of this inter-dependence between arthropods and host plants using molecular and classical phylogenetic methods. The evolution of gene sequences is a classical and accepted method of tracking the evolution of the whole organism. In two

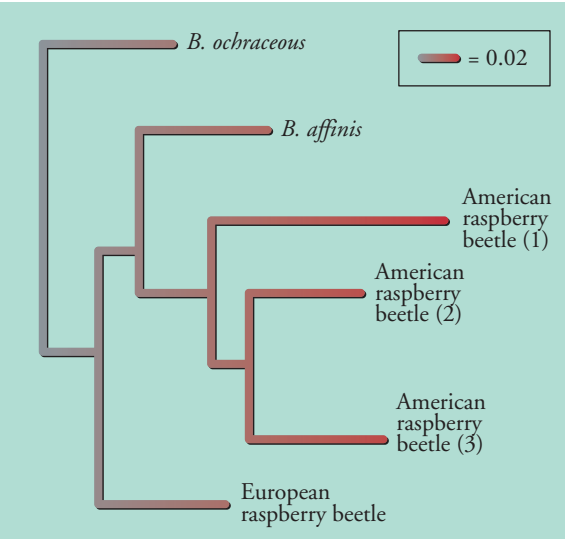


**Figure 2** A representative phylogenetic tree constructed from a maximum liklihood analysis of rDNA and mitochondrial sequences of byturid beetles. The American raspberry beetle had three biotypes indicated 1, 2, and 3.

populations which have undergone, or are undergoing speciation, changes in DNA sequences accumulate. The greater the time that passes, the more different the sequences become. Speciation will occur some time after a barrier to gene flow develops. The number of sequence changes allows the time-scale, and order of evolutionary events, to be estimated. Having done this for the insect or mite, it is then possible to compare the results to those of their host plants with the same or classical methods and determine the extent of parallel evolution.

In the byturids, there are beetles in both the old and new worlds, colonising European (*Rubus ideaus ideaus*) or American (*R. ideaus strigosus*) raspberry. The two raspberry species are inter-fertile. They, or a common ancestor, are likely to have been distributed over both continents prior to the separation of N. America from Europe. This distribution is termed holoarctic and is mirrored by many other plant and animal species including the Rosaceae (which includes raspberry) and beetle families. Therefore, it is a reasonable starting hypothesis that the two raspberry beetles on the two continents should be closely related, or even sub-species, like their hosts. However, we found that this was not the case[3], as shown in Figure 2. Instead, the American raspberry beetle is more related to an Asian species than the ecological equivalent from Europe. There is another important difference in that the America beetles have at least three distinct genetic lineages, whereas *B. tomentosus* is genetically uniform on an equivalent geographical scale, i.e. from Japan to Scotland.

In the case of the raspberry beetle pests of Europe and America, it appears that they have two distinct origins and that those of America may have colonised America from Asia. Asia is the main source of byturid diversity with 12/16 species present in this region. *B. unicolor* is also more diverse, perhaps due to the lack of competition from other closely related byturids. This genetic diversity probably renders the American beetle more adaptable as a pest.

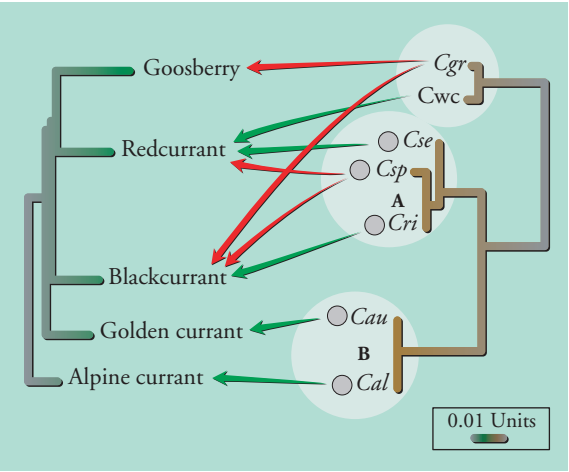


**Figure 3** The phylogenetic trees constructed from maximum liklihood analyses of the rDNA sequences of *Cecidophyopsis* mites and their host plants. The shaded circles represent those species which cause galls on the plant. The green arrows indicate the host plant for mites with single hosts and the red arrows those for mites with two hosts.

For the *Cecidophyopsis* mites, the phylogenetic analysis was taken one step further, as an assessment was also made of the genetic diversity of their host plants[4]. Therefore, it was possible to subject samples of host DNA to exactly the same analysis as the mites. This provided two molecular phylogenetic trees, which could then be compared directly (Fig. 3) to give detail about how the two groups of organisms related to each other. The main points can be summarised as follows: 1) The *Ribes* host plants appeared to have undergone a rapid diversification early in their evolutionary history and since then there has been relative stability. 2) The mites have undergone continuous evolution, with very recent diversification events giving rise to three groups. 3) There is little correlation (phylogenetic tracking) between the mite's evolutionary history and those of the host plants. 4) There is correlation in the gall forming character with the two non-gall forming mites being closely related, yet their host plants are not. 5) Two mite species appear to have more than one host, whilst the rest have a single *Ribes* host.

This work has discovered that three of the main soft fruit pests of the world have not been evolving along with their hosts, and so are likely to be able to adapt to new challenges. This is based on the following observations: 1) Host switching, such as when *C. aureum* colonises *R. aureum* in Europe although the plant originated in America. 2) Two mite species have more than one host. 3) The byturids have colonised raspberry at least twice to give rise to the European and American raspberry beetle lineages. 4) The American raspberry beetles contain three genetic lineages, which are likely to indicate extended phenotypic capabilities. This information can now be used to test new control strategies on the full range of pest potential, providing more durable pest resistance in the long term. One major aim of SCRI is sustainable agriculture using pest-resistant crops to decrease dependence on pesticides. Other benefits of this fundamental research are: 1) Information for deployment strategies (and models) for resistant crops. 2) Arthropod-plant interactions can now be studied on ecological and evolutionary time scales and we can therefore compare the effects of intensive agricultural selection with longer-term effects.

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# Plant root and microbial derived soil water repellency

P.D. Hallett, J. Douglas[1], K. Ritz, R.E. Wheatley & I.M. Young[2]

Plant roots and soil microbes have a massive influence on the physical properties of soil. The exudates and cellular biomass that they produce are active in binding together soil particles, thereby creating an aggregated structure that is superior for crop productivity and environmental buffering. These compounds are therefore an essential component of soil sustainability. Some exudates and biomass coat soil particles with films that alter surface properties considerably. One potential consequence is a hydrophobic surface that induces water repellency, thus influencing water transport and retention in soil.

Until recently, water repellency was assumed to affect a small percentage of the world's soil resources, and to be insignificant in the UK. In extreme instances, the soil is rendered infertile because repellency prohibits the uptake and retention of water that is essential for plant growth. The land area affected by this problem tends to have a sandy soil texture or be located in warm climates. In the UK, this problem is found predominantly on engineered sports-turf. Our research programme on biological and physical interactions in soil, however, has identified that repellency at much lower levels is commonplace in most soils. We believe that this property of soil is paramount in causing the preferential flow of water and pollutants, and also an essential component of soil pore structure stability.

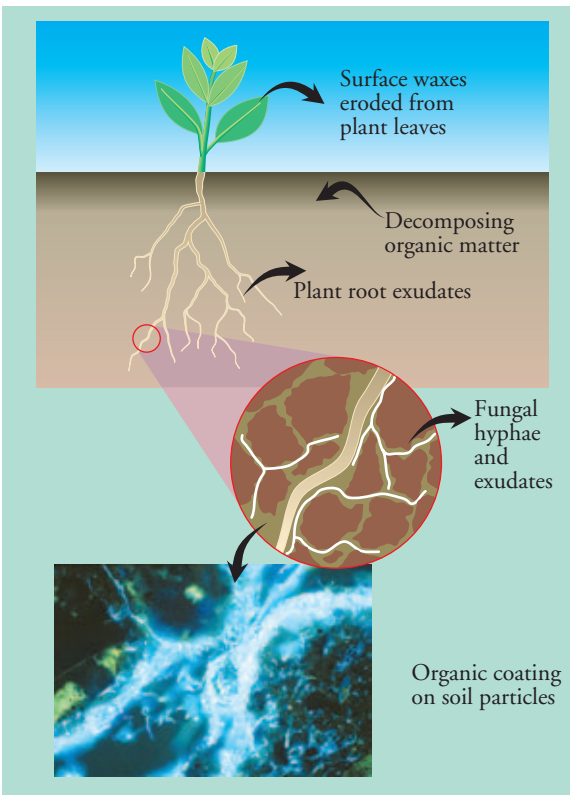


**Figure 1** The biological origin of water repellency in soil. The image showing the organic coating on soil particles in a soil thin section with the carbohydrates stained with fluorescent dye.

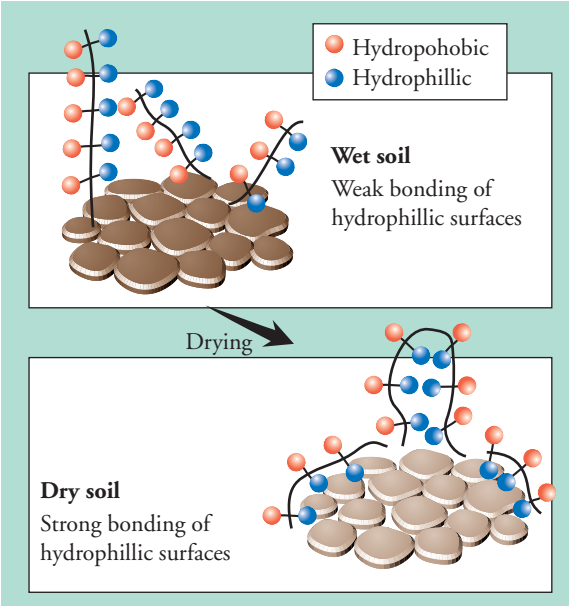


**Figure 2** The change of hydrophilic compounds to hydrophobic surface coatings in soil caused by drying. After prolonged wetting, the hydrophilic nature of these chemicals will be recovered.

## The origin of repellency

Potentially hydrophobic organic materials are produced by plant root exudates, certain fungal species, surface waxes from plant leaves, and decomposing soil organic matter (Fig. 1). Exudates are produced by plant roots and some soil microbes to enhance nutrient availability and defend against desiccation stresses. They are strongly hydrophilic when wet, but below a critical moisture threshold, the hydrophilic surfaces bond strongly with each other and soil particles, leaving an exposed hydrophobic surface (Fig. 2). The

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[2]University of Abertay, Dundee


Figure 3 Device that allows the direct measurement of water transport properties of soil and repellency, at hitherto unattainable spatial scales.

level of repellency depends on the proportion of soil particles with a hydrophobic surface coating.

### Measuring repellency

Various techniques exist for measuring soil water repellency, but they are only effective when the levels are quite high and some techniques are too crude to provide physically meaningful results. We have developed a highly sensitive testing technique that uses the fundamental properties of soil hydraulic transport to provide a quantified measure of repellency. A miniature infiltrometer probe (Fig. 3) is used to assess wetting rates from which sorptivity (i.e. the physical measurement of liquid absorption by soil) is evaluated on samples as small as a few millimetres in diameter. From these data, a repellency index, *R*, is obtained by comparing the sorptivity of water to ethanol. *R* is directly proportional to the reduction in water transport caused by repellency. If *R* is 5, for instance, repellency causes a five-fold decrease in water transport rates.

### Linking soil biology to repellency

Research examining repellency levels in soil is considerable, but very little of this work has investigated its biological origin. Our first experiments, therefore, were devised to verify whether or not repellency could be induced by biological activity. We found that relatively high levels of water repellency could be created by stimulating the biological community in soil with simple sugars (10 mg C $g^{-1}$ soil) and micronutrients. Subsequent, more detailed, work involved investigations of the effects of fertilizer application in the field, cultivation methods and specific organisms.

The addition of fertilizer to soil has obvious benefits for crop productivity, but its stimulation of both plant growth and microbial activity may also affect soil physical properties. As part of a detailed investigation linking biological and physical properties of soil under different levels of nitrogen fertilizer, we found that, after one growing season, 120 kg/ha of added nitrogen increased the repellency index, *R*, from 4.5 to 6.5 in a no-till cultivation experimental site maintained by the Scottish Agricultural College (Beechgrove Field, Penicuik). These levels of repellency are very low, and undetectable using conventional techniques. They will influence soil stability because a major disruption mechanism in soil is slaking caused by air-pressure build up in advance of wetting fronts. If the build up of air-pressure is sufficiently high, the soil explodes and the aggregated structure is lost. The difference in *R* values suggests a 45% drop in air-pressure build up due to repellency alone when a high level of fertilizer is applied. Soil stability is examined in most soil management studies, but this is the first instance where repellency has been isolated as one of the fundamental stabilising mechanisms. A negative implication of this result is that repellency induces preferential flow pathways. This may provide a rapid trans-

port pathway for environmentally sensitive solutes, such as nitrate or pesticides, to groundwater.

Intensive cultivation causes death and destruction to parts of the microbial community in soils. It can also deplete organic carbon levels *via* an enhancement of the degradation of otherwise physically-protected organic matter. On studies at several experimental sites in Europe, ploughed soil had lower *R* levels than adjacent undisturbed soil (Fig. 4). The only exception was pasture soil at the Beechgrove site mentioned previously, where the *R* value was the lowest. Currently, we are investigating the underlying mechanisms that could account for this result. The lower repellency levels that were found generally in the disturbed soil suggests that it will be more susceptible to disruption by slaking, thereby loosing its aggregated structure soon after tillage. This phenomenon is a widespread problem in agricultural soil, causing reduced plant productivity and pollutant buffering.

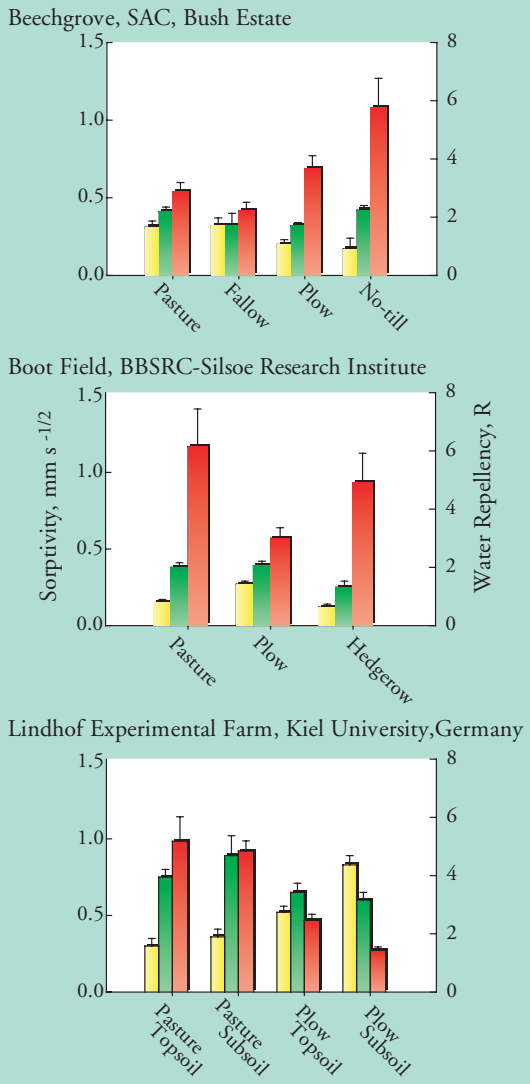


**Figure 4** Cultivation effects on water sorptivity (yellow), ethanol sorptivity (green) and water repellency (red) for a range of soils. Water sorptivity is a basic transport property of soil, so these results can be used directly in models to predict and describe water flow properties. Ethanol sorptivity is not affected by hydrophobic substances, so it can be used to assess the structure of transmission pathways in soil. The repellency levels, R, are high enough to buffer wetting stresses that cause soil slaking, but are too low to impede water availability to plants.

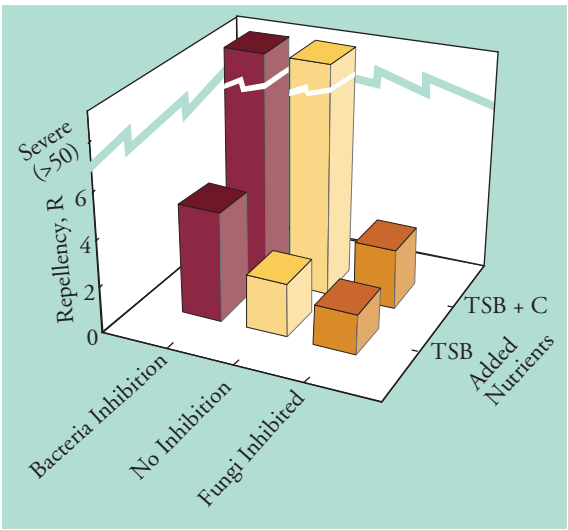


**Figure 5** Assessing the microbial groups responsible for repellency. The soil is from a golf course and has been amended with nutrients to stimulate the native microbial community; TSB refers to Tryptone Soya Broth (2 mg carbon $g^{-1}$ soil), and C refers to glucose (18 mg carbon $g^{-1}$ soil). Fungal and bacterial growth are suppressed using the biocides cycloheximide and streptomycin respectively.

The effect of different microbial groups has been studied using biocides to suppress the growth of either fungi or bacteria in soil (Fig. 5). This work confirmed previous speculation that fungi are the dominant microbial group that causes repellency. It also showed that suppressing competition from bacteria caused a higher level of repellency to develop. There are potential applications of this research for the biological control of the organisms that cause repellency by manipulating the microbial community structure. The results presented are for a golf course soil where repellency is a major problem. Treatment costs in the UK alone are about 10 000 000 Euros. We have continued this research to investigate the effect of specific fungal species on repellency.

### Linking repellency to preferential flow

Repellency causes water flow through preferential pathways in soil. This limits the soil volume available for pollutant buffering and increases the erosive effect of water across soil surfaces. We measured the spatial distribution of repellency and water flow on the surface of a large slab of grassland soil from south-east Scotland to investigate preferential flow. Our work was unique for two reasons. It was the first where low levels of repellency were measured alongside water transport. Also, the size of our infiltrometer is the smallest in existence, thus permitting sampling on a 50 mm grid, the highest resolution ever recorded for direct water flow measurements in soil.

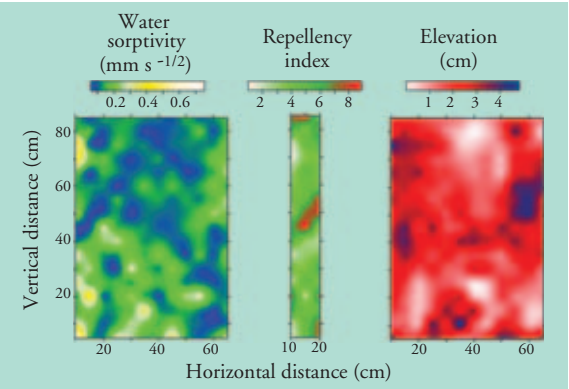


**Figure 6** The spatial distribution of water sorptivity, repellency and elevation measured in a 50 mm square grid on an intact block of soil. Patches of low water sorptivity will induce preferential flow pathways that accentuate erosion. These areas are closely linked to areas of higher repellency.

Figure 6 shows the spatial distribution of water sorptivity (i.e. wetting rate), repellency, and elevation. There are some distinctive areas where water sorptivity is impeded severely by repellency. These regions will enhance the overland flow of water, increasing its erosive effect on soil and potentially providing a nucleus for gully formation. Soil erosion is known to be highly problematic where severe levels of water repellency exist. The soil we examined had a very low level of repellency and, like most soils, appeared to take water up readily. Nevertheless, repellency will still influence the development of water channels that enhance erosion. This effect was not known previously. Enhanced overland flow due to repellency, particularly after dry periods, may also increase the risk of flooding.

### Future prospects

Our research on repellency is seeking to quantify one of the fundamental mechanisms imparted by plants and microorganisms on the formation of soil structure and water transport. We have shown so far that low levels of repellency occur in most soils, and that these levels are susceptible to physical disruption by tillage and the stimulation of organisms by added nutrients. Some of the research discussed is from larger research projects, where the microbiological properties of the soil are being described in detail.

We have already started to link repellency with other biological mechanisms involved in the formation of soil structure. The common perception is that exudates influence structure primarily by binding soil particles. By studying different exudates of biological origin, we have found certain exudates that impart repellency to be far better at stabilising soil against disruptive stresses.

The research has already isolated a previously ignored physical property of soil that has implications for soil structure dynamics and the development of preferential flow pathways. We want to exploit repellency, so that soil physical structure can be improved by prescribing appropriate plant cultivars and stimulating soil microorganisms that are superior in producing stabilising exudates. This has applications in land regeneration and is an essential component of soil sustainability. In this instance, repellency levels would be too low to affect the plant availability of water ($R$<5). High levels of repellency, $R$>50, present a problem to UK sports turf, and in other regions (e.g. Australia) impede agricultural production across very large areas. By understanding the biological origin of this problem, we will help to develop control strategies that do not require the costly application of chemical surfactants.

# Knowledge engineering - science into practice: putting knowledge where it counts

B. Marshall, G.D. Lyon, A.C. Newton & J.W. McNicol

In the SCRI annual report of 1993, we wrote about the opportunities opened up by flexible modelling, which addressed knowledge and uncertainty. A point then, which is equally important today, is the ability to reveal the wealth of strategic knowledge previously hidden in the 'black boxes' of crop models constructed in procedural languages such as FORTRAN and PASCAL. There are two types of knowledge, domain knowledge or facts, and strategic knowledge or know-how. It is in this second aspect that flexible modelling has its greatest impact, enabling the experts' know-how to be passed on to the client in forms that are understandable and context specific. Strategic knowledge is knowing how best to use the facts to make informed judgements either in short term tactical decisions or in longer term planning. Eight years on, and we have reached the market place with two products: mapp™, the Management Advisory Package for Potatoes for immediate decision making, and PCN, a longer-term planning package for the control of potato cyst nematode.

More than 5M tonnes of raw potato are produced each year in the UK. The future of the UK potato industry depends on quality. The average value of the raw product is around £40 per tonne but this can be easily doubled or trebled by achieving the right size grades of quality potatoes for baker and pre-pack markets and can even be as high as a ten-fold increase in the specialist markets of salad potatoes. Failure to achieve the right size of tuber alone is estimated to cost the industry £24M per annum. mapp™ helps to match seed rates to expected yield and market, tracks crop development from planting to burn down; manages weather data; helps decide herbicide application; enables an effective irrigation strategy; helps to limit common scab; predicts yields and changes in tuber size distribution; shows the relation between burn-down dates and profits; uses the grower's management data and sample digs to predict values that are unique to the specific crop; covers over a hundred commercial cultivars; applies to all market outlets; and provides context-sensitive help (Fig. 1).

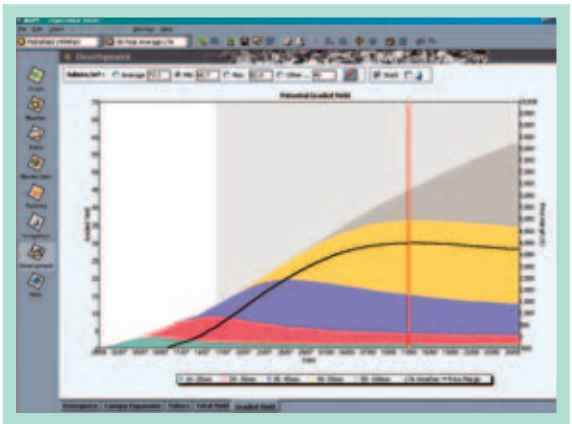

Figure 1 mapp™, the Management Advisory Package for Potatoes, predicting the development of graded yields in a seed crop with time (pale blue, <25 mm; pink, 25-35 mm; blue, 35-45 mm; yellow, 45-55 mm; grey, > 55 mm).

This user-friendly software package has made available to the industry nearly two decades of research into the physiology of potato growth, development and yield. This has been possible by combining the skills of crop and environmental physiologists, mathematical modellers and staff of the Department of Artificial Intelligence and the Artificial Intelligence Applications Institute at the University of Edinburgh with the experience of the potato industry itself. Windows-based programming provides the user-friendly interface that the customer has come to expect from modern software, and the framework for integrating contrasting programming techniques. Databases store the weather data, both current and long term average for predicting the future of the crop, as well as the husbandry data and, most importantly, the information gathered by direct observation of crop performance. Models, tried and tested in the field throughout the UK, of the evolution of tuber size distribution and water-limited yield are implemented in Visual C++. Then CLIPS, a rule-based system, provides the 'intelligence' or know-how to answer a given question and only prompt the user for missing information that is essential to answering that query. Visual Basic provides the Windows-based framework.

A multi-disciplinary team is essential and its diversity presents challenges for both coordination of and com-

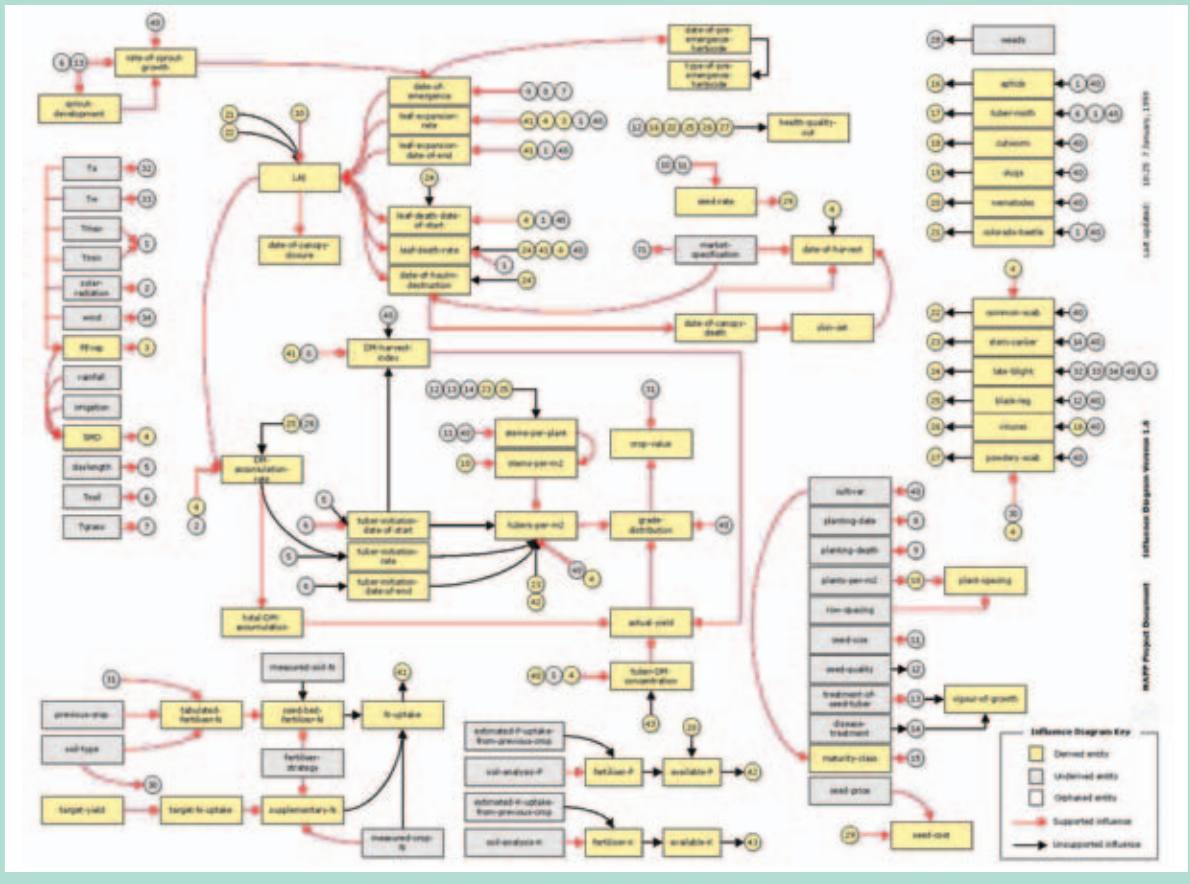

Figure 2 The Active Influence Diagram for the mapp™ project - the pivotal tool for coordination, communication and design - showing the inter-dependencies of key crop characters, weather and husbandry (red arrows indicate those influences currently addressed and black arrows are for future consideration). For clarity, not all arrows are shown in their entirety and circles with the same number indicate that they share a common connection. Clicking on any character (box) invokes a query to an underlying database that reveals the immediate and all secondary influences on the character.

munication within such a project. The creation of an Active Influence Diagram became pivotal (Fig. 2). It provides the discipline for defining the project, the ability to communicate ideas accurately and efficiently between team members, eliminates jargon and documents the structural specification. Using software developed in-house, the diagram automatically generates a database of inter-dependencies between the variables along with the explanations of each variable. The CLIPS rule-base is then constructed directly from this database. Design is an iterative process; its visualisation and automation through the active influence diagram was a major benefit to the project.

The PCN model (Fig. 3) is an example of another expert system being developed at SCRI to aid in the longer term management of the white potato cyst nematode (PCN) which is reaching epidemic proportions in which approximately 60% (and increasing) of

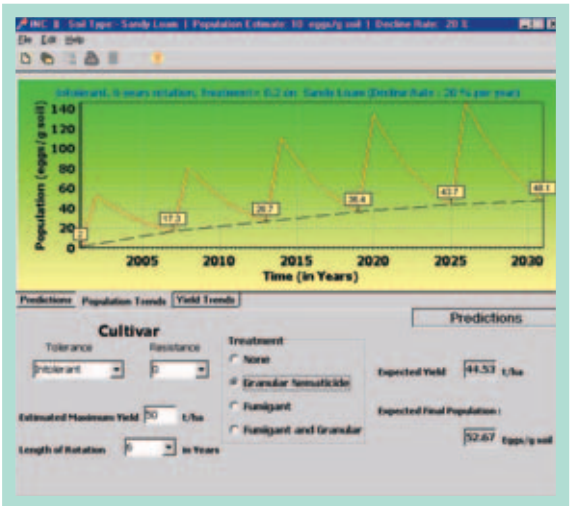


Figure 3 PCN expert system: predicting population trends in white potato cyst nematode and impact on yield for a user-specified management strategy.

potato land is infested and is currently costing the industry an estimated £50M per annum. The system shows that this situation will continue unless there is more effective integration of existing control measures.

Rotations have shortened with the introduction of potato varieties resistant to the previous nematode pest, the golden PCN, in the late 1960s. It takes approximately 30 years, typically five potato crop rotations, for very small populations to increase to damaging proportions. 94% of potatoes grown in the UK are susceptible to the white potato cyst nematode. There are no fully resistant potato varieties commercially available and those that do have a degree of (partial) resistance have limited market outlets. Granular nematicides appear to be less effective at controlling the white PCN than the golden PCN species. The growing population of white PCN can go undetected until it is too late. This new computer-based model assists the grower to predict the effect of the management strategy that they intend to implement and thus reduces the risk. It also highlights the need for good sampling strategies and then makes effective use of the data collected. Although less complex than mapp™, the development of a user-friendly expert system for PCN management has also benefited from the development of a Windows-based programming environment.

Reflecting back to the article published in the 1993 Annual Report, the incorporation of the effects of uncertainty into models for risk management has been slower to reach the market place than anticipated. Uncertainty exists both in the formulation of a model as well as in its inputs. Mathematical models are frequently constructed using some form of 'best fit' relation with estimates of the associated parameters. The fact that the observed data does not lie precisely on this relation, rather it is randomly distributed about the relation, is often ignored. Ideally, this should be captured in the model by, for example, including both the best estimates of the parameters' values and a measure of their uncertainty. The uncertainty of future weather also plays an important part in forecasting the performance of a crop. The availability of local weather records is often limited both in the number of years and detail. Accordingly, weather generators have been developed to reproduce the mean and variability of the key weather characters. They are calibrated for the specific location and rapidly generate a set of variable seasons representative of the locale. They are undergoing further refinement; capturing the correlations between the variables and paying special attention to the frequency of wet days and the distribution of rainfall amounts.

The incorporation of uncertainty has been slower because software for constructing models that capture this uncertainty or probability had to be written in-house, from first principles. Now there is both commercial and free software available for the construction of probability models, e.g. Bayesian Belief networks or 'Causal models', that do not require specialist programming skills to use, and their utility has been enhanced. Originally, probabilistic relations between a set of variables could only be represented by a set of joint probabilities. This meant that all variables had to be converted into a set of qualitative ranges, e.g. 'very low', 'low' … 'very high', before joint probabilities could be assigned. As the number of discrete ranges per variable increased, so the requirement for computer memory increased exponentially. Modern software has removed this constraint. Relations between variables no longer have to be made discrete and the belief in the value of the associated parameters can take any form of continuous probability density function. In a recent piece of research, using this modern software, we have produced a probabilistic model of the factors influencing

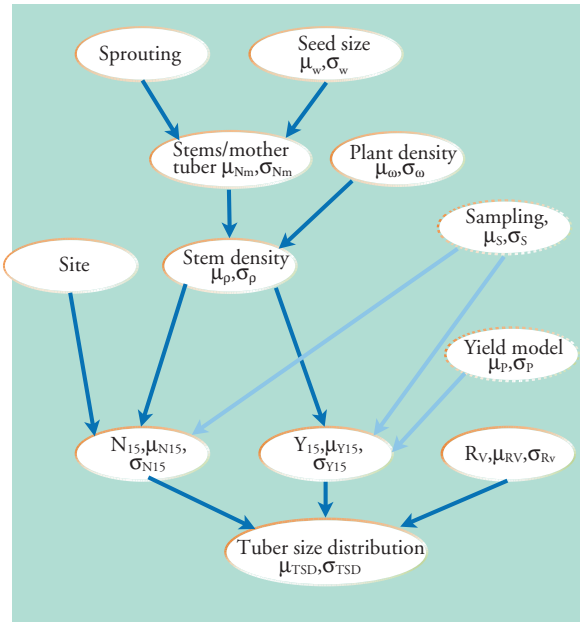


**Figure 4** Simplified causal graph of factors affecting tuber size distribution. The parameters μ and σ indicate that the variables have both a mean and variability. Likewise, the parameters that define the relations between any two nodes (ellipses) have both means and variances associated with them, which together capture both the best-fit relation and the variability about them.

tuber size distribution (Fig. 4). It requires further testing before considering release into the market place. Nevertheless, it can already provide valuable quantitative insights into where the greatest causes of uncertainty in its predictions lie.

The demand for knowledge engineering, including probabilistic modeling, is growing rapidly in many biological research areas. For example, in pathology where in the last few years many new genes have being identified, from many different host-pathogen interactions, revealing both common processes and unique pathways. Any model designed to provide an integrated understanding of pathogen recognition and defence response must include spatial and temporal aspects to the expression of these genes. The recent development of Expressed Sequence Tag (EST) technology, together with enrichment processes such as Suppression Subtraction Hybridisation (SSH), means that the discovery of genes induced in response to treatment such as pathogen recognition is outpacing our ability to carry out research to understand their function. In order to build such an understanding, many isolated host-pathogen recognition models have been published; most of these became outdated quickly by new information and proved too inflexible to update. Furthermore, many new gene discoveries simply can not be integrated with these models and require new speculative relationships to be devised. These speculations are being used to drive new experimentation, so it is essential that they are able to incorporate all the most recent data. Currently there is no such integrative mechanism. We have drawn together data from our gene discovery programmes in a spatial diagrammatic representation of pathogen recognition in potato (Fig. 5). Gaps in our understanding of plant-pathogen interactions have been filled from the published literature on other systems, including animal pathogens and other informed speculation. However, the diagram has become complex, cannot be systematically queried, and has no temporal dimension. Furthermore, it is difficult to distinguish between the sources of information. Nevertheless, it continues to be the focus of informed discussions, which have generated many testable hypotheses.

Another important potential application is the prediction and tracing of contaminants, both in ecological

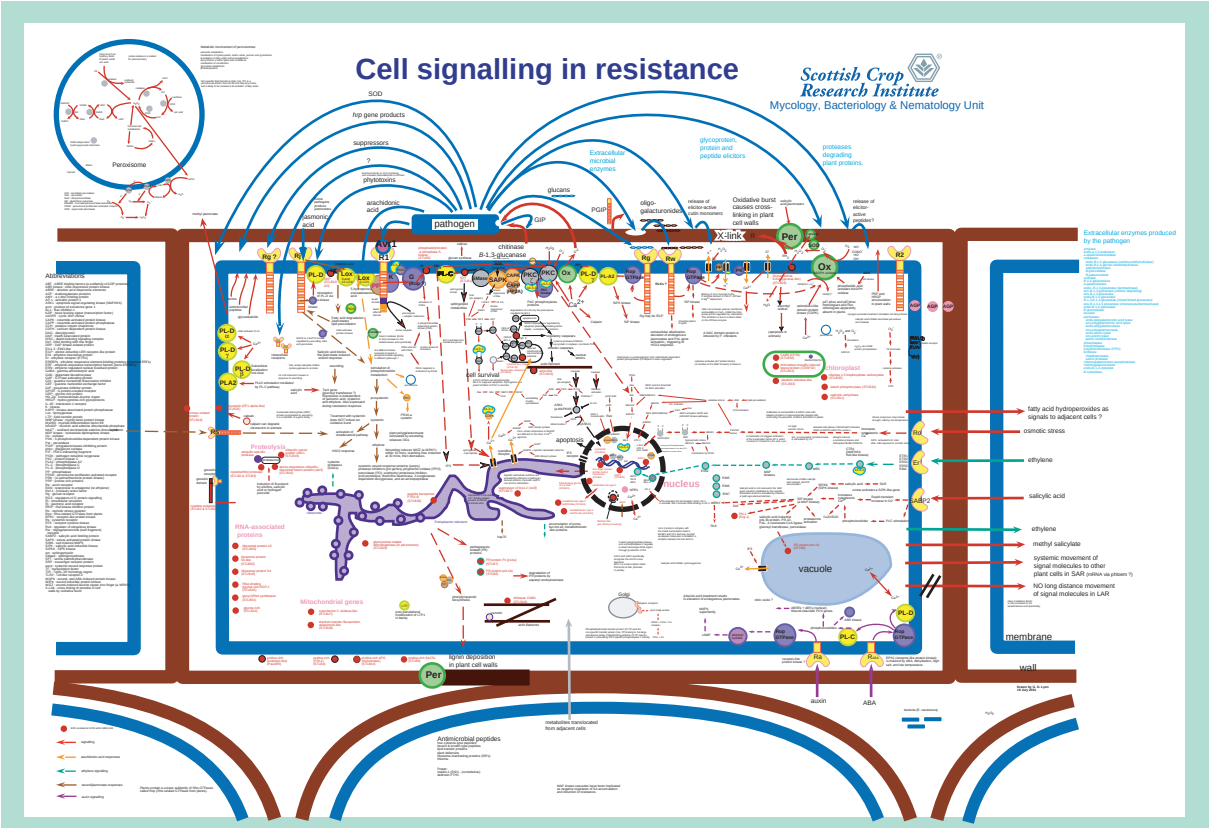


**Figure 5** A diagrammatic representation of some known and postulated relations between molecules involved in signalling events associated with the response of potato to infection by a plant pathogen.

systems and in food. For instance, SCRI's MAFF-funded work with the Central Science Laboratory on modelling oilseed rape feral populations could be the forerunner of software of equal value to ecologists, agronomists and food standards inspectors. Models based on a partial understanding of a system, such as the host-pathogen response, are used to promote debate about the nature of the system. Informally represented models, e.g. where we rely on a subjective understanding of a diagram, are limiting in the forms of analysis that we can apply to them, and in the extent of information to which they can reliably connect. We need automated techniques, which stimulate exploration and hypothesis formation while adding the precision and objectivity necessary to integrate knowledge from diverse and rapidly expanding knowledge sources. These are some of the challenges facing knowledge engineering and biologists working together.

Developing these software applications requires the skills of many people. In particular we would like to acknowledge the inputs of M Elliot, DKL MacKerron, MS Phillips and DL Trudgill at SCRI, and R Rae , D Robertson and J Tonberg of the University of Edinburgh.

# Biomathematics and Statistics Scotland

Rob Kempton & Jim McNicol

*Biomathematics & Statistics Scotland (BioSS) is devoted to the application of statistics and mathematics in the biological sciences. Its core remit is to support the SEERAD programme of biological research, which is carried out within the SABRIs, SAC and RBGE. This is achieved through a dispersed group of statisticians, mathematics and computing experts based at BioSS centres in Edinburgh, Dundee, Aberdeen and Ayr. Over the last year, much effort has been spent on planning new programmes to meet our strategic priorities in bioinformatics and statistical genomics, systems modelling and risk. Work in bioinformatics was enhanced by an alliance with the Scottish Centre for Genome Technology and Informatics, initially focused on analysis of microarrays. Our programme on risk analysis is built on developing probabilistic methods for assessing food safety and covers several potential and known hazards, including mixtures of pesticide residues, zoonotic pathogens such as E. coli O157 and salmonella, GM crops and poor diet. New competitive core funding will allow expansion of our programmes in bioinformatics and systems modelling in 2001/2.*

At SCRI, the development and application of statistical methods that recognize the importance of scale in ecological processes is being pursued in collaboration with staff from the Plants, Soils and Environment Division. These ideas are being applied in a number of important areas including the quantification of functional biodiversity in vegetation systems and the interaction between plant and microbial communities.

Work on the genetics of tetraploid species has progressed with the development of methodology for QTL mapping. This is an extension of the interval mapping method used for mapping QTLs in diploid species, but requires a novel step, namely the reconstruction of the genotypes along an entire chromosome for each offspring, and the location of crossovers. The methodology for linkage analysis and QTL mapping is currently being applied to 228 potato lines scored for more than 800 molecular markers and 40 traits (disease resistance, yield, processing quality, *etc.*), in collaboration with scientists at SCRI.

In sequence analysis, an approximate Bayesian hypothesis test for discriminating between alternative DNA mosaic structures has been developed. Our work on Hidden Markov Models (HMMs) for detecting recombination is being expanded. The earlier approach estimated all the parameters of the model in a maximum likelihood sense with the EM algorithm. We are currently exploring a Bayesian approach, where parameters are sampled from the posterior distribution with Gibbs sampling and the Metropolis-Hastings algorithm.

BioSS has also contributed to two decision support packages being developed at SCRI. The Management Advisory Package for Potatoes (mapp™) enables growers to visualize the effect on profit margin of various management decisions relating to factors such as planting date, seed rate and harvest date. The Potato Cyst Nematode (PCN) package demonstrates the effectiveness of various control strategies on both yield and post-harvest PCN populations.

# Detecting past recombination events in *Potato virus Y* genomic sequences using statistical methods

D. Husmeier & F. Wright

**Molecular evolution of RNA viruses:** Plant RNA viruses evolve more rapidly than the DNA of their plant hosts due to a higher mutation rate. Mutational processes include nucleotide substitution that replaces one nucleotide by another, and insertions and/or deletions that result in sequence length changes. In addition to mutation, inter-strain recombination can produce new mosaic strains[9]. It is important to detect recombination events because, firstly, phylogenetic studies of inter-strain relationships that assume no recombination are very likely to be incorrect, and secondly, because recombination may have important consequences for virus control strategies. We will illustrate methods to detect evidence of past recombination events in four strains of *Potato virus Y* (PVY). As we shall see, detecting recombination in this data set is not simple.

PVY is a member of the Potyvirus genus and, like other potyviruses, has a single stranded positive sense RNA genome. Four complete length strains were available from the EMBL/GenBank sequence database (accession number/ lengths: U09509/ 9698bp, D00441/ 9704bp, M95491/ 9703bp, and X97895/ 9701bp). These were labelled Singh (abbreviated to Si), Robaglia (Ro), Hungarian (Hu) and Baulcombe (Ba), respectively. The analysis was carried out on a 9692bp alignment after removing all positions with gaps (totalling 22bp). Part of the alignment is shown in Figure 1.

**Figure 1** Part of the PVY alignment with recombination breakpoint RB1 predicted at approximately position 2422. Manual inspection supports this: Hungarian and Baulcombe are very similar up to 2407, whereas Hungarian is clearly more similar to Singh and Robaglia (rather than Baulcombe) from 2467 onwards.

**Phylogenetic trees and recombination:** In the absence of recombination, the relationships among the four PVY strains would be best represented by a single phylogenetic tree, consisting of a branching order (*topology)* plus branch lengths, based on the entire alignment (see Fig. 2). However, inspection of the tree reveals that the branch length leading to Hu is very short (0.0004 substitutions per position), compared to the the branch leading to Ba (0.0713), suggesting a relative rate of nucleotide substitution of 173.8. This is a very high ratio even assuming the action of natural selection, and suggests that the phylogenetic tree model, assuming no recombination, is not appropriate.

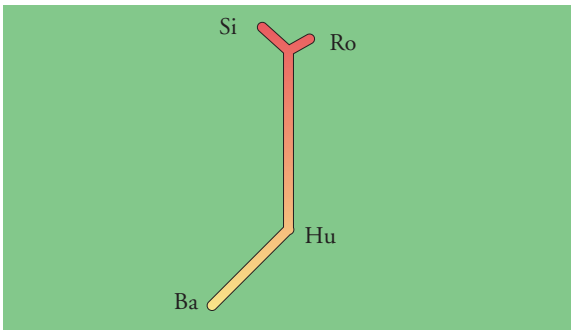


**Figure 2** Tree for alignment, assuming no recombination.

If there is recombination, phylogenetic trees can be estimated for each region of the alignment after these have been located by recombination breakpoints. This article will focus on statistical methods to detect these breakpoints.

Evidence of among-site rate heterogeneity (as seen by the presence of conserved and variable regions) is almost always found in alignments, and complicates

the detection of recombination. Methods vary in how much they deal with rate heterogeneity: some do not distinguish rate heterogeneity from topology heterogeneity, some remove the effect of differences in *mean* rate among positions of the alignment, and some try to exclude the effect of rate heterogeneity completely and look only for changes in topology.

Detecting recombination breakpoints is difficult if (1) the recombination event occurred long ago, (because nucleotide substitution accumulation will make 'ancient' recombination events difficult or impossible to detect), if (2) recombination has occurred between similar strains, and if (3) recombination breakpoints lie close to each other.

**Statistical methods for detecting recombination breakpoints:** Different methods are appropriate for small (e.g. 4 sequences), medium (e.g. 10 sequences), or large alignments (e.g. 50 sequences). A method for each of these categories has been developed by BioSS. Once recombination breakpoints have been detected, reconstructing the history of recombination events among sequences is then done by interpreting the output of phylogenetic analyses of each recombinant region.

**Hidden Markov model method:** A hidden Markov model (HMM) approach can be applied to the problem of detecting recombination in small alignments[1,2,5]. The mean distance between recombination breakpoints is modelled by the probability of a recombination event as we move along the sequence alignment. For the four sequences, there are three possible tree (unrooted) topologies (Fig. 3):

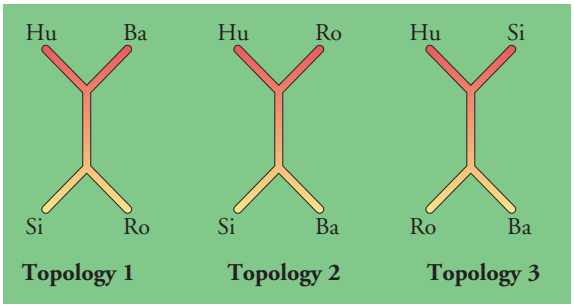


**Figure 3** The three possible topologies for the four PVY strains.

Note that topologies depict branching order only, and do not show branch lengths to scale. The transitions between the three topologies are assigned probabilities. The hidden states of the HMM represent the different phylogenetic tree topologies: we observe the sequences but cannot directly see the "hidden" tree topologies. The parameters of the model, namely the branch lengths associated with each topology and the recombination probability, are optimised in a maximum likelihood sense by applying the expectation maximization (EM) algorithm. The HMM method focuses only on topology changes in the alignment, and attempts to reduce rate heterogeneity effects. Statistical significance is assessed by (posterior) probabilities assigned to each topology for each position in the alignment.

**DSS sliding window method:** The TOPAL[6,7] program utilises fast approximate distance-based phylogenetic methods and can be used with large alignments. The method slides a fixed-size window (e.g. 500bp wide) along the alignment, comparing the left-hand window ($W_L$) with the right-hand window ($W_R$). In $W_L$, the matrix of pairwise genetic distances among the sequences is calculated, and a phylogenetic tree is then estimated by minimising the sum-of-squares ($SS_L$) between the observed distances and the distances based on the tree. A distance matrix is then calculated for $W_R$, and the $W_L$ topology is fitted to it, yielding a second sum-of-squares value ($SS_R$). When the $W_R$ topology has changed due to recombination, the $W_L$ topology will be a poor fit to the $W_R$ distance matrix. Putative recombination breakpoints can be observed by plotting the difference between $SS_L$ and $SS_R$ (DSS statistic) against the window centre. The influence of *mean* rate heterogeneity is removed from the analysis, but the DSS statistic will still be inflated when branch lengths change non-uniformly among branches as we move along the alignment. Recent improvements allow the statistical significance of DSS peaks to be estimated with parametric bootstrapping[8].

**MCMC sliding window method:** Markov chain Monte Carlo (MCMC) approaches have revolutionised Bayesian modelling and analysis, and recently MCMC techniques have been applied to phylogenetic analysis[4]. We have developed a method for detecting recombinants based on a marginal posterior distribution analysis[3]. A fixed-size window (e.g. 500 bp) is moved along a sequence alignment. For every position, the posterior probability of tree topologies conditional on the subsequence alignment selected by the moving window is determined by a MCMC simulation. On moving into a recombinant region, this marginal posterior distribution of topologies can be expected to change. This can be quantified by probabilistic divergence measures, for example, a local measure (AS) comparing the distributions on two adjacent

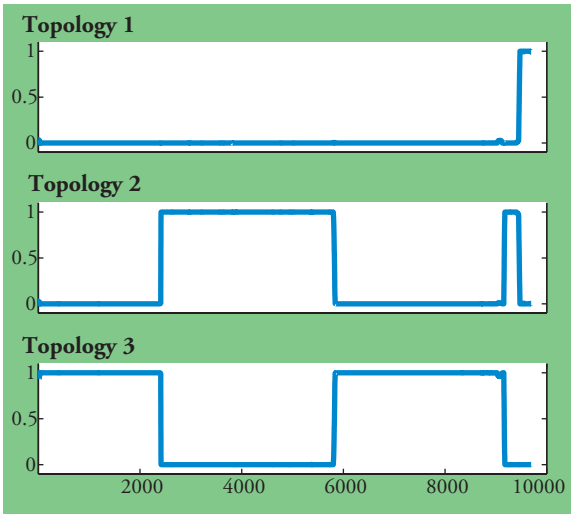


**Figure 4** Output from HMM analysis, showing the probability of each topology (as described in Figure 3) at each position in the alignment.

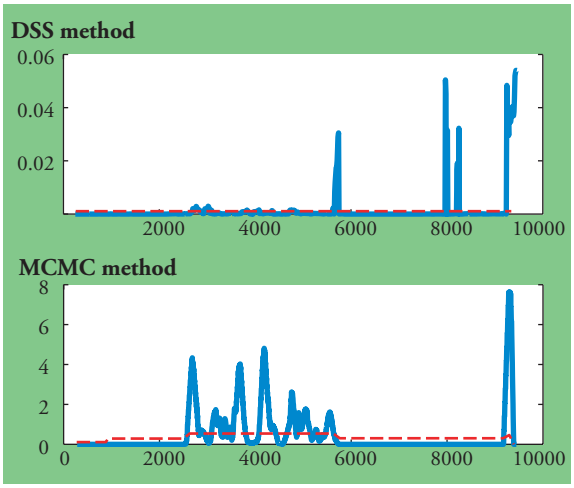


**Figure 5** Output from DSS and MCMC analyses, showing the DSS and AS statistics respectively, plotted along the alignment. The dashed line is the statistical significance threshold for peaks.

windows. This divergence measure is then plotted along the alignment. The MCMC approach is currently limited to analyses of about 10 sequences. The MCMC method attempts to focus only on topology changes and to exclude all rate heterogeneity effects.

A limited simulation study[3] has shown that the MCMC method outperforms the DSS method. We expect, in general, that the HMM method should be best at predicting the position of recombination breakpoints, although the DSS method is perhaps better than both HMM and MCMC at detecting recombination events that do not lead to topology changes.

Results of recombination breakpoint analyses: The HMM graphical output is shown in Figure 4, giving the probability of each of the three topologies for each position in the alignment. The HMM method detects four putative recombination breakpoints at approximately 2422 bp (denoted RB1), 5837 bp (RB2), 9178 bp (RB3) and 9511 bp (RB4). Note the methods do not place confidence intervals around the predicted breakpoints. In comparing positions, we have chosen 200 bp as a significant difference for this data set.

Figure 5 shows the DSS and MCMC output. The MCMC method detects RB1 (but not RB2) plus more than four breakpoints between RB1 and RB2. In addition, it detects RB3 but not RB4. The DSS method detects RB2 (but not RB1), RB3, detects some evidence of RB4, and detects six weakly significant breakpoints between RB1 and RB2. In addition, it predicts two new breakpoints at approximately 7970 bp (RBx) and 8250 bp (RBy).

Looking at phylogenetic trees for the identified regions helps interpretation. For example, Figure 6 shows the trees (with branch lengths approximately to scale) for the nonrecombinant regions before and after RB1. We can see that Hu has changed position relative to the other three strains, resulting in a change in *topology*. The topology in the 'Start-RB1' region is significantly better than the other two possible topologies. In contrast, there is no significant best topology for the 'RB1-RB2' region: the three strains Si, Ro and Hu are similar and the main feature of the tree is the long branch connecting to Ba. Within the RB1-RB2 region, the DSS and MCMC methods detect fluctuations between the three topologies.

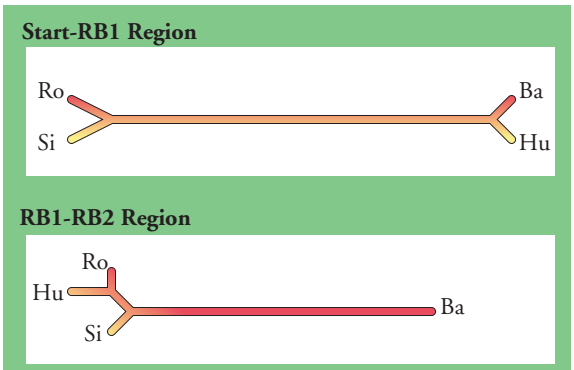


**Figure 6** Phylogenetic trees for regions before and after recombination breakpoint RB1.

Inspection of the phylogenetic tree for the recombinant RBx-RBy region (predicted by DSS) revealed a long branch connecting Si to the Ro strain suggesting a recombination event that did not change the tree topology.

Interpreting mosaic structure: We will restrict the interpretation of mosaic structure to the six recombination breakpoints (RB1 to RB4, plus RBx and RBy), plus two breakpoints found within the RB1-RB2 region by DSS and MCMC. These partition the alignment into nine nonrecombinant regions. Looking at the distribution (not shown) of pairwise genetic distances among strains within each region, we appear to have two types of strains. Between members of each type, the pairwise genetic distances are small (0.01-0.08 substitutions per position). Between pairs not belonging to the same type, the genetic distances are large (0.17 to 0.22).

Si and Ba strains can be chosen as typical members of each of the two types because they are separated by a large distance in phylogenetic trees calculated for each region in the alignment. One interpretation of the mosaic structure is that Si and Ba are similar or identical to the 'parental' types and that Ro and Hu have been produced by homologous recombination. Si-type strains are coloured yellow, and Ba-type strains green, in Figure 7.

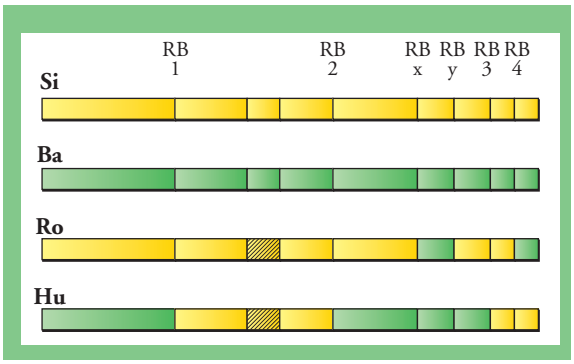


**Figure 7** Predicted mosaic structure of PVY strains.

The shaded subregion within the RB1-RB2 region was detected by both DSS and MCMC methods and appears to be due to a recombination event among the Si-type strains (involving an exchange between Ro and Hu).

Conclusions:
The analysis of the four PVY strains exposes some of the weaknesses of current methods. The PVY data showed evidence of recombination events that did not cause topology changes, and contained a region (RB1-RB2) where the dominant topology was not significantly better than the two alternatives. In addition, some recombination events appeared to have occurred between similar strains, and some recombination breakpoints are positioned close together.

The results of the three methods, while in some agreement, do differ. The RBx-RBy region appears to be a genuine recombinant region that was missed by HMM and MCMC methods due to a lack of a change in topology. On the other hand, the subregion within RB1-RB2 does involve a topology change but is possibly a false positive.

The window-based methods (DSS and MCMC) had problems detecting recombination breakpoints when these were close enough for two to fit within the window, or when they were positioned close to the end of the alignment. Although not investigated here, reducing the window size below 500 bp may help. However, the choice of window size length is not trivial: too small and a number of non-significant peaks will be obtained; too large and recombination breakpoints may be missed. We intend to reanalyse a larger PVY dataset with other window sizes.

We are currently working to improve methodology, in particular to reduce the influence of rate heterogeneity on the detection of past recombination events. An automatic method of reconstructing the evolutionary

history of a sample of sequences based on the recombination breakpoint prediction would also be useful.

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# Research services

## Analytical facilities

C.M. Scrimgeour

**Laboratory Accreditation** Within SCRI, the Gas Chromatography-Mass Spectrometry Laboratories, Stable Isotopes Facility and Lipid Analysis Unit of MRS Ltd, operate a formal Quality System certified to BS EN ISO 9001 by SGS Yarsely International Certification Services Ltd. The certification standard was upgraded from ISO 9002 to ISO 9001 in August 1999, and now includes the design and conduct of research within its scope. A generic Quality System operates in other parts of the Institute and this is summarised in the SCRI Quality Plan, a copy of which is included in the Institute's Corporate Plan. The measures required for implementation of the generic SCRI Quality System are described in a Code of Practice document, a copy of which is issued to all members of staff. The Code of Practice is also reproduced at the end of the latest version of the SCRI Laboratory Notebook. The generic system is based on the correct maintenance of work records in which specially designed hardback notebooks comprise the primary record, with other data recording systems, archival procedures, *etc.* as secondary records. The preparation of written methods or protocols (Standard Operating Procedures) and the correct use of equipment and facilities are strongly encouraged. The plan ensures full compliance with all safety regulations, and demands high standards of laboratory hygiene. If required, the Quality System can be readily upgraded to the standard required for formal certification within any activity or area. An archive facility is located within Building S and this is used for long-term storage of data as hard copy and in electronic format. Archival of data on electronic media is based on the use of compact disc writers (CD-R format) installed in several personal computers. Data can be transferred over the network or from a portable high capacity data storage disc to the computer's hard disc, and then to CD. Each CD can hold up to 650 Mbytes of data. Two copies are made, one for the owner of the data and one for the archive.

**Stable Isotope Facility** Stable isotopes are widely used for the study of plant physiology, crop genetics, ecology and food webs. Valuable information comes both from studying natural variation in stable isotope composition and from following the fate of added isotopic tracers. SCRI is equipped with a comprehensive range of modern instrumentation for stable isotope analysis of the biologically important light elements, $^{13}C$, $^{15}N$, $^{18}O$ and $^{34}S$, in a wide range of solid, liquid and gas samples.

All the instrumentation is based on continuous-flow isotope-ratio-mass spectrometers that are fully automated and operated through computer data systems. Automation allows a high through-put of samples, essential for many biological experiments where large data sets are required. For solid samples, the Europa Scientific Tracermass and 20-20 mass spectrometers are interfaced to Roboprep CN and ANCA-NT SL combustion sample converters. A Roboprep G+ gas purification unit is used for gas analysis. Plant samples of one to five milligrams are used, containing 25 to 100µg of the element of interest. Where possible, analytical protocols are devised to minimise sample preparation and fully exploit the automation.

SCRI also has expertise and resources for sample preparation from a wide range of matrices. These include plant sample drying and grinding, freeze drying and weighing facilities. Research support is aimed at developing new methods to assist the Institute's research programme.

**Organic Mass Spectrometry** The Institute's three state-of-the-art mass spectrometers, which are devoted to structural analysis of organic compounds, continue to yield valuable information on a diverse range of materials pertinent to the research remit of the Institute. The core instrument is a Hewlett Packard 5989B MS ENGINE research-grade quadrupole instrument with electron impact, chemical (positive/negative) ionisation modes and a mass range of 2000 amu. Distributed processing software permits off-line data processing and reduces analysis times. This instrument can provide mass and structural data on a wide range of organic compounds.

A further bench top instrument is dedicated principally to the analysis of naturally occurring volatile compounds. This consists of a Perkin Elmer automated thermal desorption system (ATD) linked to a VG TRIO-1000 quadrupole gas chromatograph-mass spectrometer and permits detailed characterisation of the profiles of organic volatiles generated by biological systems.

A Finnigan SSQ 710C dedicated liquid chromatography- MS instrument, with atmospheric pressure chemical ionisation (APCI) and electrospray ionisation (ESI) interfaces, is also available. This is suitable for samples whose high molecular weight, lack of volatility or polarity, make HPLC the preferred separation method. APCI and ESI are soft ionisation techniques and generally only produce molecular ions, e.g. [M-H] + or MH + , but the multicharge ionization mechanism of electrospray can extend the basic 2000 mass range of the instrument by a factor of about 20, giving a mass range of greater than 40,000 amu. This permits accurate mass determination of peptides, proteins and nucleic acids to within 0.1%, compared to the 5.0% error usually expected from SDS-PAGE determination.

Mass spectrometric analysis at SCRI covers a broad spectrum of chemical investigations generated by the research programme of the Institute. A wide range of plant metabolites has been analysed, both in the native form and as derivatives, including sterols, monoterpenes, sesquiterpenes, pentacyclic triterpenes, dimeric forms of phenolic acids, glucosinolates, long-chain wax esters, peptides, essential oils, carbohydrates, polychlorinated biphenyls and lipids, including fatty acids. The facilities are operated by experienced and expert staff, ready to tackle and solve most structural problems.

During 2001, SCRI will take delivery of two new mass spectrometers to increase substantially its capabilities and sample turnover in both high throughput

The lipid laboratory in building S.

metabolic profiling and proteomics. The first of these is a ThermoQuest LCQ-DECA, an ultra sensitive LC-$MS^n$ system with multiple quadrupole ion traps. It is capable of many more scan functions than the existing SSQ710C spectrometer, including full scan MS/MS, a tool of great utility in high throughput profiling. The same is true for protein/peptide sequencing, where rapid repeats of ion isolation and fragmentation generate sequence tags, which are searchable *via* on-board databases.

The other new instrument is a ThermoQuest TEMPUS-TOF, an innovative GC-MS system capable of rapid detection, characterisation and quantification in fast GC separations. The benefits of the design provide parallel mass analysis with a short duty cycle at high transmission. This delivers rapid acquisition and fast sampling of narrow peaks at high sensitivity with high sample throughput.

A fundamental requirement of successful gas chromatographic-mass spectrometric investigation is the development of robust chromatographic separations. Together with the MRS Lipid Analysis Unit, there are six gas chromatographs for high throughput analysis and the development of new separation protocols.

# Media Kitchen

W. Ridley

The Media Kitchen, which was established in 1996, provides a wide range of sterile microbiological, mycological and plant tissue culture, media and disposable plasticware for the Institute's laboratory staff. It operates as a research facility under the central administration overhead, to minimise bureaucracy, with each user site being 'shadow-tolled' for its throughput of consumables, *etc.*

Buying in bulk has shown huge savings for the Institute and prices have, in the main, held since 1996. The Media Kitchen is staffed by two full-time and one part-time worker, and the facility is supported by the efforts of Walter Burry and James McMillan (part-time), who were recruited from The Helm Project in Dundee. Orders are delivered on a daily basis to 13 pick-up and drop-off locations to top-up each Unit's supplies. Agar plates and any other specific media can be ordered either by telephone, by e-mail or by visiting the Media Kitchen. These requests are usually met within 24 hours. The support workers, in addition to filling tip-boxes and Eppendorf pots, also collect and recycle the glassware and collect, autoclave and dispose of microbiological materials.

The outputs of the Media Kitchen, year by year, can be seen in Figure 1. It would appear from the figures that 'saturation point' may have been reached. However, the reorganisation of the Institute into Divisions and Units, the Open Days, the re-wiring of the Institute and the Media Kitchen's move to new premises are all factors that will have made a significant impact on the year's overall output.

It became apparent during 1998/99 that, if the Media Kitchen were to continue to see growths in production, much larger premises were required. To this end, a new location was sought and, towards the end of 2000, the Media Kitchen was moved to an area at least six times the size of the original, with facilities that will, in due course, enable us to achieve accreditation according to ISO 9000. This will prove invaluable as regards future grants and contracts.

The work carried out by the Media Kitchen staff frees the innovative scientists, visiting workers, trainee students and support technicians from the repetitive and time-consuming tasks associated with media preparation. This secures a standardised quality of service throughout the Institute, at the same time saving the additional expense of each Unit maintaining a sterilisation and media preparation facility.

Given the large number of visiting scientists and students (of all standards and backgrounds) who work at SCRI, the provision of a standardised, quality-assured media and sterile disposable-ware facility, with its daily delivery service and daily removal of waste microbiological materials, has proved to be invaluable both to researchers and to those monitoring costs and assessing value for money.

| | 1997 | 1998 | 1999 | 2000 |
|---|---|---|---|---|
| Boxes of tips (100/box) | 13,933 | 14,300 | 19,738 | 19,653 |
| Eppendorf tubes (*c.* 200/pot) | 2,600 | 2,620 | 4,211 | 4,279 |
| Agar plates | 37,011 | 43,600 | 56,084 | 52,064 |
| Other items * | 24,654 | 45,080 | 47,928 | 51,850 |

* Item = 5ml LB or 1 L 20 x SSC for example: anything bottled and capped.

**Figure 1** Media kitchen output.

# Division of Finance & Administration

Douglas Watt

The Division is responsible for the provision of the 'non scientific' services to the Institute and encompasses the Units of Engineering and Maintenance; Estate, Glasshouse & Field Research, Finance and Human Resources; Information Technology; and Scientific Liaison and Information Services, employing a total of 76 staff.

The work of the Division is geared towards providing a service to the scientific community to ensure that they have the resources and ability to carry out the research commissioned by the various bodies. The variety and sophistication of the work carried out at the Institute has increased significantly in recent years and the staff have responded by adapting working patterns, learning new skills and taking on additional responsibilities.

The Division is an integral part of the Institute and the staff interact very closely with the scientific staff, often providing a breadth and depth of practical experience that is not available elsewhere. This is particularly true of the Estate, Glasshouse & Field Research Unit who actually have to get their hands dirty and battle with the vagaries of the Scottish weather to produce consistent, high quality results for scientists, whose work is increasingly geared towards the laboratory. The Engineering Unit has to work with a large variety of buildings and equipment and it is to their credit that the Institute is able to provide such a breadth of facilities within a structure that, other than glasshouses, has not changed substantially in the last ten years.

The function of the IT Unit has become central to an Institute that now has more computers than people, and is increasingly reliant on the Internet and massive databases for much of its information. This is an area in which the Institute will require to allocate more resource to ensure that the staff are able to keep pace with the volume of information and the need to disseminate their work to an ever larger and information hungry 'customer' base.

This is similar to the problems faced by the Scientific Liaison and Information Services Unit who are tasked with promoting science, and more particularly the science of the Institute, to as wide an audience as possible. The quality of the displays, posters and presentation is remarkable given the size and resources of the Unit and they are expected to be able to answer questions and provide detailed information on all aspects of the Institute's work, usually at very short notice.

Underpinning all of this is the Finance and Human Resources Unit, which tries to ensure that all the administrative processes run as smoothly as possible and that the scientists do not exhaust the resources of the Institute. Similarly, the HR staff support the Institute staff, without whom the Institute would not exist, and significant progress has been made in pushing forward the Investor in People initiative, developing the training programme and setting up a sophisticated, computerised HR system.

The Division has to carry out its work within tight financial constraints but the staff approach their work with an enthusiasm and dedication that reflects their commitment to the work of the Institute.

# Scientific Liaison and Information Services

W.H. Macfarlane Smith

We live in the age of the 'spin doctor' and any group, however small and unrepresentative of the majority, with the inclination and the money to do so, can mount campaigns against governments, major corporations and even individuals, which can be misguided and malicious. Frequently, they have a large but unjustified impact. In doing this, these groups are sometimes supported vigorously by certain sectors of the media who either have cast themselves in the role of champion of the underdog, or who have had no idea of the science carried out but see a negative approach towards it as something which will curry favour with readers, listeners or viewers. In such circumstances, it is not uncommon for objectivity and scientifically proven facts to disappear totally. Happily, this is balanced by others in the media who are learned, well informed or both, and who now seek to give a much better balance to the portrayal of such areas of human endeavour as biotechnology and other advanced scientific developments. Nevertheless, it has become more and more necessary for scientists to allocate increasing amounts of their valuable time to explain and justify their work. Pressure groups often exploit the concept of public accountability and see no comparable need to explain and justify their objections. Genuine debate has been replaced by 'sound bites'.

Given that this is the way of things in the UK today, it is not surprising that my Unit, SLIS, is having to spend increasing amounts of time on improving 'public awareness of science'. In just my own activities, this takes many different forms, from explaining the Institute's research to biological science students, to giving public lectures (33), taking part in radio and television programmes (14 and 3 respectively) and press interviews (17). A number of Institute staff, including my immediate colleague, Tim Heilbronn, are similarly involved in these interactions with sections of the public and media. Other equally direct means of taking the Institute's work to the general public are used through participation in science festivals, garden shows, *etc.,* by having our own Open Days, and hosting individual and groups of visitors. There is also a need to educate the educators and, to this end, we not only bring teachers to SCRI but go out to talk to staff in schools. Scientific and other publications are also vital for spreading knowledge of the Institute's work and the 222 refereed and 247 non-refereed papers and articles in the past year not only help to achieve this but provide ample evidence of the productivity and success of our scientists. Finally, there is the presentation of our science at the widest range of scientific conferences affordable, to ensure that our findings are given at first hand and not by someone whose knowledge of our work and its interpretation is distorted by a pre-determined agenda. Other avenues such as participation in the British Society of Plant Breeders Communications Working Party, are used increasingly to inform. A measure of this impact is the over 330 press references to SCRI's work during the year 2000.

There is no lack of public interest in SCRI, with over 1700 people attending our Open Days, and well over 2600 in total visiting the Institute in the last year through arrangements made with SLIS. Additional numbers arrange visits directly with our scientists for discussion and consultation.

It is not enough simply to take out information to the various venues and assume that understanding will be automatic. A high quality of pictorial presentation is required and staff have had to acquire and use robust and 'easy to understand' presentational skills, especially where certain confrontational members of the media and general public are involved. On this latter point, media training by experienced television journalists has given senior staff both an awareness of the processes of TV and radio presentation, and the ability to make their case in a concise fashion. On the former, it is gratifying that our high standards of visual presentation have been recognised by the award of Silver Medals at both the National Garden Show for Scotland and the highly successful Dundee Flower

Show. Particular interest has been expressed in our displays on ladybirds, their benefits and their predators, and in our research on beneficial nutritional factors in crops, especially soft fruit. The latter was the Institute's principal display at this year's Edinburgh International Science Festival.

Politicians have not been slow to appreciate the importance of SCRI's research, and the Institute has been visited by representatives of all the major political parties at MP, MSP, MEP and local council levels. As a further aid to increasing awareness of the science carried out at SCRI and other publicly funded research organizations in Scotland, SEERAD has co-ordinated regular meetings of Information Officers from all the SABRI's, SAC and RBGE. These are used both to obtain information which can be presented concisely to politicians and other interested parties, and to extend further the presentation of the scientific achievements of these bodies to the general public through publications such as reSEARCH, the newsletter of the Agricultural and Biological Research Group of SEERAD.

Other specialist interests have been served through the organisation of various conferences. SLIS staff have played major roles in the organisation of conferences on 'Crop Protection in Northern Britain', on a 'Nitrogen Forum for Scotland' and the 8th International *Rubus* and *Ribes* Symposium, and continue to be involved in the planning for the XVIth Eucarpia Congress in Edinburgh.

There is an increasing need to give support for the commercialisation of research findings such as the Institute's recently developed diagnostic (jointly with Grampian University Hospitals Trust) for *E.coli* O157. The commercial potential of this work was recognised by an award from the Altran Foundation worth £600,000. Since then, there have been substantial media and commercial interests. Other public events such as the presentation of the Institute of Horticulture Norah Stucken Award to SCRI for achievements in soft fruit breeding at the IoH AGM in Edinburgh, provide opportunities to inform and explain.

As the demands on SLIS staff increase, greater efficiency gains have to be sought. The purchase of a Hewlett Packard 5000 colour printer allows the production of high quality, large format material such as exhibition display panels, to a size and clarity, and reduced production time, than was possible previously. The ongoing development of digital cameras, together with the availability of suitable software for image manipulation, had reached a stage where a switch to this system of photography was justified. A Fuji SI professional digital camera, with the ability to produce high resolution images (6.3 megapixels), is now in use and capable of dealing with a wide range of subject material. Originally, a transition period of several years was planned, but the high quality of end product is such that, taken together with the reduced turn around times, the majority of staff now specify this technology. There is the further benefit that requests from the media for photographs of plants, equipment or members of staff can be met very quickly using electronic transfer of images.

The Library has continued to take on board the progression from the printed format to electronic delivery of information. Publishers and subscription agents are also wrestling with the new medium which has caused several crises leading to cessation of the delivery of electronic journal information, and a rise in price which bears no relation to ordinary inflation or, indeed, to the expenditure plans for the Institute. We remain concerned about the integrity of the archive and our continued access to it.

A large proportion of the Library services can now be delivered directly to the scientist's desk. However, it was necessary to undertake a major upgrade of the Library facilities. This will be completed in 2001 and will provide new shelves for journals and books, additional space and, so, better arrangement of the Library stock, and increased computer access, with four PC's available for use by SCRI staff.

The end-users in agriculture and horticulture continue to take a great interest in the Institute's research and, indeed, support aspects of it through their membership of SSCR (see also page 177). Information is disseminated through leaflets and an annual newsletter produced by SLIS, and Crop Walks for soft fruit and potatoes (the latter in conjunction with SAC and BPC) that attracted 80 and 350 visitors respectively in the year 2000.

The future is an interesting but challenging one, where new technology will have to be brought into play fully, to satisfy the many and varied demands on the Unit.

# Information Technology

S. Clark

The year 2000 began with the re-organisation of the IT unit, following the Institute restructuring in the previous year. The Bioinformatics element was separated, with Dr David Marshall, the then head of IT, moving on to commit all his efforts to this field within the Genomics Unit. Dr Bruce Marshall was appointed Head of Unit, with his time split 50% between IT and his scientific activities in the Unit of Vegetation Systems. A new post was created for the role of PC specialist, in line with the recommendations of the review carried out by BBSRC in 1999, which brought the compliment of IT staff to four full and one part time.

During the past few years, the network infrastructure, which was installed in 1991, was beginning to show serious signs of strain, due to larger numbers of computers and the increasing complexity of the technology. A case was made to upgrade much of the old infrastructure to a modern, industry standard, and a capital grant of £300,000 was awarded by SEERAD. A network specification was planned with the Engineering and Maintenance Unit, and Computer Cable Networks, Scone, was appointed as contractor to carry out the installation. The new network covers the six major laboratory blocks and uses Cat 6 class cabling, which is connected to workgroup switch stacks in each building. These stacks operate at 100MBS and the backbone, which uses existing fibre optic cable, has been upgraded to run Gigabit ethernet. A new Cisco 6506 switch was purchased, which acted as the central switch for the Gigabit ethernet, and the connection to the main servers was also upgraded to Gigabit. This work has involved major disruption to the Institute, as whole corridors have had to be cleared, but due to the good co-operation between IT, Engineering and Maintenance, the contractors, and individual scientists, this work has been carried out with very few problems.

An IT interface group, consisting of the members of the IT Unit and representatives from each Division, was set up. The purpose of this group is to communicate the work and plans of the IT Unit to the scientific staff and to get feedback from the other Units on any IT issues they might have.

The issue of computer viruses continues to grow; much time, which could be better spent, being used to deal with actual virus infections and setting up an Institute-wide system for virus detection and disinfection. Due to the Institute not adopting certain mail clients, we were largely unaffected by some of the well-publicised virus outbreaks which have crippled many companies and educational establishments over the last year.

The SCRI Intranet system has now become one of the major methods of communication within the Institute, replacing much of the function of the notice boards and other means of communication.

# Finance and Human Resources Unit

I.F. Harrington & I. Paxton

The Finance and Human Resources Unit (FHR), has a staff of 16 who are responsible for not only the day-to-day, smooth running of the Institute but also for longer-term assessments and planning. The Institute has more than 350 staff who are an integral part of the organisation and SCRI recognises that the performance of the Institute is dependent upon its success in developing and realising the full potential of staff at all levels, and at all stages of their careers. Staff costs also account for more than 66% of the Institute's recurrent expenditure, which is the single largest category of expense and an important factor in the financial performance of the Institute.

Not surprisingly, therefore, the work of the HR section within the Unit is vital to the success of the Institute. The section co-ordinates the recruitment of Institute staff (during the year 25 posts were advertised, attracting 361 applications from external and internal sources), and carries out the administrative induction for new members of staff as well as supervising probation procedures during their first year of employment. It is responsible for monitoring absences, with particular emphasis on those attributed to ill-health. HR handles requests for job evaluation at the request of both individuals and Institute management, and administers the training budget, which is maintained at 1% of the Institute's salary costs to reflect the importance of staff training and development, on behalf of the Institute Training Committee. In addition, it collates training requests from staff and arranges training with the appropriate training providers. HR are also involved in monitoring the progress of the 36 research students based at the Institute, and in providing assistance to new staff, visitors and students with accommodation and other welfare related matters. Throughout the year, HR staff were involved with the Institute's Investor in People Initiative, and it is planned to achieve IIP status by the end of 2001.

Each month, the Finance team within the Unit pays in excess of 350 members of staff, processes in excess of 1100 purchase invoices from suppliers, and raises over 20 sales invoices. The Institute is project driven and over 200 projects are maintained and monitored at any one time. Institute staff are also involved in co-ordinating projects, including the finance, with other research bodies throughout the world. The team also maintains over 2100 items on its fixed asset register, ranging from PC Computers to laboratory buildings.

The Institute has invested in new computerised Financial, HR and Payroll systems, and has implemented the BACS payment system to reduce the number of cheque payments, in an effort to streamline systems and improve the quality and quantity of information provided to staff, customers and suppliers.

The Institute continues to operate under considerable financial pressure as the Grant-in-Aid that it receives from the Scottish Executive Environment and Rural Affairs Department (SEERAD) has declined in real terms. The control of the Institute's finances is therefore a critical activity to aid in the continuing production of successful science by the Institute. Capital grant received from SEERAD is still an important source of finance, but the Institute has to attract an increasing proportion of its funds from other, 'external' sources to provide the necessary infrastructure to maintain and expand the quality and quantity of science within the Institute.

# Estate, Glasshouse & Field Research Unit

G. Wood

The Estate, Glasshouse and Field Research Unit fulfils the fundamental and crucial rôles of producing and maintaining plant material for the Institute's scientific research and contractual undertakings. A wide variety and large number of plants are made available throughout the year for work both in contained/controlled environments and in the field. The landscaping of amenity areas within the various sites and the maintenance of all estate and field boundaries are additional responsibilities.

The Unit has 24 staff and provides a fully equipped and professionally expert service to fulfil the requirements of its clients with regard to the preparation of land, growing medium, sowing, drilling, planting, propagation, plant maintenance, harvest and clearance of residues for the Institute's field and glasshouse research objectives. It may have responsibility for an entire package from start to finish, or can provide prepared land and/or controlled environment régimes for inputs to be undertaken in varying degrees by scientific clients.

The work undertaken spans sub-cellular to whole-plant aspects within the range from high-technology research on the genetic modification of plants, virus manipulation and testing; vegetation dynamics, bioremediation, woody perennials, fibres and biomass; defining data parameters for deriving mathematical models of crops; through studies on the effects of nutrient, pest, disease, weed environment on crops; to 'traditional' variety trials and maintenance of germplasm and nuclear stocks.

Within the controlled environment and glasshouse facilities, more and more accent is being placed on molecular/cellular/GMO/transgenic work and, as a consequence, on the specialist protocols and facilities required to undertake this type of work. A new research glasshouse complex, with varying degrees of containment provision and sophisticated environment control, came on stream during the year.

The range of plant material handled by the Unit, in both glasshouse and field trials, continues to increase. It includes cereals (winter and spring barley/oats/wheat), field bean, grass, clover, forage brassica (swede, turnip, kale, rape), oilseed rape, potato, blackcurrant, cane fruit (raspberry, black- and hybrid-berry), strawberry, *Lilium, Narcissus,* novel fruits (blueberry, cranberry, *Rosa spp*, sea buckthorn, *Sambucus*), fibres and other industrial crops (hemp, reed canary grass, *Miscanthus*, nettles, lupins, willows), reserve collection of top fruit stocks (apple, pear, plum, damson), breeding programme apple selections, mixed tree species windbreaks and woody perennials, *Rubus* and *Ribes* nuclear stocks of germplasm, cassava, coffee, groundnuts, maize, and a miscellany of virus-indicator/screening species test plants.

The Institute has 172 ha of free draining, loamy soil at Mylnefield, Gourdie, and Pilmore Holding/Lonsdale. The land rises from 15 m to 140 m elevation, faces south to south-west and is exposed to westerly winds. Windbreaks of both hardwood and conifers are planted at intervals across the prevailing wind track. Each year, more than one third of the total land area is used for experimental crops, and trials are also carried out at other off-station sites. The general crop husbandry is based on a long-term (15+ years) plan of land use and is consistent with good practice and sound business management. Unless otherwise specified by experimental requirements, the land is maintained at pH 6.5, high P and K status, not deficient in trace elements, no evidence of previous trial cropping, free from perennial weeds and volunteer crops and, as far as possible, free from soil-borne pests and diseases.

Land is divided into packages of approximately 10 ha, providing areas for arable (annual) crop trials with a 5-year break between crops of the same type, and soft fruit (perennial) trials with a 6-year break. Smaller isolated/designated areas of land are provided for specialist requirements. The Unit is equipped with a range of

up-to-date field, experimental plot and glasshouse machinery to fulfil the work programme, and machinery can be modified as necessary in the workshop to suit the requirements of plot work. Water for field irrigation is provided from boreholes through underground ringmains with hydrants every 100 m. There are crop drying, handling and storage facilities.

The Unit maintains virus-free nuclear stocks of *Rubus*, *Ribes* and *Fragaria*. The *Rubus* and *Ribes* collection, which contains over 100 cultivars, is the basis of commercial production within the UK and a source of healthy plant material for research and commercial organisations world-wide. It is continually augmented by new cultivars and seedling lines from the HRI, SCRI, and contracted clients' breeding programmes.

The 11,000 $m^2$ of heated glasshouses provide fully automated services for all-the-year plant production which now exceeds 500,000 units annually. Glasshouse cubicles range in size from 12 $m^2$ to 350 $m^2$, providing specialist support for the varied research packages and for specific purposes including quarantine and isolation. In addition, there are 5000 $m^2$ of cold glasshouses, polytunnels and net structures.

Facilities for growing plants under controlled temperature, light and humidity regimes range from small 300 litre cabinets to large walk-in rooms. During the year, two new 600 litre tissue culture and seven 1000 litre growth cabinets plus four large growth rooms were provided in the header house area of the new research glasshouse complex.

# Engineering and Maintenance Unit

S. Petrie

The Engineering and Maintenance Unit has 25 staff of all grades and experience and offers a technical design and maintenance service throughout the Institute. The main Institute buildings were built in the early eighties and before, therefore preservation of Institute assets is of paramount importance and careful, skilled inspections are carried out frequently. Corrective maintenance work takes place to ensure the expected performance and life of equipment, vehicle, plant or building is achieved.

The Unit is divided into sections that specialise in a variety of engineering disciplines such as electrical, electronic, refrigeration, heating and mechanical engineering. In addition, it provides an engineering design and maintenance service to cover scientific and ancillary equipment, and building services, including heating, ventilation and air conditioning. There is also a farm workshop section providing maintenance facilities for a substantial fleet of tractors and agricultural machinery. The workshops are generally well equipped to deal with the maintenance tasks assigned to them. The Unit also provides a general stores facility along with the cleaning and security service.

The rapidly changing and wide ranging scientific aims of the Institute ensure that laboratory alterations will always be a part of the Engineering Unit's work. With this in mind, the specialist services to laboratories must be as flexible and adaptable as possible. Over the last few years, systems have been introduced which allow the Unit to respond quickly and efficiently when changes are necessary, thus reducing laboratory disruption to a minimum. With planning, scientists can confidently bring new and diverse projects to the Institute knowing that a team is on hand to ensure the facilities will meet their requirements.

During 2000, as in previous years, many areas within the Institute were refurbished or enhanced, including laboratories in Buildings B, D, E, V and AF. Much of this work was carried out by our own staff and has resulted in high quality facilities being available in each of these areas.

An important part of the refurbishment is the need to comply with the increasingly complex Health & Safety legislation and the Unit strives to ensure that the Institute is able to provide a safe and effective working environment. Unit staff ensure that they are conversant with changes in legislation and approved working practices by attending specific courses and seminars, as well as working closely with suppliers. This extends to the training of staff in safe working practices, particularly when using hazardous chemicals and equipment.

One of the main tasks undertaken during the latter part of 2000 was the installation of a new data and voice cabling system capable of meeting Category 6 standards and the needs of the Institute into the foreseeable future.

A major part of the contract involved installing suitable containment to cope with the vastly increased number of cables and this required staff to be decanted whilst the work was being carried out. To minimise disruption, the contract was divided into 11 sections, each section being completed within two weeks, and staff moving back prior to the commencement of the next section. The most pleasing aspect of the project was the high level of co-operation between staff, engineering personnel and the contractor, which helped to make what was a difficult and daunting installation much easier than anticipated. The resultant benefits to network users and system administrators are faster access with more reliable and flexible connections.

The Unit is also involved in the installation and maintenance of increasingly sophisticated and expensive research equipment and the Unit staff have responded by learning and developing new skills and abilities in response to the ever-increasing demands.

The Unit is responsible for negotiating utility contracts with electricity, gas, water and telephone companies and makes use of the appropriate consultancies to ensure that the most efficient tariff is used.

# Mylnefield Research Services Ltd

N.W. Kerby & J.B. Snape

Mylnefield Research Services Ltd (MRS) was established in 1989 as the commercial arm of the Scottish Crop Research Institute (SCRI) to enhance competitiveness and understand and fulfil the needs of industry. MRS not only markets the unique resources and expertise of SCRI, but also undertakes near-market research and development. MRS places particular emphasis on developing partnerships and forging strong relationships with customers.

As a knowledge-transfer company, MRS endeavours to promote the contribution of life sciences and technology to wealth creation and the quality of life.

We aim to improve competitiveness and enhance the future prosperity of SCRI by reducing reliance on Government funding.

Responsibilities of MRS include:
Marketing SCRI's scientific expertise and promoting SCRI as a centre of scientific excellence
Evaluating commercial potential of innovations
Developing commercially viable concepts
Protecting and managing Intellectual Property (IP)
Licensing
Diversifying the funding base
Assisting research proposals
Negotiating contracts
Managing contracts
Contract research

**Mission Statement** To develop commercially the Scottish Crop Research Institute's scientific expertise, resources and intellectual property, and to improve the quality of services to achieve new standards of excellence.

**Finance** From the time of incorporation, MRS has been self-sufficient in providing its own accommodation and staffing, achieved without start-up funding, Government subsidy or venture capital.

**Financial Year 2000** The Financial year 1999/2000 was the most successful to date, with income exceeding £2.5m, an increase in excess of 40% on the financial year 1998/1999. £1.63m was transferred to SCRI in direct and indirect costs. This included Gift Aid of £205k. A further £20k was gift aided to The Mylnefield Trust, leaving a profit before tax of £37k.

**Financial Year 2001** Due to the uncertainties of the 'agbio' and 'agchem' sectors (see Box below) a downturn in income of approximately 15% was forecast for the financial year 2000/2001. Nevertheless, we are delighted to report a very similar level of income to 1999/2000 of £2.47m. This was due, in part at least, to a 40% increase in royalty income. MRS transferred to SCRI £1.55m which included £362k gift aid to SCRI and The Mylnefield Trust. The operating profit increased from 9% to 13% making it possible to transfer such sums. Furthermore, administrative expenses were reduced by 11% compared with the preceding year. Income is derived predominantly from contract research and, to a lesser extent, royalties and analytical services such as The Lipid Analysis Unit.

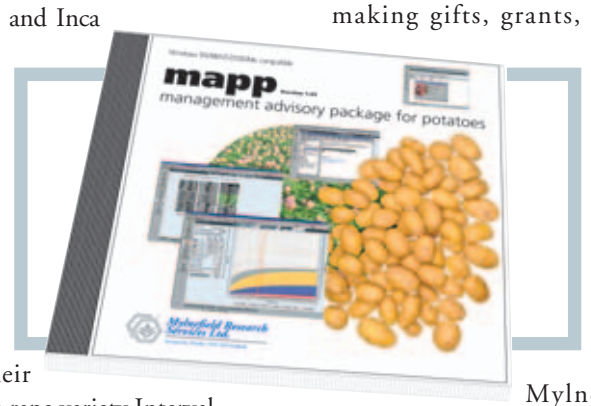
**Royalty Income** Royalty income in 2000/2001 exceeded £200k for the first time. The strawberry variety Symphony was again the star performer with particularly impressive growth in Germany and the Netherlands, as well as good sales in the UK. The blackcurrant varieties Ben Hope and Ben Gairn

**Overview of 'Agbio'** The life-sciences business strategy, the application of technologies developed in both 'pharma' and 'agbio' to form global life sciences companies, has undergone major revision recently. The deployment of genomics and bioinformatics technologies across human healthcare, agriculture, animal health and nutrition would appear to make economic sense. However, global stock markets have had neither patience nor vision to support this strategy. Pressure to consolidate in both the pharmaceutical and agricultural industries is strong, and amidst the mergers and demergers, 'agbio' and 'agchem' activities were consolidated into separate free-standing companies. Monsanto merged with Pharmacia and Upjohn in April 2000 and spun-out its agricultural assets into a separate company. In November 2000, the 'agribusiness' and 'agchem' business of Novartis and Astra-Zeneca were spun-out to form Syngenta. Aventis (formed by mergers of AgrEvo (Hoechst merged with Schering AGI) and Rhone-Poulenc) is currently divesting its interests in 'agbio' and creating Agreva. The consolidation, rationalisation and demerger of 'agbio' interests from life-sciences companies creates uncertainty and continues to impact on the business activities of MRS. This has been compounded by the uncertainties surrounding the commercialisation and regulation of GM crops in Europe.

According to Ernst & Young's Eighth Annual Life Sciences Report 2001, the prospects for EU plant research remain bleak because of poor integration of parallel schemes and declining national funding.

exceeded all expectations following their first full season in the UK and have attracted considerable interest from other European countries. The strength of the blackcurrant portfolio was emphasised by strong performances from established varieties such as Ben Alder, Ben Tirran and Ben Connan. A modest increase in the royalty income obtained from raspberries was recorded, with Glen Ample proving to be the most popular of our varieties by far. There was a slight decline in income from the blackberry variety Loch Ness; however, a lot of interest in the selections currently undergoing on-farm trials has been generated, and the future looks promising.

Hopefully the under-performance of our potato varieties in recent years has been reversed. The chipping variety Spey is increasing in popularity and there are high hopes for new varieties such as Lady Balfour and Montrose in the next few years. In addition, two new *Solanum phureja* varieties, Mayan Gold and Inca Sun, will be launched commercially in 2002.




Our portfolio of brassica varieties continues to perform well, with Caledonian kale establishing itself as a market leader in the game cover sector. The swede varieties Invitation and Kenmore both increased their market share, as did the forage rape variety Interval.

**mapp™: Management Advisory Package for Potatoes** is a new and exciting innovation developed by the Scottish Crop Research Institute in partnership with MRS, the British Potato Council and Scottish Enterprise Tayside. It is an interactive computer package which helps with critical decisions on potato production. It is designed for use by growers and advisors and was launched in January 2001.

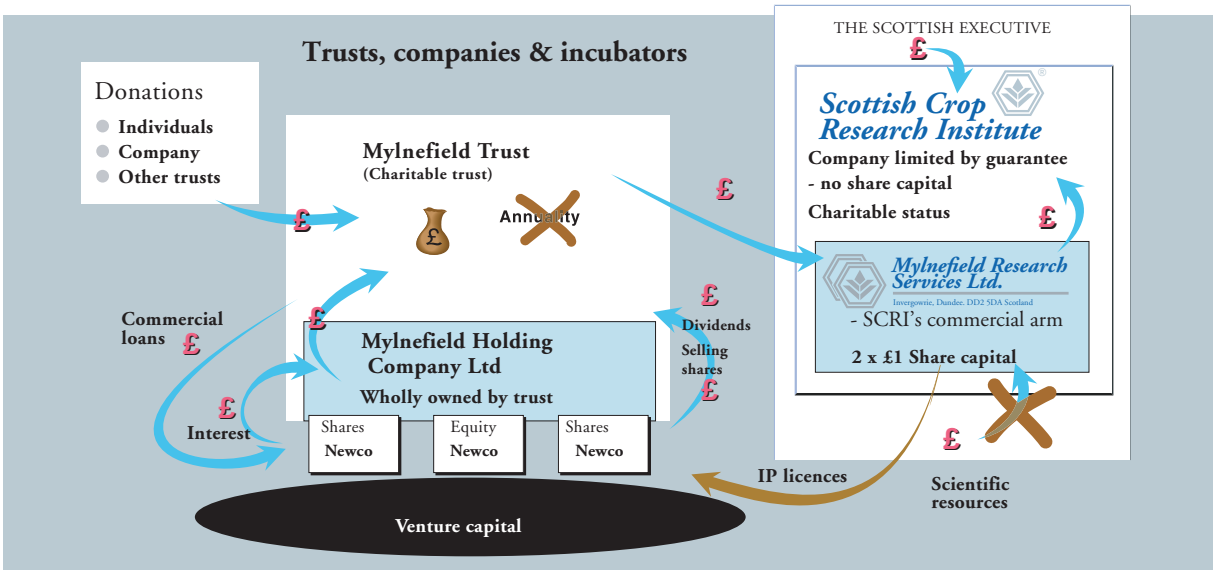
## New Organisational Structures

**The Mylnefield Trust** During 2000, The Mylnefield Trust was registered as a charity with objects to:
-promote research and scientific work in the life, environmental and related sciences, in particular production of agricultural, horticultural and forestry crops, methods of limiting or eradicating pests and diseases, wood sciences and biomathematics, methods of increasing production or growth, improving cultivation and research into improved cultivars;
-promote the dissemination of such research.

The Trust will support scientific research at SCRI by making gifts, grants, loans or payments to the Institute, subject to the above objectives being met.

In April 2000, MRS gift-aided £20,000 to The Mylnefield Trust and a further £5,200 was received in anonymous donations.

**Mylnefield Holdings Ltd** In February 2000, Mylnefield Holdings Ltd was incorporated as a wholly owned subsidiary of The Mylnefield Trust. A new company Phygene Ltd was also incorporated as a wholly owned subsidiary of Mylnefield Holdings, but has yet to trade.




**Investor In People** MRS is committed to investing in its employees so that it can compete effectively and achieve new standards of excellence.

In December 2000, MRS achieved accreditation under the Investor In People scheme. This is a significant award and the company recognises the work undertaken by the staff in achieving it. The company is fully committed to the aims of Investor In People in developing the full potential of its employees so that they can perform effectively, efficiently and gain long-term satisfaction from their work.

**Employees** In 2000/2001, Margaret Barton was appointed as a replacement for Linda Butler (one of the first employees of MRS). Subsequently Margaret resigned and we are pleased to welcome Hayley Wilson as Secretary to MRS. John Marshall joined MRS in November 2000 specifically to market mapp™.

New scientific appointments included Alison Blake to the Lipid Analysis Unit and Nieves Medina Escobar as a Molecular Biologist on an externally-funded project. Additionally, several short-term appointments were made during this reporting period.

MRS would like to congratulate Jane Fairlie on achieving her B.Sc. at The Open University and Lesley Beaton for gaining a Diploma of Management Studies at The University of Abertay, Dundee.

**Acknowledgements** MRS gratefully acknowledges its sponsors and the co-operation of SCRI scientists and administrative staff for their contribution to the success of the company.

# Scottish Society for Crop Research

D.L. Hood

Trustees: - Mr A G M Forbes
Mr G B R Gray OBE (Resigned)
Mr I E Ivory
Mr A Pattullo

Chairman: - Dr D A S Cranstoun

Vice Chairman: - Mr J M Drysdale (Resigned)

Members of Committee of Management: -
Mr J Arbuckle (Resigned)
Mr D Craib (Resigned)
Mr T D Gray
Mr A Logan
Mr L M Porter
Mr G Rennie
Dr S Wale
Mr J S Whitehead

Secretary and Treasurer: - Mr D L Hood

Registered Office: - c/o Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA

Membership Numbers: - 266

The Scottish Society for Crop Research is a registered Friendly Society, formed in 1981 by the amalgamation of the Scottish Society for Research in Plant Breeding and the Scottish Horticultural Research Association.

The Society provides a link between the Scottish Crop Research Institute and farmers, processors and other interested bodies: -

- by organising field walks and meetings for the exchange of information

- by financing science based publications for the benefit of the membership

- through the formation of crop-based sub-committees which maintain contact with members on specialised topics.

The Society continued to support projects initiated in previous years. Farm scale trials for 'Malting Quality Analysis in Winter Barley Mixtures' have been planted.

The Soft Fruit industry has suffered severe setbacks and the Society has prepared itself to be a catalyst in attracting further essential funding from public and private sources. The alternative is that work extending for some 40 years may come to an end.

These projects, together with the award of travel grants to enable Institute members of staff to attend conferences and seminars overseas to discuss their research, promotes both the Institute and the Society. Reports from those awarded travel grants are summarised in the Newsletter.

Mr Michael Gibson, Scottish Board Member of the Food Standards Agency, who took as his topic 'Food for Thought', addressed the Society Annual General Meeting in July.

The numbers attending the Society's Soft Fruit Walk fell, reflecting the decline in the industry. The growing of soft fruit has contracted considerably in recent years, with fewer and fewer farmers continuing production. Of those remaining in the industry, a good proportion continue to support this event, which gives the growers a chance to see the main areas of soft fruit research, though commercial confidentiality precludes some of SCRI's research work from being shown.

'Potatoes in Practice', the Society Potato event, organised in conjunction with the British Potato Council, the Scottish Agricultural College, and the Institute, took place in August. Exhibitors of machinery and traders combined to continue the impetus from previous years and make this the largest event of its kind in Scotland.

The Crop Sub-Committees, including Cereals, met diligently and informed the Management Committee of their approved projects for the coming season.

The Management Committee met twice during the year, in May and November, and were pleased to have the guidance of the Trustees for these meetings. The Society mirrors the age of the population, with the resignation of two long serving Management Committee members, Mr Arbuckle and Mr Craib. Mr G B R Gray OBE also resigned during the year and was presented with a Certificate of Honorary Membership. Mr Gray was a member of the Scottish Society for Research in Plant Breeding, one of the constituent founding Societies, and was elected a

Dr David Cranstoun presents George Gray with Honorary Membership of the Society.

Trustee of the Scottish Society for Crop Research on its inauguration in 1982.

Society membership continues its decline as farming communities shrink. Fortunately the Society's activities are not dependent on subscription income. Farming controversies such as GM crops, BSE, and now Foot and Mouth in the Spring of 2001, are likely to hasten the departure of good agriculturists through no fault of theirs.

The Management Committee welcomes suggestions for research topics and comment from members and others and urges them to contribute to the Society by becoming a member if not already one, and thereafter possibly joining one of the Crop Sub-Committees or indeed the Management Committee.

Contributed articles, together with photographs of interest, are welcomed for the Society Newsletter, and should be forwarded for the attention of the Secretary and Treasurer.

# Health & Safety

M.J. De,Maine

The Institute was the subject of an inspection (in September 2000) by a team of Occupational Health & Safety specialists drawn from the BBSRC and SABRIs. The inspection report was generally favourable but several recommendations were made to improve health and safety management. Action on these is continuing and progress is monitored directly by the Deputy Director with regular reports being made to SCRI's Governing Body.

Over the year, the pesticide store has been refurbished with fire-proof, non-absorbent steel shelving replacing the old wooden shelves, and the floor has been bunded to contain accidental spillage of hazardous liquids. To aid escape during a power failure, emergency lighting has been installed in D building. The Institute engineer, Mr Petrie, has been appointed as Fire Officer. Fire risk assessments are taking place for all the buildings on the site with five completed to date.

Training in fire safety precautions has been introduced for all users of laminar flow cabinets, as working with these is seen as a high fire risk. The training involves a presentation on best practice (for fire safety and scientific purposes), for work in a laminar flow cabinet, practical use of a fire extinguisher and an inter-active, self-study, CD-ROM-based, fire-awareness course devised by Hereford and Worcester Fire Brigade, 'In the Line of Fire'.

Professor Powell was appointed Chairman of SCRI's Health, Safety and Welfare Committee. The Safety Co-ordinator was a member of two BBSRC/SABRI teams inspecting health and safety management at the Macaulay and Moredun Research Institutes. He also attended the BBSRC/SABRI Safety Officers 6-monthly meetings and chaired the two-monthly meetings of SCRI internal safety advisors and SCRI safety post-holders. The two-year cycle of inspections of the BBSRC/SABR Institutes will recommence in 2002.

Defibrillator training of SCRI first-aiders with Red Cross trainer, Peter Carver (centre).

A Survivalink portable defibrillator has been purchased and a team of SCRI first-aiders has been trained in its use by the Red Cross. It is an 'intelligent' machine which can determine from a casualty's heart rhythm whether a defibrillating shock is required. The machine will not deliver a shock if it is not appropriate but will continue to monitor the casualty's heart rhythm, providing vital data which can be down-loaded by the emergency services to assist with treatment.

There were no serious injuries sustained by any member of staff at work and no RIDDOR-reportable incidents occurred during the year.

# Staff Association

J. Fairlie

The Scottish Crop Research Institute has had a very active Staff Association for the past 8 years and, currently, there are approximately 200 members. The primary aim of the Association is to raise funds for charity through raffles, prize draws, functions and donations from companies. Staff are encouraged to nominate both local and national charities to be beneficiaries, and at the Annual General Meeting, the members choose the charity.

For the year 2000, Roxburghe House in Dundee (a local hospice), was chosen. Mrs Elizabeth Goss, Service Manager, was presented with a cheque for £1708 in March 2001, and this money will be used to improve the gardens for the patients.

The Staff Association organises several outings for staff, including golf competitions, angling competitions, surfing, snowboarding and ten-pin bowling. Currently, there is a weekly yoga class, and there are netball, volleyball, softball, badminton and football teams, who are provided with equipment and strips from Association funds. This year, there have been inter-divisional netball and football tournaments, and a fun swimming gala. In June, the Association hosts a summer BBQ for staff and their families, and a Ceilidh, Disco and children's party are held in December.

There is a monthly draw for cinema tickets and a meal for two, as well as periodic prize draws. Local companies, restaurants, and theatres generously donate the prizes for these draws. This year's prizes include a flying lesson from Dundee airport. Staff discount schemes are also organised by the Staff Association and these include local shops, hairdressing salons, purchase of seeds and plants, as well as Corporate Sports membership at Dundee University Sports Union. The Association also holds corporate membership of the National Trust for Scotland, which provides four membership cards for staff use. 'Which' magazine is provided for the SCRI library.

Jane Fairlie presents a cheque to Elizabeth Goss.

Membership fees for the Association remain at £1.50 per month and the office bearers and committee are elected annually at the AGM. The charity for the year 2001 is DEBRA, which raises funds for children with a rare skin disorder.

# Publications

Publications are classified in the following manner:

J Papers describing original research in refereed journals.
R Critical reviews in journals, book chapters and reviews in books - providing each has been edited externally.
P Published proceedings of contributions to conferences or learned societies (including published abstracts).
T Technical reports, other publications.
O Popular articles, other publications.

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**Zhang, X., Bengough, A.G., Crawford, J.W., Young, I.M., Chessell, J.M. & Deeks, L.** 2000. Modelling water flow and solute transport in heterogeneous soil. *Eurosoil 2000,* Reading, September 2000. P

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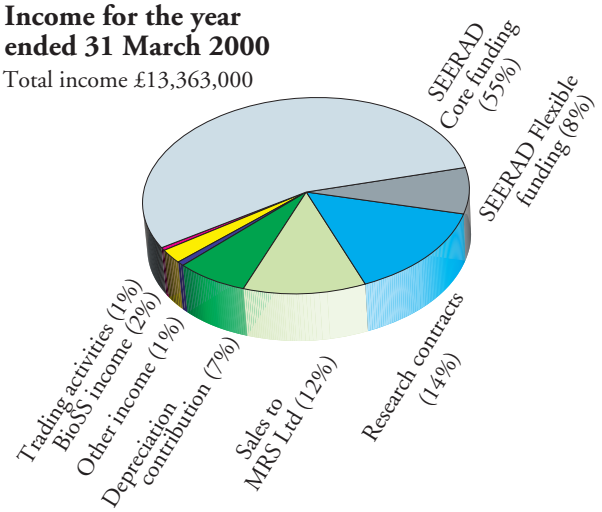
# Summary of the Accounts

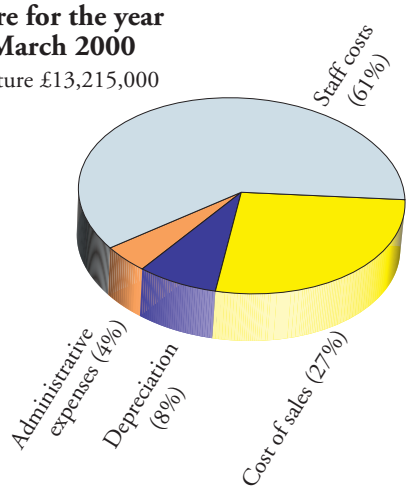
**Income for the year ended 31 March 2000**

Total income £13,363,000




**Expenditure for the year ended 31 March 2000**

Total expenditure £13,215,000




**Balance sheet at 31 March 2000** **Total value £21,403,000**

**Assets**

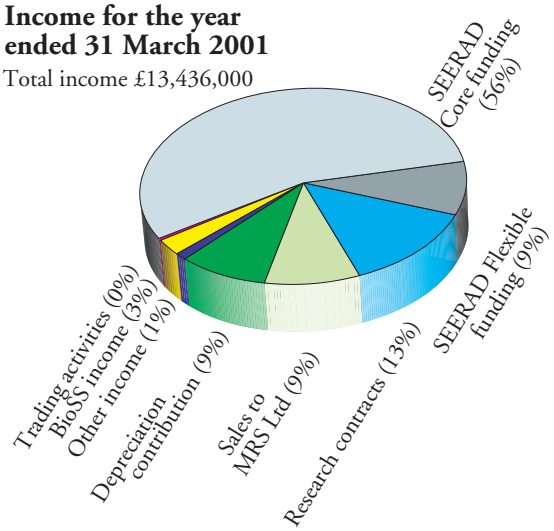
| | |
|---|---|
| Fixed assets | 90 % |
| Stocks | 0 % |
| Debtors | 10 % |

**Liabilities**

| | |
|---|---|
| Capital reserve | 89 % |
| Income & expenditure account | 3 % |
| Current liabilities | 8 % |

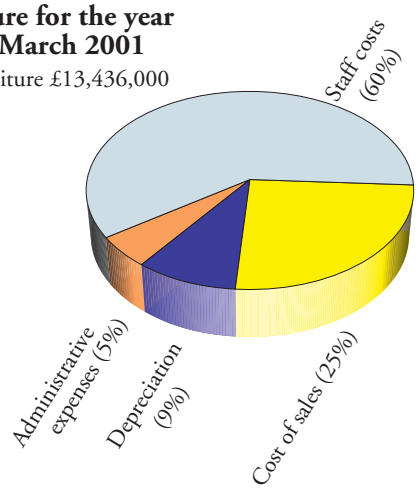
**Income for the year ended 31 March 2001**

Total income £13,436,000




**Expenditure for the year ended 31 March 2001**

Total expenditure £13,436,000




**Balance sheet at 31 March 2001** **Total value £21,451,000**

**Assets**

| | |
|---|---|
| Fixed assets | 92 % |
| Stocks | 0 % |
| Debtors | 8 % |

**Liabilities**

| | |
|---|---|
| Capital reserve | 91 % |
| Income & expenditure account | 3 % |
| Current liabilities | 5 % |

# The Governing Body

**Chairman: J.E.Godfrey, B.Sc., F.R.Ag.S.,** gained his degree in agriculture from the University of Reading. He is a Director of family farming companies in Lincolnshire and Yorkshire. He is Chairman of Willisham Group plc, and is a member or adviser to numerous committees including the Royal Agricultural Society of England. He is a Trustee of the International Potato Center (CIP) in Peru and a Director of World Potato Congress Inc. He was appointed to the Governing Body of SCRI in 1991, became Vice Chairman in 1997 and Chairman in 1999.

**E. Angus, MBE, M.Sc., Fio.D.**, has been actively involved in the start-up of several knowledge economy companies since retiring from Napier University in 1999, where he held the post of Business Director for the University and Managing Director of Napier University Ventures Limited. His degree in corporate leadership was gained after studying business incubation systems and processes in the US, UK, the Continent and Scandinavia. His strategic management experience at Board level in food, textiles and distribution companies, span a period of 25 years and he was awarded the honour of an MBE for his contribution to exporting in 1977. He was appointed to the Governing Body of SCRI in 2000.

**Professor J.J.F. Belch, M.B., Ch.B., F.R.C.P., M.D.**, is Professor of Vascular Medicine at the University of Dundee, where she is interested in the causes, manifestations and treatment of disease of the blood vessels and circulation. Additionally she is a member of the Medical Research Council Advisory Board, a member of the Scottish Office Acute Services Review Sub-Committe on Peripheral Arterial Disease, and UK Chairman of the Forum on Angiology. Her interests in terms of crop research relate to the antioxidant content of food, specific fatty acid types within oils, and the relationships of these to vascular disease. She was appointed to the Governing Body of SCRI in 1998.

**Professor R.J. Cogdell, B.Sc., Ph.D., F.R.S.E.**, was awarded his two degrees by Bristol University, and completed his post-doctoral research in the USA. He joined the Botany Department of Glasgow University (now the Institute of Biomedical and Life Sciences) in 1975, and currently holds the Hooker Chair of Botany there. He was awarded a Humbolt Research Prize in 1995. He was appointed to the Governing Body of SCRI in 1997, and was recently re-appointed. He is a member of the Chairman's Committee, and Chairs the Science Sub-Committee. He is a Director of MRS and a Trustee of the new Mylnefield Trust.

**Dr K. Dawson, B.Sc., Ph.D., D.I.C.P.,** is Technical Director of CSC CropCare, the largest privately owned crop consultancy service in the North of the UK. He trained as an agricultural and environmental scientist and was awarded his degrees by the University of Newcastle-Upon-Tyne and the University of Reading. He joined the Scottish Agricultural College in 1982 and, after a spell as Northern Technical Advisory Manager for BASF(UK) Ltd, formed CSC CropCare in 1987. He is an elected director of BASIS(UK) Ltd and a member of the Government's Pesticide Forum. He also has been closely associated with the Scottish Natural Heritage TIBRE programme, utilising new technology for agronomic and environmental benefit. His main interests are in crop protection and Integrated Farming Management. He was appointed to the Governing Body of SCRI in 2000.

**Dr M. Eddie, B.Agr., Ph.D.**, gained both his degrees from The Queen's University, Belfast. After employment as a research scientist by the Ministry of Agriculture, Northern Ireland for 4 years, he joined Unilever plc where he spent 25 years mainly in their agribusiness operations, eventually becoming Chairman, in sequence, of two agribusiness companies. The first was based in Scotland and the second in Malaysia. After retirement from Unilever in 1999, he was appointed to the Governing Body of SCRI in March 2000.

**Professor M.J. Emes, B.Sc., Ph.D.**, is Director of the Research and Graduate School in the Faculty of Biological Sciences, University of Manchester, where he is responsible for over 120 academic staff and the training of 400 postgraduate students. His own research activities are focused on understanding the control of plant metabolism, particularly mechanisms of regulating starch synthesis in cereals. He has extensive experience of BBSRC grants committees and is a member of the Governing Council of the John Innes Centre. He is also an editor of the Journal of Experimental Botany. He was appointed to the Governing Body of SCRI in 2000.

**Professor J. Evans, OBE, B.Sc., Ph.D., D.Sc., F.I.C.For.**, is Professor in Tropical Forestry (part time) at Imperial College, London, and was formerly Chief Research Officer (S) with the UK Forestry Commission from 1989 to 1997. He is Chairman of the Commonwealth Forestry Association and is Chair of DfID's Programme Advisory Committee for Forestry Research. Professor Evans also holds an honorary Chair of Forestry at the University of North Wales, Bangor. He is the author of eight technical books, including the newly published Forests Handbook and the standard text on tropical forest plantations. Professor Evans owns and manages his own small woodland. He was appointed to the Governing Body of SCRI in 1998.

**Wendy Goldstraw, B.Sc., P.G.Dip.B.A., M.C.I.P.D.**, gained her degrees from the University of Edinburgh, before joining the Post Office as a management trainee. After a number of roles in human resources and line management, she was latterly General Manager for Post Office Counters Ltd for Scotland and Northern Ireland, with responsibility for 2800 Post Offices. She was an executive member of both the Scottish and Northern Ireland Post Office Boards, and served as a Director of Edinburgh Chamber of Commerce and also on the Scottish Committee of the Institute of Directors. She has been a member of the Accounts Commission for Scotland since 1994. She was appointed to the Governing Body of SCRI in 2000.

**K. Hopkins, F.C.A.**, joined Reeves & Neylan, Chartered Accountants, in Canterbury, Kent, in 1971, from a farming background. He moved to open the Scottish Practice in 1978 and was appointed a partner in 1981. 'The Scottish Partnership' (a separate business since April 1996) acts for over 500 farmers in Scotland, and specialises in the establishment of farmer-led agricultural cooperatives. His firm now has three offices, Forfar, Perth and Dundee, and employs over 60 staff. Mr Hopkins specialises in capital taxes, agricultural law and cooperatives, development and expansion of business, writes for the agricultural press, and lectures throughout Scotland. He is Treasurer for District 1010 of Rotary, a member of the Institute of Directors, and Chairman of the charity Childlink Scotland. He was appointed to the Governing Body of SCRI in 1997.

**Professor B. King, M.Sc., Ph.D., F.I.W.Sc., C.Biol., F.I.Biol.**, is Principal and Vice-Chancellor, University of Abertay Dundee, having joined it in 1992 from the Robert Gordon University, Aberdeen, where he was Assistant Principal and Dean of the Faculty of Health and Food. He is a Non-Executive Trustee of Tayside Primary Care NHS Trust, Board Member of Scottish Enterprise Tayside, Governor of the Unicorn Preservation Society, and a member of the Institute for Learning and Teaching in Higher Education. He is a member of the International Research Group on Wood Preservation and of the Biodeterioration and British Mycological Societies. He was appointed to the Governing Body of SCRI in 1998.

**I. McLaren, S.D.A.**, is a partner in a family owned farming business, specialising in potato and cereal production. He is also a partner in a retail dairy business, a garage business, and a visitors' centre. He is Chairman of a leisure complex, the Dewar's Centre in Perth, and a member of the Perth & Kinross Agricultural Forum, and was a member of the Home-Grown Cereals Authority from 1988 to 1997. He was appointed to the Governing Body of SCRI in 2000.

**Emeritus Professor Sir John S. Marsh, C.B.E., M.A., P.G. Dip. Ag. Econ., F.R.A.S.E., F.R.Ag.S., C.Biol., F.I.Biol.**, was Professor of Agricultural Economics, University of Aberdeen, from 1977-1984, then Professor of Agricultural Economics, University of Reading from 1984-1997. He is a Former Director of the Centre of Agricultural Strategy and Chairman of the Agricultural Wages Board, and is currently Chairman of RURAL Council, Governor of the Royal Agricultural College, and Member of the Agriculture, Horticulture and Forestry Foresight Panel. He was made a Knight Bachelor in the Queen's Birthday Honours List in 1999 for his wide-ranging contributions to agriculture and agricultural research. He was appointed to the Governing Body of SCRI in 1998.

**Professor A.R. Slabas, B.Sc., D.Phil.**, is Head of Plant Molecular Biology Research in the Department of Biological Sciences, University of Durham, where he leads a team involved in various aspects of lipid metabolism ranging from novel gene identification to structural studies. His more recent interests are in proteomics and the plant cell wall. He has extensive collaboration with Industry, including Biogemma, Zeneca, Linnaeus and Unilever. He has served as a panel member of the UK Technology Foresight Programme 'Crops for Food and Industrial Use'; the Eukaryotic Cell Link Management Committee; and the BBSRC Inovative Manufacturing Committee. He joined the Governing Body in 1995.

**P. Whitworth** retired from United Biscuits plc as Technical Director, Snacks. During his 38 years with the company, he was associated with all aspects of the

development and production of biscuits, potato crisps and savoury snacks. He joined the board of the European Snacks Association (ESA) in 1988, and served as President of the Association from 1994 to 1996. He was a founder Director of ECSA Research Ltd (ERL), which through an ECLAIR funded project sought to improve the quality of crisping potatoes using genetic manipulation. A major part of this work has been carried out at SCRI. Having retired from the board of ERL, he was appointed to the Governing Body of SCRI in 1997 and is now a member of the Chairman's Committee and a Director of MRS.

# Staff list as at 31 March 2001 (except where indicated)

| | | |
|---|---|---|
| Director | Professor J.R. Hillman, B.Sc., Ph.D., D.Sc., F.L.S., C.Biol., F.I.Biol., F.I.Hort., F.R.S.E.[2,3,4] | Band 1 |
| Deputy Director | Professor W. Powell, B.Sc., M.Sc., Ph.D., D.Sc. [5,6,7,21] | Band 2 |
| Company Secretary | D. Watt, L.L.B., C.A. | Band 3 |
| Assistant to Director | T.J.W. Alphey, B.Sc., Ph.D., C.Biol., M.I.Biol. | Band 4 |

## Division of Biochemistry and Cell Biology

Head : H.V. Davies, B.Sc., Ph.D., C.Biol., F.I.Biol.[6,8] Band 3

Deputy Head : K.J. Oparka, B.Sc., Ph.D.[6] Band 3 (IMP)

### Unit of Plant Biochemistry (PB)

| | |
|---|---|
| Head : R. Viola, B.Sc., Ph.D.[11,22] | Band 4 |
| Deputy Head : D.W. Griffiths, M.A., Ph.D., C. Chem., M.R.S.C. | Band 4 |
| H.V. Davies, B.Sc., Ph.D., C.Biol., F.I.Biol.[6,8] | Band 3 |
| B.A. Goodman, B.Sc., Ph.D., C.Chem., F.R.S.C. | Band 4 |
| D. Stewart, B.Sc., Ph.D. | Band 4 (Prom. Jul 00) |
| M.A. Taylor, B.Sc., Ph.D.[10] | Band 4 |
| N. Deighton, B.Sc., Ph.D., C.Chem., M.R.S.C. | Band 5 (SPD) |
| G. Dobson, B.Sc., Ph.D. | Band 5 (SPD) |
| G.J. McDougall, B.Sc., Ph.D. | Band 5 (SPD) |
| H.A. Ross, H.N.C., Ph.D., C.Biol., M.I. Biol. | Band 6 (PD) |
| F. Falconer, H.N.C. | Band 8 |
| D. McRae, O.N.C. | Band 8 |
| P. Dobson | Band 10 (P/T) |
| J.A. Sungurtas | Band 10 (P/T) |
| J.F. Wilkie | Band 10 |
| C. Torrie | Band 11 (P/T) (HELM) |
| Quality Assurance Officer : T. Shepherd, B.Sc., Ph.D. | Band 6 (PD) |

Unit Administrators :

| | |
|---|---|
| E.L. Stewart | Band 8 |
| C. Goldmann, M.A. | Band 8 (P/T) (Appt. Feb 01) |

SEERAD FF

| | |
|---|---|
| P.P.M. Iannetta, B.Sc., Ph.D. | Band 6 (PD) |

EU

| | |
|---|---|
| P. Baumgartner, B.Sc., M.Sc. | Band 6 (PD) (Appt. Apr 00) |
| E. Pascual, B.Sc., Ph.D. | Band 6 (Appt. Oct 00) |
| M. L. Ruiz del Castillo, B.Sc., Ph.D. | Band 6 (Appt. Dec 00) |
| L.V.T. Shepherd, B.Sc., M.Sc., Ph.D. | Band 6 (PD) |
| R. Razzo, B.Sc. | Band 9 (Appt. Jan 01) |
| J.A. Stewart, H.N.D., B.Sc. | Band 10 (Appt. Dec 00) |

GlaxoSmithKline

| | |
|---|---|
| R.D. Hancock, B.Sc., Ph.D. | Band 6 (PD) |
| M.R. MacLeod, B.Sc., Ph.D. | Band 6 (PD) |

### Unit of Cell Biology (CB)

| | |
|---|---|
| Head : K.J. Oparka, B.Sc., Ph.D.[6] | Band 3 (IMP) |
| I.M. Roberts, H.N.C., Dip.R.M.S. | Band 4 |
| P. Boevink, B.Sc., Ph.D. | Band 5 (SPD) |
| G.H. Duncan, H.N.C. | Band 5 |
| S. Glidewell, M.A., M.Sc., Ph.D. | Band 6 (PD) |
| K.M. Wright, M.A., Ph.D. | Band 6 (PD) |
| F. Carr | Band 8 (P/T) |
| Unit Administrator : F. Watt | - |

SEERAD FF

| | |
|---|---|
| T. Gillespie, B.Sc. | Band 6 (PD) |
| A. Roberts, B.Sc., Ph.D. | Band 6 (PD) |
| J. Moir, B.Sc. | Band 7 (P/T) (w.e.f. 1/5/01) |
| J. Watters, H.N.D., B.Sc. | Band 7 (Tr. from GE Oct 00) |

EU

| | |
|---|---|
| L. Simon-Buela, M.Sc., Ph.D. | Band 6 (PD) |

## Division of Genetics

Acting Head : G.C. Machray, B.Sc., Ph.D.[1] (w.e.f. 1-7-01) Band 4

### Unit of Gene Expression (GE)

| | |
|---|---|
| Head : G.C. Machray, B.Sc., Ph.D.[1] | Band 4 |
| Deputy Head: C.G. Simpson, B.Sc., Ph.D. | Band 5 (SPD) |
| J.W.S. Brown, B.Sc., Ph.D.[9] | Band 3 (IMP) |
| S. Millam, B.Sc., Ph.D.[9] | Band 5 |
| G. Thow, B.Sc., Ph.D. | Band 6 (PD) |
| G. Clark, H.N.C., B.Sc. | Band 7 |
| B. Harrower, H.N.D., B.Sc., M.Sc. | Band 7 |
| J. Middlefell-Williams, H.N.C. | Band 7 |
| A. Booth, H.N.C. | Band 8 |
| D. Davidson | Band 8 (P/T) |
| J.D. Fuller | Band 9 |

DNA Sequencing / Genotyping Facility

| | |
|---|---|
| Clare McQuade, B.Sc. | Band 7 |

Media Kitchen

| | |
|---|---|
| W. Ridley | Band 7 |
| E. Warden, O.N.C. | Band 9 |
| W. Burry | Band 11 (HELM) |
| M. Burton | Band 11 (P/T) |
| J. McMillan | Band 11 (P/T) (HELM) |

Unit Administrator :

| | |
|---|---|
| E.L. Stewart | Band 8 |

SEERAD FF

| | |
|---|---|
| S.N. Jennings, B.Sc. | Band 9 (P/T) (Appt. Dec 00) |

BBSRC GAIT

| | |
|---|---|
| J. Wardrop, B.Sc., Ph.D. | Band 6 (PD) |

BBSRC DTI LINK

| | |
|---|---|
| A. Ibrahim, B.Sc., Ph.D. | Band 6 (PD) |

[1] Visiting Professor in the University of Strathclyde
[2] Visiting Professor in the University of Dundee
[3] Visiting Professor in the University of Edinburgh
[4] Visiting Professor in the University of Glasgow
[5] Honorary Senior Lecturer in the University of St. Andrews
[6] Honorary Senior Lecturer in the University of Dundee
[7] Honorary Professor, Oregon State University
[8] Professor, Universities of Cordoba and Malaga
[9] Honorary Lecturer in the University of Dundee
[10] Honorary Lecturer in the University of Glasgow
[11] Associate Professor, University of Parma
[12] Adjunct Professor, Cornell University
[13] Visiting Professor, Agricultural University of Athens
[14] Visiting Professor, University of Zhejiang, China
[15] Honorary Lecturer in the University of Aberdeen
[16] Honorary Research Fellow in the University of Dundee
[17] Visiting Professor, University of Kyoto, Japan
[18] Honorary Professor of Botany, Florida International University
[19] Honorary Fellow in the University of Edinburgh
[20] Honorary Lecturer in the University of Strathclyde
[21] Honorary Professor, Heriot-Watt University, Edinburgh
[22] Visiting Professor, University of Naples, Italy
[23] Honorary Professor, Seoul Women's University
[24] Adjunct Professor, Moscow State University

## Unit of Genomics (Genom)

| | |
|---|---|
| **Head:** R. Waugh, B.Sc., Ph.D.[9] | Band 4 |
| **Deputy Head:** D.F. Marshall, B.Sc., Ph.D. | Band 4 |
| J. Graham, B.Sc., Ph.D. | Band 4 (SPD) (Prom. Jul 00) |
| G. Bryan, B.Sc., M.Sc., Ph.D. | Band 5 (SPD) |
| J. Russell, B.Sc., Ph.D. | Band 5 (Appt. Jun 00) |
| D. Caldwell, B.A. | Band 6 (Appt. Sep 00) |
| P. Davie, O.N.C. | Band 8 |
| N. McCallum, B.Sc. | Band 7 (Appt. Jan 01) |
| J. McNicoll, H.N.C., B.Sc. | Band 7 |
| M. Macaulay, H.N.C., B.Sc. | Band 7 |
| N. Bonar, H.N.C. | Band 8 |
| S.L. Linton, B.Sc. | Band 8 (w.e.f. 1/4/01) |
| S. Mudie | Band 8 |
| K. Smith, Dip. H.E. | Band 8 |
| G. Simpson | Band 9 |
| **Unit Administrator:** | |
| S. Forsyth | Band 8 |
| **SEERAD FF** | |
| P. Hedley, B.Sc., Ph.D. | Band 6 (SPD) (Appt. Nov 00) |
| H. Liu, M.Sc. | Band 7 (Appt. Oct 00) |
| A. Purvis, B.Sc., Ph.D. | Band 6 (PD) (Appt. Oct 00) |
| K. McLean, B.Sc. | Band 7 |
| **BBSRC** | |
| L. Cardle, B.Sc., Ph.D. | Band 6 (PD) |
| **EU** | |
| D. Milbourne, B.Sc., Ph.D. | Band 6 (PD) |
| L. Ramsay, B.Sc., Ph.D. | Band 6 (PD) |
| I.M. Tierney, B.Sc., M.Sc. | Band 6 (Appt. Mar 01) |
| **HORTLINK** | |
| H. McCafferty, B.Sc., Ph.D. | Band 6 (PD) |
| A. Stevenson | Band 10 (P/T) |
| **Leverhulme** | |
| A. James, B.Sc. | Band 6 (PD) |

## Unit of Applied Genetics (AG)

| | |
|---|---|
| **Head :** J.E. Bradshaw, M.A., M.Sc., Ph.D.[9] | Band 4 |
| **Deputy Head :** W.T.B. Thomas, B.Sc., Ph.D. | Band 4 |
| G.R. Mackay, B.Sc., M.Sc., C.Biol., F.I.Biol.[6] | Band 3 |
| R.J. McNicol, B.Sc.[6] | Band 3 |
| R.M. Brennan, B.Sc., Ph.D. | Band 4 |
| M.F.B. Dale, B.Sc., Ph.D.[9] | Band 4 |
| R.P. Ellis, B.Sc., Ph.D.[9] | Band 4 |
| B.P. Forster, B.Sc., Ph.D.[9] | Band 4 |
| G. Ramsay, B.Sc., Ph.D. | Band 5 (SPD) |
| J.S. Swanston, B.Sc., Ph.D., C.Biol., M.I.Biol. | Band 5 (SPD) |
| M.J. De,Maine, B.Sc., M.Phil. | Band 5 |
| H.E. Stewart, C.Biol., M.I.Biol. | Band 6 (PD) |
| R.M. Solomon-Blackburn, B.A., M.Sc. | Band 6 |
| J. Lyon | Band 7 |
| G.E.L. Swan | Band 7 |
| D. Todd, B.Sc., M.Sc. | Band 7 |
| R.N. Wilson, N.C.H. | Band 7 |
| G.R. Young, H.N.C. | Band 7 |
| S.L. Gordon, H.N.C. | Band 8 |
| R. Keith | Band 8 |
| P.E. Lawrence | Band 9 |
| M. Myles, O.N.C. | Band 9 |
| S.J.Neilson, Dip. Biol. Sci. | Band 9 |
| A. Bertie | Band 10 |
| A.M.S.McInroy | Band 10 |
| **Unit Administrators :** | |
| S. Forsyth | Band 8 |
| **SEERAD FF** | |
| C. Thompson, B.Sc., Ph.D. | Band 6 (PD) (w.e.f 1-4-01) |
| **EU** | |
| J. Moir, B.Sc. | Band 7 (P/T) |
| H. Baldie | Band 8 |
| D. Jean Harkins | Band 9 |
| **H-GCA** | |
| P. Rajasekaran, B.Sc., Ph.D. | Band 6 (PD) |

# Division of Pathology

**Head :** P.F. Palukaitis, B.Sc., Ph.D.[6,12,13,23] Band 3 **Deputy Head :** H. Barker, B.Sc., Ph.D. Band 4

## Unit of Virology (Vir)

| | |
|---|---|
| **Head :** H. Barker, B.Sc., Ph.D. | Band 4 |
| **Deputy Head :** M.A. Mayo, B.Sc., Ph.D., C.Biol., M.I.Biol.[6] | Band 3 (IMP) |
| A.T. Jones, B.Sc., Ph.D.[6] | Band 3 (IMP) |
| P.F. Palukaitis, B.Sc., Ph.D.[6,12,13,23] | Band 3 |
| J.M.S. Forrest, B.Sc., Ph.D. | Band 4 |
| S.A. MacFarlane, B.Sc., D.Phil. | Band 4 (Prom. Jul 00) |
| D.J. Robinson, M.A., Ph.D.[9,14] | Band 4 |
| M. Taliansky, Ph.D., D.Sc.[24] | Band 4 |
| L. Torrance, B.Sc., Ph.D.[9] | Band 4 |
| J.A.T. Woodford, B.A., M.A., Ph.D., F.R.E.S.[9] | Band 4 |
| A. Kumar, B.Sc., Ph.D. | Band 5 (SPD) |
| B. Reavy, B.Sc., D.Phil. | Band 5 (SPD) |
| A. Ziegler, B.Sc., Ph.D. | Band 5 (SPD) (Prom. Jul 00) |
| T. Canto, B.Sc., Ph.D. | Band 6 (PD) |
| M. M. Swanson, B.Sc., Ph.D. | Band 6 |
| G.H. Cowan, H.N.D., M.Sc. | Band 7 |
| S. M.S. Dawson, H.C. | Band 7 |
| A. Dolan, H.N.C. | Band 7 (P/T) |
| W.J. McGavin, B.Sc. | Band 7 |
| K.D. McGeachy, H.N.C. | Band 7 |
| G.L. Fraser | Band 8 (P/T) |
| **Unit Administrator :** | |
| F. Watt | - |
| **SEERAD FF** | |
| N. Wood, B.Sc., Ph.D. | Band 6 (PD) |
| L. Jorgensen, H.N.D. | Band10 |
| **EU** | |
| F. Cillo, Ph.D. | Band 6( Appt. Feb 00) |
| J.S. Miller, B.Sc., Ph.D. | Band 6 (PD) |
| E. Davidson, B.Sc. | Band 6 (Appt. Oct 00) |
| H. Grant, B.Sc. | Band 7 (Appt. Jun 00) |
| M. Liney | Band 7 |
| **Leverhulme** | |
| S.H. Kim, B.Sc., Ph.D. | Band 6 (Appt. Mar 00) |

## Unit of Mycology, Bacteriology and Nematology (MBN)

| | |
|---|---|
| Head : J.M. Duncan, B.Sc., Ph.D.[6] | Band 3 |
| Deputy Head : M.S. Phillips, B.Sc. | Band 4 |
| D.J.F. Brown, B.A., Ph.D., D.Sc., C.Biol., F.I. Biol., F.R.S.N., F.S.O.N.[9,14] | Band 4 |
| G.D. Lyon, B.Sc., M.Sc., Ph.D., D.I.C.[9] | Band 4 |
| A.C. Newton, B.Sc., Ph.D. | Band 4 |
| B. Williamson, B.Sc., M.Sc., Ph.D., D.Sc.[9] | Band 4 |
| P.R.J. Birch, B.Sc., Ph.D. | Band 5 (SPD) |
| V. Blok, B.Sc., M.Sc., Ph.D. | Band 5 (SPD) |
| D.E.L. Cooke, B. Sc., Ph.D. | Band 5 (SPD) |
| J.T. Jones, B.Sc., Ph.D. | Band 5 (SPD) |
| I.K. Toth, B.Sc., Ph.D.[15] | Band 5 (SPD) |
| A.K. Lees, B.Sc., Ph.D. | Band 6 (PD) |
| L.J. Hyman, B.A., M.Sc. | Band 6 |
| R. Lowe | Band 6 |
| R. Neilson, H.N.C., M.Sc., Ph.D. | Band 6 (PD) |
| J. Heilbronn, H.N.C., B.Sc. | Band 7 |
| A. Smith, B.Sc. | Band 7 (P/T) |
| N.A. Williams, H.N.C. | Band 7 |
| A.M. Holt | Band 8 (P/T) |
| S.S. Lamond | Band 8 |
| L. Sullivan, B.Sc. | Band 9 (Appt. Oct 00) |
| A.J. Paterson, H.N.D. | Band 10 (P/T) |
| Unit Administrators : | |
| M. Murray | Band 8 |
| F. Watt | - |
| SEERAD FF | |
| A. Avrova, B.Sc., Ph.D. | Band 6 (PD) (Appt. Feb 01) |
| K. Bell, B.Sc., Ph.D. | Band 6 (PD) |
| S. Whisson, B.Sc., Ph.D. | Band 6 (PD) |
| L. Castelli, B.Sc., M.Sc. | Band 7 |
| SEERAD/BPC | |
| P. van de Graaf, B.Sc., M.Sc., Ph.D. | Band 6 (PD) |
| J.L. Brierley, B.Sc., Ph.D. | Band 6 (Appt. Mar 01) |
| Commercial Funding | |
| P. Neave | Band 10 |
| EU | |
| M. Armstrong, B.Sc., Ph.D. | Band 6 (PD) |
| B. Banks | Band 6 (Appt. May 00) |
| J. Wishart, B.Sc., Ph.D. | Band 6 (PD) (Appt. May 00) |
| D.C. Guy, H.N.D. | Band 7 |
| W. Morris, B.Sc. | Band 7 |
| A. Prior, B.Sc. | Band 7 (P/T) |
| G. Ellis | Band (P/T) 10 (Appt. Jan 01) |
| BPC | |
| M. Elliot, B.Sc. | Band 7 |
| MAFF/BPC | |
| D. Cullen, B.Sc., Ph.D. | Band 6 (PD) |

# Division of Plants, Soils and Environment

Head : G.R. Squire, B.A., Ph.D. — Band 4

Deputy Head : K. Ritz, B.Sc., Ph.D.[16,17] — Band 4

## Unit of Soil-Plant Dynamics (SPD)

| | |
|---|---|
| Head : K. Ritz, B.Sc., Ph.D.[16,17] | Band 4 |
| Deputy Head : A.G. Bengough, B.Sc., Ph.D. | Band 5 (SPD) |
| B. Boag, B.Sc., Ph.D.[6] | Band 4 |
| B.S. Griffiths, B.Sc., Ph.D. | Band 4 |
| L.L. Handley, B.A., B.Ed., M.Sc., Ph.D.[18] | Band 4 |
| R.E. Wheatley, B.Sc., Ph.D. | Band 4 |
| T. Daniell, B.Sc., Ph.D | Band 5 (SPD) (Appt. Oct 00) |
| J. Liu, B.Sc., M.Sc., Ph.D. | Band 5 |
| C.M. Scrimgeour, B.Sc., Ph.D.[9] | Band 5 |
| P.D. Hallett, B.Sc., Ph.D. | Band 6 (PD) |
| D.C. Gordon, H.N.C. | Band 6 |
| W.M. Stein, H.N.C., B.Sc. | Band 6 |
| S. Caul, H.N.C. | Band 7 |
| S. Verrall, H.N.C. | Band 7 (P/T) |
| Unit Administrator : | |
| S. Inglis | Band 8 |
| SEERAD FF | |
| L. Deeks, B.Sc., Ph.D. | Band 6 (PD) |
| X. Zhang, B.Sc., Ph.D. | Band 6 (PD) |
| J. Squires, B.Sc., Ph.D. | Band 7 (Appt. Nov 00) |
| S. Verrall, H.N.C. | Band 7 (P/T) |
| BBSRC | |
| V. Stubbs, B.Sc., Ph.D. | Band 6 (PD) (Appt. Feb 01) |
| K.. Zhang, BSc. Ph.D. | Band 6 (PD) (Appt. Jun 00) |
| K. Harris, B.Sc. | Band 8 |
| DTI LINK | |
| N. Nunan, B.Sc., Ph.D. | Band 6 (PD) |
| K. Wu, B.Sc., Ph.D. | Band 6 (PD) |
| EU | |
| C. Fernie, B.Sc. | Band 6 (Appt. Apr 00) |

## Unit of Vegetation Systems (VS)

| | |
|---|---|
| Head :D.K.L. MacKerron, B.Sc., Ph.D. | Band 4 |
| Deputy Head : B. Fenton, B.Sc., Ph.D., C.Biol., M.I.Biol. | Band 5 (SPD) |
| G.R. Squire, B.A., Ph.D. | Band 4 |
| D.L. Trudgill, B.Sc., Ph.D., C.Biol., F.I.Biol., F.S.O.N.[5] | Band 3 |
| A.N.E. Birch, B.Sc., Ph.D., C.Biol., M.I.Biol., F.R.E.S. | Band 4 |
| S.C. Gordon, H.N.C. | Band 5 |
| M. Maule, B.Sc., Ph.D | Band 6 (Appt. Oct 00) |
| G. Malloch, D.C.R., B.Sc. | Band 7 |
| G. Wright, H.N.C. | Band 7 |
| Unit Administrators : | |
| S. Inglis | Band 8 |
| M. Murray | Band 8 |
| SEERAD FF | |
| I.E. Geoghegan, M.Sc. | Band 7 |
| MAFF | |
| J. Hillier, B.Sc., Ph.D. | Band 6 (PD) |
| S. Hockaday, B.Sc., M.Sc., Ph.D. | Band 6 (PD) |
| DETR | |
| C. Hawes, B.Sc., Ph.D. | Band 6 (PD) |
| M. Young, H.N.D., M.Sc., Pg.Dip.IT. | Band 6 (PD) (Appt. Jun 00) |
| J. Reay, B.Sc. | Band 7 |
| A. Cocker, B.Sc. | Band 8 (Appt. Jun 00) |
| J.K. Beaton, B.Sc. | Band 9 (Appt. Mar 01) |
| M.A. McKinlay, H.N.C., H.N.D. | Band 9 (Appt. Mar 01) |

# Division of Finance & Administration

Head : D. Watt, L.L.B., C.A. Band 3

## Unit of Finance and Human Resources

| | |
|---|---|
| Financial Controller : I.F. Harrington, C.A. | Band 4 |
| Assistant Secretary : D.L. Hood, B.Admin., Dip. Ed., L.T.I., A.I.I.M. | Band 6 |
| Personnel Officer : I. Paxton, H.N.C., M.Sc., M.I.P.D. | Band 6 |
| Director's Secretary: A. Pack | Band 7 |
| C. Skelly | Band 7 |
| D.L. Beharrie, Dip. Ed. | Band 8 |
| R.G. Davidson | Band 8 |
| P. Duncan | Band 8 |
| L. Ellis, H.N.C. | Band 8 (Appt. Oct 00) |
| K.L. Grant, B.A. | Band 8 |
| B.V. Gunn | Band 8 |
| S. Phillip, B.A. | Band 8 |
| S. Bell | Band 9 |
| L. Fiddes | Band 10 |
| J. Keith | Band 10 |

## Unit of Scientific Liaison and Information Services (SLIS)

| | |
|---|---|
| Head : W.H. Macfarlane Smith, B.Sc., Ph.D., C.Biol., M.I.Biol., F.I. Mgt. | Band 4 |
| Deputy Head : T.D. Heilbronn, B.Sc., M.Sc. | Band 6 |
| T.G. Geoghegan, A.B.I.P.P., A.M.P.A. | Band 5 |
| I.R. Pitkethly, H.N.D. | Band 6 |
| S.E. Stephens, B.Sc., M.A., A.L.A. | Band 6 |
| S.F. Malecki, A.B.I.P.P. | Band 7 |
| U. M. McKean, M.A., Dip. Lib. | Band 7 |
| Safety Coordinator : M.J. De,Maine, B.Sc., M.Phil. | Band 5 |

## Unit of Information Technology (IT)

| | |
|---|---|
| Head : B. Marshall, B.Sc., A.R.C.S., Ph.D.[16] | Band 4 |
| Operations Manager : S. Clark, H.N.C., M.Sc. | Band 5 |
| P. Smith, B.Sc. | Band 6 |
| L. Davidson, B.A. | Band 8 (Appt. Oct 00) |

## Unit of Engineering & Maintenance Department (EM)

| | |
|---|---|
| Head: S. Petrie, B.Sc. | Band 4 |
| D. Gray, H.N.C. | Band 6 |
| A. Low | Band 7 |
| I.C. McNaughton, H.N.C. | Band 7 |
| K.A. Henry | Band 8 |
| R.D. McLean | Band 8 |
| G.C. Roberts | Band 8 |
| R. White | Band 8 |
| J. Anderson | Band 9 |
| D. Byrne | Band 9 |
| E. Lawrence | Band 9 |
| C.G. Milne | Band 9 |
| W. Scott | Band 9 |
| C. Conejo | Band 10 |
| J. Flight | Band 10 |
| D.J. Redford | Band 10 (Appt. Jun 00) |
| D.L.K. Robertson | Band 10 |
| J. Rowe | Band 10 |
| M.J. Soutar | Band 10 |
| Unit Administrator : Wendy A. Patterson, H.N.D. | Band 8 (P/T) |

## Unit of Estate, Glasshouse & Field Research (EGFR)

| | |
|---|---|
| Head : G. Wood, B.Sc., Ph.D., F.E.T.C. | Band 4 |
| Glasshouse Manager : P.A. Gill, H.N.D., N.E.B.O.S.H. | Band 5 |
| G.R. Pitkin, H.N.D. | Band 6 |
| J.R.K. Bennett | Band 7 |
| W.D.J. Jack, B.Sc. | Band 7 |
| D.S. Petrie | Band 7 |
| D.G. Pugh | Band 7 |
| A.W. Mills | Band 8 |
| R. Ogg | Band 8 |
| G. Pugh | Band 8 |
| A.M. Thain, H.N.C. | Band 8 |
| J.T. Bennett | Band 9 |
| B. Fleming | Band 9 |
| C. Cuthill, N.C. | Band 10 (Appt. Nov 00) |
| A. Dobson, H.N.C., H.N.D. | Band 10 |
| I. Fleming | Band 10 |
| A.C. Fuller | Band 10 |
| M. Grassie, H.N.C., B.E.D. | Band 10 |
| P. Heffell, O.N.C. | Band 10 |
| J. Mason | Band 10 |
| T.A. Mason, N.E.B.S.M. | Band 10 |
| D.I. Matthew, B.Sc. | Band 10 |
| A.D. Munro, H.N.D. | Band 10 |
| J.K. Wilde | Band 10 |
| J. Abernethy | Band 11 (P/T) (HELM) |
| J-M Ford | Band 11 (P/T) (Appt.Feb 01) |
| M. Torrie | Band 11 (P/T) (HELM) |
| Unit Administrator : Wendy A. Patterson, H.N.D. | Band 8 (P/T) |

# Biomathematics and Statistics Scotland (BioSS)

*King's Buildings, University of Edinburgh*

| | |
|---|---|
| Director : R.A. Kempton, M.A., B.Phil.[19] | Band 3 |
| C.A. Glasbey, M.A., Dip. Math. Stats., Ph.D., D.Sc., M.I.S.I.[19,20] | Band 3 (IMP) |
| G.J. Gibson, B.Sc., Ph.D. | Band 4 |
| E.A. Hunter, B.Sc., M.Phil.[19] | Band 4 |
| M. Talbot, F.I.S., M.Phil. [19] | Band 4 |
| I.J. McKendrick, B.Sc., Ph.D. | Band 5 (SPD) |
| J. M. Dickson, B.Sc. | Band 5 |
| G.R. Marion, B.Sc., M.Sc., Ph.D. | Band 5 (SPD) (Appt. Dec 00) |
| A.M. Roberts, B.Sc., M.Sc. | Band 5 |
| J.A.N. Filipe, B.Sc., M.Sc., Ph.D. | Band 6 (PD) |
| A.D. Mann, B.Sc. | Band 6 |
| M.A.M. Kirkwood, D.A. | Band 7 (Prom. Feb 00) |
| D. Glancy | Band 10 (P/T) |
| A.G. Stewart | Band 10 (P/T) |
| Administration Officer : E.M. Heyburn, M.A. | Band 7 |
| J. Clabby | Band 8 (P/T) |

*Ayr Unit*

| | |
|---|---|
| Head : S. Brocklehurst, B.Sc., Ph.D. | Band 5 (SPD) |
| I.M. Nevison, M.A. | Band 6 (PD) |

*Aberdeen Unit, RRI*

| | |
|---|---|
| Head : G.W. Horgan, B.A., M.Sc., Ph.D. | Band 5 (SPD) |
| C.D. Mayer, M.Sc., Ph.D. | Band 5 (SPD) (Appt. Oct 00) |
| G. Zuur, M.Sc., Ph.D. | Band 6 (PD) |

*Aberdeen Unit, MLURI*

| | |
|---|---|
| Head : D.A. Elston, B.A., M.Sc. | Band 4 |
| M.J. Brewer, B.Sc., Ph.D. | Band 5 (SPD) (Appt. Feb 01) |
| J.M. Potts, B.Sc., M.Sc., Ph.D. | Band 6 (PD) |
| E.I. Duff, B.Sc. | Band 6 |
| M.E.H. Hodgson, B.A., Ph.D. | Band 6 |

*Dundee Unit*

| | |
|---|---|
| Head : J.W. McNicol, B.Sc., M.Sc. | Band 4 |
| C.A. Hackett, B.A., Dip. Math. Stats., Ph.D. | Band 5 (SPD) |
| F.G. Wright, B.Sc., M.Sc., Ph.D. | Band 5 (SPD) |
| G.S. Begg, B.Sc., Ph.D. | Band 6 (PD) |

SEERAD FF

| | |
|---|---|
| D.J. Allcroft, B.Sc., M.Sc. | Band 6 |
| M.L. Durban-Reguera, B.Sc., Dip. Math. Stats., Ph.D. | Band 6 |
| S.J. Ferris, B.Sc., M.Sc. | Band 7 |

BBSRC

| | |
|---|---|
| D. Husmeier, B.Sc., Ph.D. | Band 6 (PD) |
| L.Broadfoot, B.Sc. | Band 6 |

# Mylnefield Research Services Limited (MRS)

Managing Director : N.W. Kerby, B.Sc., Ph.D., C.Biol., F.I.Biol.
Commercial Manager : J.B. Snape, M.A., M.Sc., Ph.D., C.Biol., M.I.Biol.
Administrative Executive Officer : A. Ross, H.N.C., C.P.P.
Administrative Assistant : L. Beaton, H.N.C., D.M.S.
Personal Secretary/Administrative Assistant : H. Wilson.

N. Aziz, B.Sc.
A. Blake, B.Sc.
E. Brown, B.Sc., M.Sc.
E. Campbell, M.Sc.
S. Chapman, B.Sc., Ph.D.
M. Dorward
N. Escobar, B.Sc., Ph.D.
J.E. Fairlie, O.N.C., B.Sc.
S. Haupt, Dip.Biol.
I. Hein, M.Sc.
S.N. Jennings, B.Sc.
M. Jones, B.Sc., M.Sc., Ph.D.
T. Marrtila
J. Marshall, B.Sc.
S. Mitchell, B.Sc.
C. M. Reid, B.Sc.
V-M. Rokka, Ph.D.
S. Rowbottom, O.N.C., H.N.C.
L. Smolenska, B.Sc.
R. Toth, B.Sc., Ph.D.
T. Valentine, B.Sc., Ph.D.
M. Woodhead, B.Sc., Ph.D.
V. Young, B.Sc.

# Resignations

| Name | Unit | Band | Month |
|---|---|---|---|
| R. Clarke | Vir | 6 | August 00 |
| K. Connolly | MBN | 10 | December 00 |
| M. Durban-Reguera | BioSS | 6 | August 00 |
| D. Feeney | SPDU | 7 | September 00 |
| S.J. Ferris | BioSS | 6 | March 01 |
| J.A.N. Filipe | BioSS | 6 | September 00 |
| G.J. Gibson | BioSS | 4 | August 00 |
| M.E.A. Hodgson | BioSS | 6 | August 00 |
| A. James | Genom | 6 PD | December 00 |
| H. McCafferty | Genom | 6 PD | September 00 |
| R. Meyer | AG | 6 | June 00 |
| I. Muckenschnabel | PB | 6 | September 00 |
| M. Nicolson | MBN | 8 | June 00 |
| L. Ramsay | Genom | 6 PD | December 00 |
| S. Santa Cruz | CB | 4 | August 00 |
| W. Smith | AG | 7 | December 00 |
| A. Stevenson | Genom | 10 (PT) | May 00 |
| G Streftaris | BioSS | 6 | March 01 |
| Z. Zhang | Genom | 6 PD | July 00 |

# Staff Retirements

| Name | Unit | Band | Month |
|---|---|---|---|
| D. Crabb | SPDU | 8 | September 00 |
| N. McInroy | EM | 10 | June 00 |
| M. Talbot | BioSS | 4 | November 00 |
| I.M. Morrison | PB | 4 | April 00 |
| R. Pugh | EM | 9 | March 00 |

# Deaths

| Name | Unit | Band | Month |
|---|---|---|---|
| I. Black | IT | 7 | February 01 |

# Voluntary and Flexible Retirements

| Name | Unit | Band | Month |
|---|---|---|---|
| M. Campbell | AG | 8 | March 01 |
| I.M. Chapman | AG | 5 | March 01 |
| G. Menzies | SLIS | 7 | March 01 |
| A. Wilson | AG | 8 | March 01 |
| J. Oldershaw | EM | 11 | March 01 |

# Honorary Research Professors

Professor P. Broda, M.A., M.Sc., Ph.D., D.Sc., Hon.D.Sc.
Professor H. Griffiths, B.Sc., Ph.D.
Professor F. Gunstone, B.Sc., Ph.D., D.Sc., F.R.S.C., F.T.S.E., C.Chem.
Professor B.D. Harrison, C.B.E., B.Sc., Ph.D., D.Ag.For., F.R.S., F.R.S.E.
Professor N. L. Innes, O.B.E., B.Sc., Ph.D., D.Sc., C.Biol., F.I. Biol., F.R.S.E., F.I. Hort.
Professor P.H. Nye, M.A., B.Sc., F.R.S.
Professor B. Sleeman, B.Sc., Ph.D., D.Sc., C.Math., F.I.M.A., F.R.S.E.
Professor J. Sprent, O.B.E., B.Sc., D.Sc., Ph.D., A.R.C.S., F.R.S.E.
Professor Sir W. Stewart, B.Sc., Ph.D., D.Sc., C.Biol., F.I.Biol., F.R.S., F.R.S.E.
Professor C.E. Taylor, C.B.E., B.Sc., Ph.D., F.R.S.E., C.Biol., F.I.Biol.

# Honorary Research Fellows

A. Blackwell, B.Sc., Ph.D., M.R.C.V.S.
J. Bown, B.Sc., Ph.D.
R.A. Brown, B.Sc., M.Sc., Ph.D.
W.W. Christie, MBE, B.Sc., Ph.D., D.Sc., C.Chem., F.R.S.E., F.R.S.C.
J.G. Harrison, B.Sc., Ph.D.
R.J. Jarvis, M.A., D.Phil.
H.M. Lawson, B.Sc., M.Agr.Sc., Dip.Agric., F.I.Hort.
J. McColl, M.B.E., S.H.M., N.D.H., S.D.H.
A.F. Murant, B.Sc., A.R.C.S., Ph.D., D.I.C., C.Biol., F.I.Biol., F.R.S.E.
M.C.M. Pérombelon, M.B.E., B.Sc., M.Sc., Ph.D.
D.A. Perry, B.Sc., Ph.D.
W.M. Robertson, H.N.C., F.L.S.
P.D. Smith, B.Sc., Ph.D., C.Math., F.I.M.A.
N. White, B.Sc., Ph.D., C.Biol., M.I.Biol.

# Postgraduate Students

| Name | Unit | Subject |
|---|---|---|
| D.J. Allcroft | BioSS | Mathematical modelling of short-term behaviour in farm animals. |
| N. Aziz | GE | Gametic transformation and its potential in gene targeting. |
| G. Banks | SPD | Spatial variation in the nitrification process in arable fields. |
| J. Bolandandam | Vir | Study of resistance mechanisms to potato leafroll virus in diploid and tetraploid potato. |
| K. Boutsika | MBN | Development of molecular diagnostic protocols for detecting 'spraing' tobravirus disease of potato and its vector trichodorid nematodes. |
| E.C. Brown | MRS | *Tobacco rattle virus* as a viral vector for gene silencing. |
| K. Caldwell | Genom | Grain hardiness linkage disequilibrium in barley. |
| A. Campbell | BioSS | Inferential tools for stochastic epidemic modelling. |
| J. Chessel | SPD | Reactive transport in soil. |
| Q. Chen | MBN | *Xiphinema americanum*-group virus-vector nematodes: development of a diagnostic protocol. |
| E. Davidson | PB | Isolation and characterisation of new plant-derived mannose-specific lectins and their use in the diagnosis and mechanistic studies of the infection of mammals with a range of bacteria and viruses. |
| J. Durban | BioSS | Bayesian methods for marine mammal population assessment. |
| H. El-Menaie | AG | Salinity tolerance in barley in Kuwait. |
| S. Fonseca | BioSS | Stochastic geometry of muscle fibre development. |
| C. Furlanetto | MBN | Genes encoding oesophageal gland secreted proteins involved in host-parasite and/or nematode-virus interactions of *Xiphinema index.* |
| K. Harris | SPD | The visualisation of interactions between soil physical conditions and fungi. |
| J. Heilbronn* | MBN | Characterisation of signalling genes induced by *Erwina* in potato. |
| I. Hein | MRS | Gene discovery in cereals. |
| G. Henderson | SPD | Modelling soil-water/structure functions. |
| M. Holeva | MBN | A molecular study of the type III secretion system and its surrounding DNA in the potato pathogen *Erwinia carotovora* subsp. *atroseptica.* |
| R. Holeva | MBN | Development of a pre-plant soil test for *Tobacco rattle virus* and virus vector Trichodorid nematodes |
| S. N. Humphris | SPD | The role of volatile organic compounds in the biological control of wood dry rot by *Trichoderma* spp. |
| S. Hussain | MBN | Epidemiology of *Phytophthora infestans.* |
| E. Isidore | Genom | Construction of an ultra high density linkage map of potatoes. |
| V. Ivandic | AG | Simple sequence repeats in relation to adaptation in barley. |
| E. Karanastasi | MBN | Plant virus sequences involved in particle assembly and transmission by nematodes. |
| D. Kiezebrink | SPD | Modelling soil and water structure functions to assess the efficiency of pesticides in agricultural soils against plant-pathogenic nematodes. |
| H.L. Kuan | SPD | What is the link between microbial diversity and soil resilience? |
| S.G. Lane | Vir | Studies on recombinant antibodies to water pollutants. |
| A. Le Fevre | BioSS | Statistical aspects of lameness in dairy cattle. |
| F. Lioliopoulou | Vir | Studies on molecular interactions between PMTV and its vector, *Spongospora subterranea* f.sp. *subterranea.* |
| H. Liu | Genom | Molecular dissection of developing barley grain. |
| L. Mackinnon | GE | Transformation of hemp – a multi-purpose fibre crop. |
| G. Malloch* | VS | Genetic variation in the family Byturidae. |
| M. Maule | BioSS | Stochastic modelling in plant epidemiology and ecology. |
| H. McGovern | SPD | The influence of soil biota on soil structural conditions. |
| A. Nonyane | BioSS | Improving statistical design and analysis of repeated measures using prior information. |
| R. Nsubuga | BioSS | Statistical study of the epidemiology of *E. coli* O157 infection in cattle. |
| E. Pachepsky | VS | Modelling phenotypic and genotypic interaction in species-rich grassland. |
| B. Pande | Genom | Linkage mapping in 4x potatoes. |
| E. Pascual | CB | Oxidation processes in coffee. |
| A. Popovich | Vir | Development of a rapid screening system for gene function. |
| A. Prior | MBN | Functional characterisation of a secreted protein from potato cyst nematode, *Globodera pallida.* |
| A. Richardson | PB | Coniferyl alcohol oxidases in lignifying tissues of higher plants. |
| C. D. Robinson | BioSS | Bayesian methods for segmenting X-ray CT images of sheep. |
| K. Rutherford | BioSS | Fractal analysis of animal behaviour. |
| G. Shilvanth | MBN | Enhancement of resistance to *Botrytis* grey mould of chickpea using PGIP genes. |
| L. Smolenska | Vir | The use of potato virus X for high level production of foreign proteins in plants. |
| E. Souleyre | PB | Carbohydrate metabolism during ripening in the fruit of strawberry. |
| K. Stanley | VS | Towards an understanding of the molecular mechanisms of lectin toxicity to aphids through gut glycoprotein interactions. |
| K. Stewart | MBN | Breakdown of *Mlo* resistance under stress. |
| N. Vassilakos | MBN | Genetic determinants of complimentarity and exclusivity of vector transmission of tobraviruses. |
| E. Vellios | MBN | Molecular elucidation of interaction between plant tobravirus gene products and virus-vector trichodorid nematodes. |
| W. Wei | AG | Mechanisms of salt tolerance in barley. |
| J. Wishart | MBN | Characterisation of *Meloidogyne* species using molecular and immunological techniques. |
| C-P. Witte | PB | Modification of urea metabolism in transgenic potato. |
| J. C. Wood | BioSS | Mathematical modelling of *E. coli* infection. |
| M. Wood | BioSS | Use of wavelet methods in crop cultivar recognition by image analysis. |
| C. Zhang | GE | Improvement of Chinese wheat cultivars. |

* Permanent member of staff

# Service on External Committees or Organisations

| Name | Position | Committee or Organisation |
|---|---|---|
| T.J.W. Alphey | Secretary | Committee of Heads of Agricultural and Biological Organisations in Scotland |
| | Secretary | Scottish Management Advisory Committee |
| A.N.E. Birch | Convenor | International Organisation for Biological Control 'Breeding for Resistance to Insects & Mites' Steering Committee |
| | Member | IOBC Global Working Group on 'Transgenic Organisms in Integrated Pest Management and Biological Control' |
| | Member | European Science Foundation GM Crop Workshops |
| V.C. Blok | Member | AAB Nematology Group |
| J.E. Bradshaw | Chairman | Potato Section, EUCARPIA |
| | Committee Member | BBSRC Brassica IGF Steering Committee |
| R. Brennan | Adviser | Glaxo SmithKline Blackcurrant R&D Committee |
| | Secretary | Organising Committee for 8th International *Rubus* & *Ribes* Symposium |
| S. Brocklehurst | Member | HRI Ethical Review Committee |
| D.J.F. Brown | Co-chairman | Russian Society of Nematologists |
| | Member | American Society of Nematologists Ad Hoc Committee, International Federation of Nematologists |
| | Member | European & Mediterranean Plant Protection Organisation Ad Hoc Committee, *Xiphinema americanum* group nematodes |
| J.W.S. Brown | Assessor | RNA Programme of University of Vienna |
| D. Cooke | Chair | ISPP *Phytophthora* Committee |
| H.V. Davies | Member | EU Scientific Committee on Plants |
| M.J. De,Maine | Member | BBSRC Joint Committee on Health & Safety |
| | Member | SABRI Safety Officers' Group |
| G. Dobson | Treasurer | Royal Society of Chemistry, Lipid Chemistry Group |
| J.M. Duncan | Committee Member | Global Initiative on Late Blight (GiLB) |
| R.P. Ellis | Member | BSPB Cereal Crop Group |
| | Representative of BSPB | SAC Cereal Recommended List Consultative Committee |
| | Representative of BSPB | GENRES CT108 EU Research Project |
| | UK Representative | European Co-operative Programme/Plant Genetic Resource |
| | Chairman | ECP/PGR Barley Working Party |
| D.A. Elston | Member | ITE Biometrics Network |
| | Member | RSS Highland Local Group Committee |
| | Member | Statistical and Technical Working Group, UK Environmental Change Network |
| B.P. Forster | Co-ordinator | International Barley Chromosome Committee |
| | Member | COST 824, Gametic Embryogenesis |
| | Assistant Co-ordinator | International Triticeae Mapping Initiative (ITMI) |
| | National Representative | COST 851, Gametic Cells and Molecular Breeding |
| C.A. Glasbey | Member | EPSRC Mathematics College |
| | Member | Council of Royal Statistical Society |
| | Chairman | RSS Statistical Image Analysis and Processing study group |
| | Chairman | RSS Edinburgh Local Group Committee |
| | Member | RSS Research Committee |
| | Member | Mathematical Sciences Committee of British Association for the Advancement of Science |
| | Member | RSS2001 Conference Committee |
| | Member | Management committee of SAC-BioSS CT Scanning Unit |
| | Member | Management group of Scottish Centre for Genome Technology and Informatics |
| C.A. Hackett | Member | International Biometric Society (British Region) Committee |
| T.D. Heilbronn | Finance / Administrator | Association for Crop Protection in Northern Britain |
| | Member | Organising Committee for EUCARPIA 2001 |
| | Member | Organising Committee for 8th International *Rubus* & *Ribes* Symposium |
| | Member | Organising Committee for Crop Protection in Northern Britain 2002 |
| J.R. Hillman | Chairman | SCRI/SASA/SAC Liaison Group |
| | Chairman | Tayside Biocentre Group |
| | Deputy Chairman | Board of Directors, Mylnefield Research Services Ltd |
| | Member | Board of the Mylnefield Trust |
| | Member | Board of Mylnefield Holdings Ltd |
| | Chairman | Committee of Heads of Agricultural and Biological Organisations in Scotland |
| | President | Agriculture and Food Section, the British Association for the Advancement of Science |
| | Member | ECRR Board of Management |
| | Member | SNSA Adviser to Committee |
| | Member | Court of University of Abertay Dundee and its Audit Committee |
| | Member | Senate, University of Dundee |
| | Member | University of Strathclyde Sub-Board for the Degree of B.Sc. in Horticulture |
| | Member | Tayside Economic Forum |
| | Member | Perth & Kinross Agricultural Forum |
| | Member | Board of Directors, BioIndustry Association |
| | Adviser | International Foundation for Science, Stockholm |
| D.L. Hood | Secretary & Treasurer | Scottish Society for Crop Research |
| G.W. Horgan | Member | RSS Highland Local Group Committee |
| E.A. Hunter | Member | RSS local committee |
| | Member | S A C – Edinburgh, Animal Experiments Committee |
| | Member | Scientific Committee of the ASU Conference, Lille, France |
| | Founding Member | International Sensometrics Society |
| | Member | Inter-departmental Statisticians Group (IDSG) |
| | Member | Herbage VCU group |
| A.J. Jones | Member | ICTV Study Group on Comoviridae |
| | Member | ICTV Study Group on Ilaviruses |
| J.T. Jones | Member | Council of the British Society of Parasitologists |

| Name | Position | Committee or Organisation |
|---|---|---|
| R.A. Kempton | Member | International Biometric Society Awards Fund Committee |
| | Member | International Statistical Institute Risk Analysis Committee |
| | Member | Fisher Memorial Committee |
| | Member | Steering Group, Biometric Network for Sub-Saharan Africa |
| | Member | Edinburgh Centre for Rural Research |
| | Member | Advisory Committee on Forest Research |
| | Member | Working Group on Risk Assessment for Mixtures of Pesticides, Food Standards Agency |
| A. Lees | Board Member | British Society for Plant Pathology |
| S.A. MacFarlane | Member | Association of Applied Biologists, Virus Group |
| | Member | Society for General Microbiology, Virus Group |
| W.H. Macfarlane Smith | Member | BSPB Oilseed & Industrial Crops Group |
| | Member | ECRR PR Officers' Group |
| | Member | NPTC Plant Variety Development Panel |
| | Member | Organising Committee EUCARPIA 2001 |
| | Member | SEERAD Information Officers Group |
| G.C. Machray | External examiner | University of East Anglia |
| G.R. Mackay | President | EUCARPIA |
| | Chairman | Organising committee of XVIth Congress of EUCARPIA |
| | Member | UK Potato Quarantine Unit Review Committee |
| D.K.L. MacKerron | Secretary | Potato-Crop Sub-Committee, SSCR |
| | Chairman | Potato Crop Network, GCTE Focus 3 |
| | Chairman | Section Physiology, EAPR |
| | Member | Steering Committee of the Seed Potato Forum |
| D.F. Marshall | Panel Member | BBSRC GAIT Initiative |
| | Member | BBSRC AgriFood Committee |
| | Member | SIMBIOS Management Committee |
| M.A. Mayo | Secretary | International Committee on Taxonomy of Viruses (ICTV) |
| | Member | Advisory Committee on Releases to the Environment (ACRE) |
| | Chair | ICTV Satellites Study Group |
| | Member | IUBS/IUMS International Commission on Bionomenclature |
| | Member | ICTV Luteoviridae Study Group |
| U.M. McKean | Joint Chair | Scottish Agricultural Librarians' Group |
| I.J. McKendrick | Member | The Forest of Bowland Louping III Eradication Project |
| | Member | MRI Animal Experimentation and Ethics Committee |
| R. Neilson | Member | Governing Board, European Society of Nematologists |
| I.M. Nevison | Member | HRI Ethical Review Committee |
| | Member | SAC - Auchincruive Animal Experiments Committee |
| A. Newton | Member | Crop Protection in Northern Britain 2002 Organising Committee |
| | Member | United Kingdom Cereal Pathogen Virulence Survey Committee |
| | Board Member | European and Mediterranean Cereal Rusts Foundation |
| W. Powell | Co-ordinator | International Triticeae Mapping Initiative Management (ITMI) |
| | Member | BBSRC Initiative on Gene Function (IGF). |
| | Member | Genetical Society Committee (Quantitative Genetics), British Society of Plant Breeders, Working Party on Biotechnology |
| | Member | Advisory Board for Scottish Informatics Mathematics Biology & Statistics (SIMBIOS) Centre (2001) |
| | Member | External Review Team for ICARDA (Syria), IITA (Nigeria), VIB (Belgium) and CIP (Peru) |
| | External examiner | Genetics (B.Sc.) and M.Sc. at UCW Aberystwyth |
| | External examiner | Genetics (M.Sc.) at the University of Birmingham |
| G. Ramsay | Member | UK Potato Quarantine Unit Review Committee |
| K. Ritz | Network Group Member | BBSRC Plant & Microbial Sciences Committee |
| | Member | ICTV Umbravirus Study Group |
| A.M.I Roberts | Secretary | Statisticians Group, UK Plant Varieties and Seeds Committee |
| | Member | Technical Working Party on Automation and Computing Programs, International Union for the Protection of Plant Varieties |
| | Member | *Ad hoc* Subgroup on Molecular Techniques for Oilseed Rape, International Union for the Protection of PlantVarieties |
| D.J. Robinson | Member | ICTV Tobamovirus & Tobravirus Study Group |
| S.E. Stephens | Joint Chair | Scottish Agricultural Librarians' Group |
| | Member | Information Services Group - Scottish Library Association Committee |
| | Member | Tayside and Fife Library and Information Network |
| | Working Group Chair | British Research Institutes Serials Consortium (BRISC) |
| | Secretary | Research Councils Library and Information Consortium (RESCOLINC) |
| | Chair | BBSRC Research Institutes Librarians Committee (BRILCOM) |
| M. Talbot | Chairman | Statisticians Group, UK Plant Varieties and Seeds Committee |
| M.E. Taliansky | Member | Plant Virus Subcommittee, International Committee on Taxonomy of Viruses (ICTV) |
| | Chairman | Umbravirus Study Group, International Committee on Taxonomy of Viruses (ICTV) |
| | Member | Statistics Committee, International Seed Testing Association |
| | Member | Technical Working Party on Automation and Computer Programs International Union for the Protection of Plant Varieties |
| | Member | RSS Statistical Computing Committee Statistics Committee, International Seed Testing Association |
| L. Torrance | Member | ICTV Plant Virus Sub Committee |
| | Chair | ICTV Furovirus and Allies Study Group |
| I. Toth | Board Member | British Society of Plant Pathology |
| | Committee Member | Crop Protection in Northern Britain 2002 |
| D. Watt | Member | Scottish Management Advisory Committee |
| | Company Secretary | Mylnefield Research Services (MRS) Ltd |
| | Secretary | Mylnefield Trust |
| R. Waugh | Member | ITMI Overall Planning Committee |
| B. Williamson | External Examiner | Ph.D. Examination Committee, University of Wageningen, The Netherlands (Plant Pathology) |
| | External Examiner | Ph.D. Examination Committee, University of Bristol (Biochemistry) |
| | Treasurer | Association for Crop Protection in Northern Britain |
| | Member | Committee for 12th International *Botrytis* Symposium |
| | Treasurer | Organising Committee for 8th International *Rubus* & *Ribes* Symposium |
| F.G. Wright | Member | BBSRC Computational Molecular Biology Review Panel |

# Short-Term Workers and Visitors

| Name | Country of origin | Unit | Month/yr of arrival | Length of stay |
|---|---|---|---|---|
| C. Agufa | Kenya | Genom | Aug 00 | 6 weeks |
| A. Alander | Finland | VS | Jun 00 | 3 months |
| E. Alvarez | Colombia | MBN | Jun 00 | 1 week |
| I. Andreev | Russia | Vir | Mar 01 | - |
| S. Anwar | UK | MBN | Jul 00 | 3 months |
| A. Ariza | UK | PSE | Feb 00 | 3 months |
| T. Baumgartl | Germany | PSE | Aug 00 | 2 weeks |
| A.J. Boe | UK | PSE | Feb 00 | 3 months |
| S. Cacciola | Italy | MBN | Sep 00 | 2 weeks |
| G. Cargill | UK | Genom | Jun 00 | 4 months |
| K. Chalmers | Australia | Genom | Jul 00 | 4 months |
| Y. Chen | UK | PSE | Dec 01 | 6 months |
| S.K. Choi | S. Korea | Vir | Jun 00 | 3 months |
| C. Dolan | UK | MBN | Mar 00 | 2 weeks |
| S. Fiengold | Argentina | Genom | Nov 00 | 2 weeks |
| J. de la Fuente | Spain | PB | Sep 00 | 2 months |
| R. Gallagher | UK | PB | Feb 01 | 3 months |
| A. Greiner | France | PB | Jul 00 | 3 months |
| T. D'Hertefeldt | Sweden | VS | Oct 00 | 1 year |
| T. Higuchi | Japan | SPD | Jul 01 | 2 weeks |
| A. Howden | UK | AG | Jul 00 | 2 months |
| K. Hrubikova | Slovakia | GE | Jan 00 | 1 year |
| J-S. Hsieh | Taiwan | AG | Aug 99 | 1 year |
| A. Hyde | UK | PB | Feb 01 | 3 months |
| M. Iijima | Japan | SPD | Jul 01 | 2 weeks |
| M. Ivanova | Russia | Vir | Feb 01 | 1 month |
| A. Jarmolowski | Poland | GE | Dec 00 | 1 week |
| C. Johnstone | UK | MBN | Oct 00 | 6 months |
| T. Jokela | Finland | VS | Jun 00 | 3 months |
| C. Kelly | UK | GE | Oct 00 | 5 months |
| S. Kirk | UK | MBN | May 00 | 1 week |
| M. Kolber | Hungary | Vir | Jul 00 | 2 weeks |
| Z. Kremenovic | Bosnia | AG | Nov 00 | 2 weeks |
| L.P. Kumar | India | Vir | Sep 00 | 1 year |
| P. Langridge | Australia | Genom | Jul 00 | 3 weeks |
| D. Lewandowska | Poland | GE | Sep 00 | 1 month |
| H-S. Lo | Taiwan | MBN | Aug 00 | 1 month |
| J. Loke | Colombia | MBN | Jun 00 | 1 week |
| A. Lund | USA | Genom | Jun 00 | 2 months |
| F. Moh | Malaysia | PB | Jul 00 | 2 months |
| L. Molstad | Norway | SPD | Nov 00 | 2 weeks |
| J. Nechtwatal | Germany | MBN | Nov 00 | 1 week |
| K. Nyerges | Hungary | Vir | Jul 00 | 2 weeks |
| A. Orr | USA | SPD | Aug 00 | 6 months |
| T. Pennanen | Finland | SPD | Jan 00 | 1 year |
| T. Pettit | Australia | BioSS | Dec 00 | 5 weeks |
| A. van der Ploeg | The Netherlands | PB | Jul 00 | 6 months |
| G. Quirk | UK | AG | Jul 00 | 3 months |
| S.K. Raj | India | Vir | Oct 00 | 6 months |
| C. Schultze Gronover | Germany | MBN | Jul 00 | 2 weeks |
| I. Snowball | UK | PSE | Feb 01 | 3 months |
| K.Thirumala-Devi | India | Vir | Feb 01 | 2 weeks |
| K. Varga | Hungary | MBN | Nov 00 | 1 month |
| A. Ward | UK | MBN | Jul 00 | 10 weeks |
| T. Wenzler | Germany | Vir | Sep 00 | 6 months |
| A. Whittet | UK | AG | Jun 00 | 10 weeks |
| L. Wilson | UK | AG | Jul 00 | 1 month |
| N. Wilson | Ireland | BioSS | Jan 01 | 2 months |

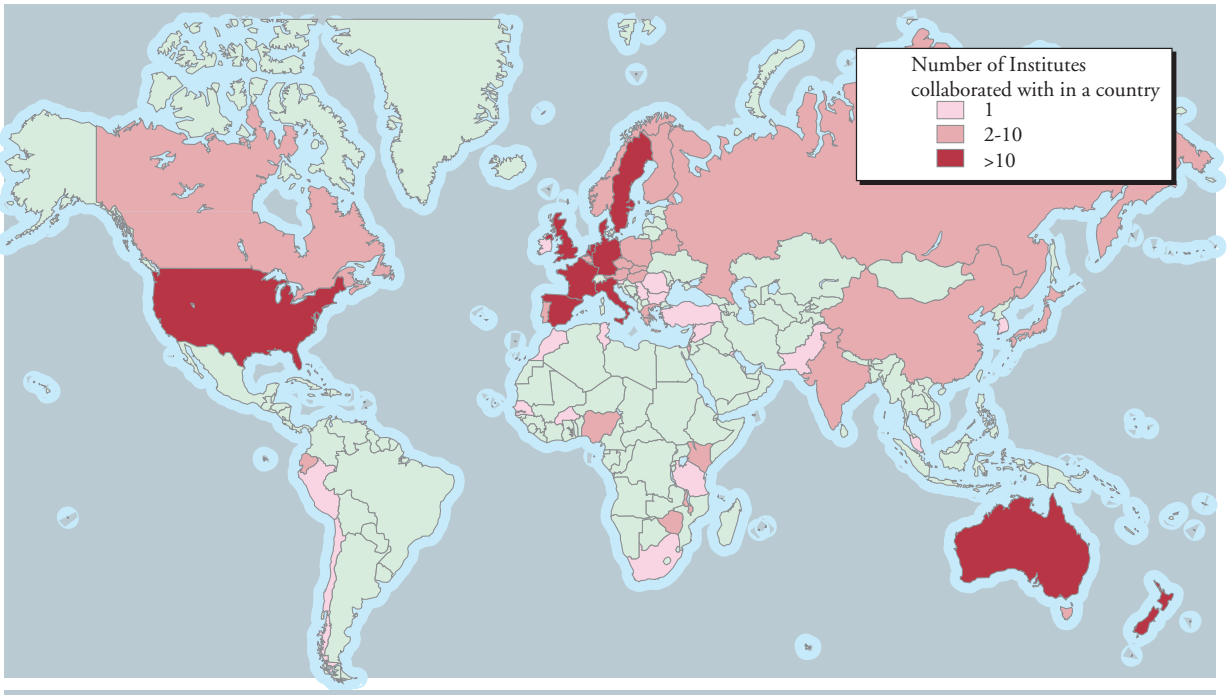
# Editorial Duties

| Name | Position | Journal Title |
|---|---|---|
| H. Barker | Editor | *Annals of Applied Biology* |
| A.G. Bengough | Editor | *Annals of Botany* |
| A.N.E. Birch | Editor | *IOBC Bulletin, 'Breeding for Resistance to Insects and Mites'* |
| B. Boag | Editorial Board | *Annals of Applied Biology* |
| | Editorial Board | *Nematologia Mediterranea* |
| | Editorial Board | *Journal of Nematology* |
| R. Brennan | Associate Editor | *Journal of Horticultural Science & Biotechnology* |
| D.J.F. Brown | Honorary Chief Editor | *Russian Journal of Nematology* |
| | Editorial Board | *Nematologia Mediterranea* |
| | Editorial Board | *Helminthologia* |
| | Editorial Board | *Journal of Nematode Morphology and Systematics* |
| J.W.S. Brown | Advisory Group | *The Plant Journal* |
| M.F.B. Dale | Editor | *Annals of Applied Biology* |
| H.V. Davies | Editorial Board | *Phytochemistry* |
| J.M. Duncan | Associate Editor | *Journal of Horticultural Science & Biotechnology* |
| B. Fenton | Associate Editor | *Proceedings of the European Association of Acarologists* |
| C.A. Glasbey | Associate Editor | *Biometrics* |
| | Associate Editor | *Journal of Royal Statistical Society, Series B* |
| B. Griffiths | Editorial Board | *Pedobiologia* |
| C.A. Hackett | Editorial Board | *Heredity* |
| J.R. Hillman | Publication Committee | *Journal of Horticultural Science* |
| | Editorial Board | *Agricultural Systems* |
| | Editorial Board | *Journal of Agricultural Science* |
| G.W. Horgan | Statistical Editor | *British Journal of Nutrition* |
| E.A. Hunter | Editorial Board | *Food Quality & Preference* |
| P. Iannetta | Associate Editor | *Acta Horticulturae* |
| A.T. Jones | Editor | *AAB Descriptions of Plant Viruses* |
| D.K.L. MacKerron | Associate Editor | *Journal of Horticultural Science* |
| | Editorial Board | *Euphytica* |
| M.A. Mayo | Editorial Board | *Virology* |
| | Editor | *Archives of Virology* |
| | Editorial Advisory Board | *Encyclopedia of Virology* |
| | Editorial Advisory Board | *Encyclopedia of Life* |
| J.W. McNicol | Statistical Editor | *Annals of Applied Biology* |
| A.C. Newton | Editor | *Cereal Rusts and Powdery Mildews Bulletin* |
| | Editorial Board | *Plant Pathology* |
| P. Palukaitis | Senior Editor | *Molecular Plant-Microbe Interactions* |
| | Editorial Board | *Journal of General Virology* |
| | Associate Editor | *Virology* |
| | Advisory Board | *Plant Pathology Journal* |
| M.S. Phillips | Associate Editor | *Journal of Nematology* |
| W. Powell | Associate Editor | *Molecular Ecology* |
| K. Ritz | Subject Editor | *Soil Biology Biochemistry* |
| | Editorial Board | *FEMS Microbiology Ecology* |
| D.J. Robinson | Editorial Board | *Journal of Virological Methods* |
| | Editor | *AAB Descriptions of Plant Viruses* |
| I. Toth | Associate Editor | *Molecular Plant Microbes (MPMI)* |
| D.L. Trudgill | Advisory Board | *European Journal of Plant Pathology* |
| | Editorial Board | *Nematology* |
| B. Williamson | Deputy Chairman | *Annals of Applied Biology* |

# Awards and Distinctions

| Name | Unit | Degree/Award/Distinction/Appointment |
|---|---|---|
| SCRI | - | Institute of Horticulture Norah Stucken Award for achievements in soft fruit breeding |
| D.J.F. Brown | Vir | Foreign Member of the Russian Academy of Natural Sciences |
| P. Palukaitis | Vir | Honorary Professor, Seoul Women's University |
| W. Powell | Dep. Dir. | Honorary Professor, Heriot-Watt University, Edinburgh |
| M.E. Taliansky | Vir | Adjunct Professor, Moscow State University |
| R. Viola | PB | Visiting Professor, University of Naples, Italy |
| J.R. Hillman | Director | British Potato Council Potato Industry Award, 1999 |
| J.R. Hillman | Director | World Potato Congress 2000 Industry Award |
| I. Toth & L. Hyman | MBN | Altran Foundation Award for Innovation |
| I. Geoghegan | VS | Fellowship, British Association Millennium Awards |
| S. Malecki | SLIS | Fellowship, British Association Millennium Awards |
| E. Davidson, | PB | Ph.D., University of Dundee |
| G. Henderson | SPD | Ph.D., University of Dundee |
| D. Kiezebrink | SPD | Ph.D., University of Dundee |
| S.G. Lane | Vir | Ph.D., University of Dundee |
| M. Maule | BioSS | Ph.D., University of Edinburgh |
| E. Pascual | UPB | Ph.D., University of Dundee |
| A. Richardson | PB | Ph.D., University of Dundee |
| C. Robinson | BioSS | Ph.D., University of Edinburgh |
| G. Shilvanth | MBN | Ph.D., University of Dundee |
| E.J.F. Souleyre | PB | M.Phil., University of Abertay, Dundee |
| D. Todd | AG | M.Sc., University of Dundee |
| J. Fairlie | MRS | B.Sc. Open University |
| S. Neilson | AG | Diploma in Biological Sciences, Open University |
| M. Young | VS | Postgraduate Diploma in IT, University of Abertay, Dundee |
| L. Beaton | MRS | Diploma of Management Studies, University of Abertay, Dundee |

# International Collaboration and Consultancies




Research is executed within an international framework that encourages information transfer. The extent of SCRI's international commitment in recent years (since 1993) is reflected in the collaborative research that was undertaken with c. 320 Institutions in 59 countries. In 2000 alone, SCRI collaborated with 95 organisations in 39 countries and, within the UK, collaborated with over 100 organisations.

# SCRI Research Programme
## 2000-2001

SEERAD funded research programme showing: SEERAD project number; Title (prefixed ROA for ROAMEd core-funded projects; FF for Flexible Fund projects); Scientific Project Leader. In addition to this list, there are research projects undertaken on behalf of various bodies, including other governmental bodies, commerce and levy boards.

| | | |
|---|---|---|
| SCR/479/96 | ROA Maintenance, improvement, evaluation and exploitation of biodiversity in germplasm collections of potato | Mackay G R |
| SCR/494/97 | ROA Genetic control of pathogenicity, host specificity and race structure at the molecular level in the fungal pathogens *Phytophthora infestans*, *Phytophthora fragariae* and related *Phytophthora* species | Duncan J M |
| SCR/497/97 | ROA Studies on mechanisms of host gene-mediated and pathogen-derived transgene-mediated resistance to viruses to improve the deployment of new types of resistance for germplasm enhancement | Barker H |
| SCR/508/98 | ROA Cell biology of plant-virus interactions | Oparka K J |
| SCR/509/98 | ROA Molecular dissections of plant viral movement proteins | Oparka K J |
| SCR/510/98 | ROA Molecular mechanisms involved in the aphid transmission of luteoviruses, potyviruses and the nematode transmission of tobraviruses | Mayo M A |
| SCR/512/98 | ROA Produce and maintain pathogen-tested stocks of *Rubus, Ribes* and *Fragaria* germplasm and index for infection material imported into SCRI | Jones A T |
| SCR/513/98 | ROA Gene expression and manipulation in barley | Machray G C |
| SCR/514/98 | ROA Isolation and functional characterisation of genes important in the host-parasite interaction of plant parasitic nematodes including *Globodera, Meloidogyne* and *Xiphinema* spp. | Jones J T |
| SCR/518/98 | ROA Towards the construction of a physical and functional map of the interval between GP21 and GP179 on potato linkage group V | Waugh R |
| SCR/520/98 | ROA Mapping and isolation of *Rhynchosporium secalis* recognition and early signalling genes in barley | Newton A C |
| SCR/521/98 | ROA Genetic modification of soft fruit crops and risk assessment for their introduction | Graham J |
| SCR/525/99 | ROA Interactions between the structure of soil habitats and biological processes | Bengough G |
| SCR/526/99 | ROA Integrative mapping of the long arm of barley chromosome 5H | Thomas W T B |
| SCR/527/99 | ROA Development of a graphical database for the visualisation of genotypic and phenotypic data in barley | Marshall D F |
| SCR/528/99 | ROA Use of an accelerated marker assisted selection scheme to introgress novel variation for economically important traits into cultivated barley | Thomas W T B |
| SCR/532/99 | ROA Plant membrane lipid compositions and stress, with respect to genetically modified *Arabidopsis* and rape with primary lipid defects and cold tolerance in blackcurrant | Dobson G |

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| SCR/533/99 | ROA Molecular and genetic studies of the basis of virulence/avirulence in plant parasitic nematodes | Phillips M S |
| SCR/534/99 | ROA Isolation and functional analysis of plant genes involved in resistance responses to nematodes | Blok V C |
| SCR/536/00 | ROA Development and application of chemical strategies to facilitate genetic and molecular marker studies of factors affecting quality traits in potatoes | Davies H V |
| SCR/537/00 | ROA Biochemical approaches to define novel targets for the genetic improvement of malting barley | Davies H V |
| SCR/538/00 | ROA Optimising production and biodiversity of arable plants and invertebrates at patch and landscape scales | Squire G |
| SCR/539/00 | ROA Self organisation of plant and canopy architecture in barley and feral brassicas: trade offs between production and defense | Squire G |
| SCR/540/00 | ROA Genetics of cultivated potatoes | Bradshaw J E |
| SCR/541/00 | ROA Genetic approaches to evaluation and utilisation of soft fruit germplasm | Bradshaw J E |
| SCR/542/00 | ROA Consequences of soil biodiversity for the functioning and health of agricultural soils in relation to C cycling dynamics and resilience | Griffiths B S |
| SCR/544/00 | ROA Consequences of soil biological diversity for the functioning and health of agricultural soils in relation to N cycling processes | Ritz K |
| SCR/545/00 | ROA Detection, diversity and epidemiology of important viruses and their vectors in berryfruit crops and strategies for their effective control | Jones A T |
| SCR/546/00 | ROA Development and use of molecular markers to study the epidemiology of late blight (*Phytophthora infestans*) of potato in Scotland | Cooke D |
| SCR/547/00 | ROA Biodiversity in the antioxidant status and composition of *Rubus* and other soft fruit germplasm | Stewart D |
| SCR/549/00 | ROA Characterisation of molecular interactions between soft rot erwinias and potato | Lyon G D |
| SCR/550/00 | ROA Control of meristematic activity in plants: dormancy in potato tubers as the model system | Viola R |
| SCR/551/00 | ROA Post-transcriptional control of gene function | Brown J W S |
| SCR/552/00 | ROA Barley 'deletion' mutation grid | Waugh R |
| SCR/553/00 | ROA Characterising plant responses to viral infection | Palukaitis P |
| SCR/554/00 | ROA Protein-protein interactions and the role of virus proteins in disease processes | Torrance L |
| SCR/505/97 | FF Molecular approaches to manipulate the development and composition of strawberry fruit | Davies H V |
| SCR/516/97 | FF Genetic mapping and molecular cloning of novel sources of resistance to *Globodera pallida* | Waugh R |
| SCR/522/98 | FF Development of *Rubus* genotypes with transgenic resistance to *Raspberry bushy dwarf virus* | Jones A T |
| SCR/523/98 | FF Investigation of the mechanisms of disease induction and host-specificity in major bacterial and fungal potato pathogens | Birch P R J |

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| SCR/524/98 | FF Unravelling the pathways of protein transport in plant and animal cells using virus-based vectors | Oparka K J |
| SCR/535/99 | FF Impacts of a conventional and an organic crop insecticide spray treatment on life history traits of two-spot ladybirds | Birch A N E |
| SCR/555/00 | FF Cereal transcriptome resources | Waugh R |
| SCR/556/00 | FF Comparison of the molecular bases of pathogenicity in the model oomycetes *Peronospora parasitica* and *Phytophthora infestans* through a genomics approach | Birch P R J |
| SCR/567/00 | Appraisal of options for aphid monitoring and control to manage virus transmission in Scottish seed potato crops | Woodford J A T |
| SCR/568/00 | Significance and mechanisms of landscape-scale gene flow | Ramsay G |
| SCR/569/00 | *Phytophthora* diseases of soft fruit: determining their prevalence and the source of new outbreaks in Scotland | Duncan J M |
| SCR/570/00 | Mechanical properties of primary cell walls by micro-stretching *in vivo* | Bengough A G |
| SCR/808/94 | FF Development of molecular biological and physiological techniques in studies of the interaction between microbes, nutrient cycling and vegetation among a range of agriculturally important pastures, to enable scaling from microcosm to field | Ritz K |
| SCR/816/95 | FF Phenotypic and genotypic bases of population dynamics in heterogeneous, species-rich grassland | Squire G |
| SCR/818/95 | FF Genetic engineering of crop plants for resistance to insect and nematode pests: effects of transgene expression on animal nutrition and the environment | Jones A T |
| SCR/822/97 | FF The application of the free-living nematode *C. elegans* to the development of control procedures for nematode parasites of animals and plants | Jones J T |
| SCR/823/97 | FF Significance of physical heterogeneity for scaling of solute chemistry in soils from fine scale to subcatchment | Bengough G |
| SCR/824/97 | FF Efficacy studies on a plant virus-based expression system and on alternative delivery routes for peptides and proteins with pharmaceutical, therapeutic and related uses for improving animal health, nutrition and welfare | Brown J W S |
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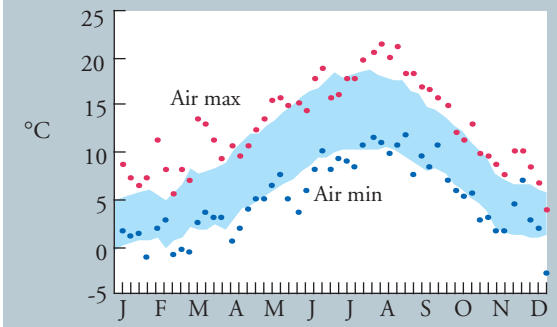
| SCR/833/00 | Microsatellites as population genetic markers | Powell W |

# Meteorological Records

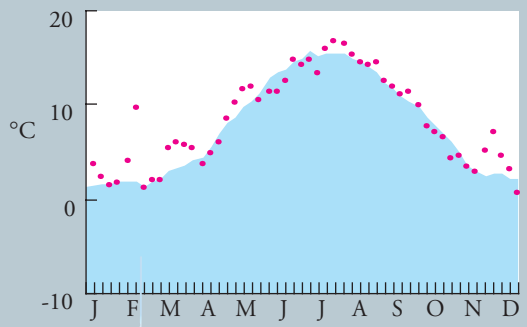
G. Wood

Detailed meteorological records are kept regularly at SCRI. The graphs shown are for weekly values for 2000 and the long term average for 1961-1990( ).

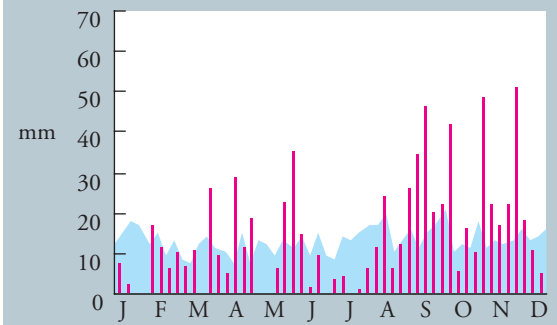
**Air temperature**



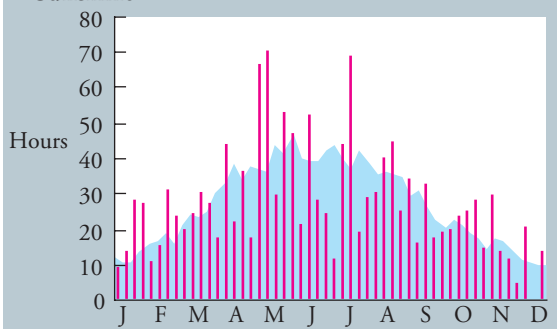

**10cm soil temperature**



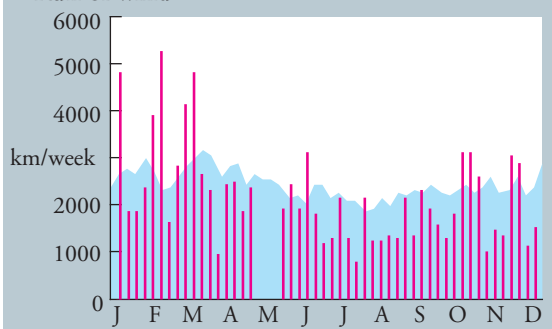

**Rainfall**




**Sunshine**




**Run of wind**



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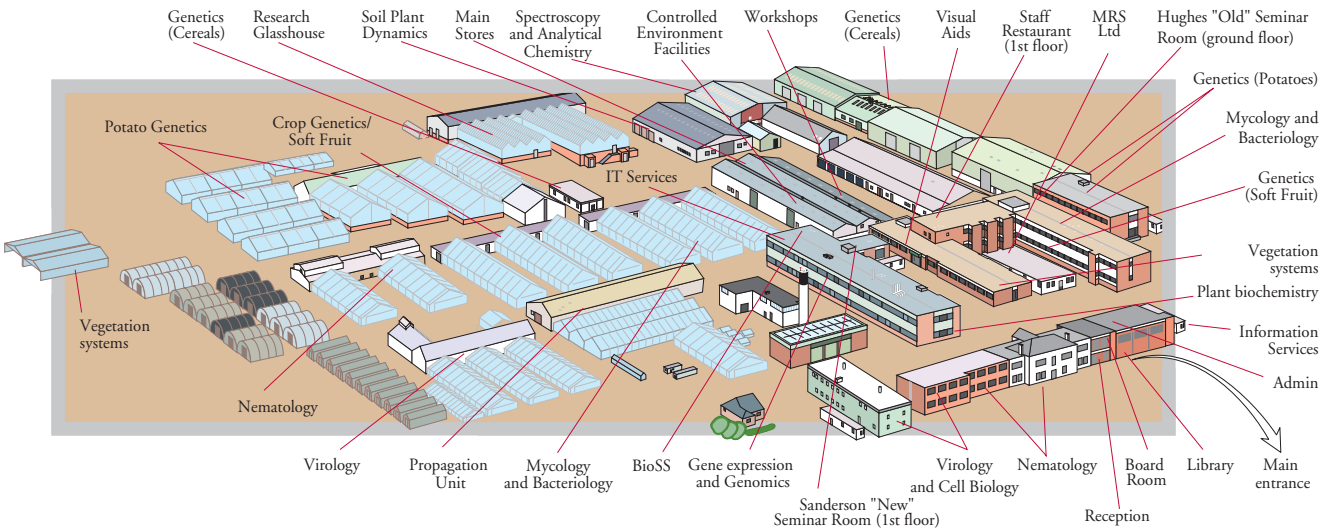
# List of Abbreviations

| | |
|---|---|
| AAB | Association of Applied Biologists |
| ACRE | Advisory Committee on Releases to the Environment |
| ADAS | Agricultural Development and Advisory Service |
| BBSRC | Biotechnology & Biological Sciences Research Council |
| BCPC | British Crop Protection Council |
| BioSS | Biomathematics and Statistics Scotland |
| BPC | British Potato Council |
| BSPB | British Society of Plant Breeders |
| BTG | British Technology Group |
| CAPS | Cleaved Amplified Polymorphic Sequence |
| CEC | Commission of the European Communities |
| CHABOS | Committee of Heads of Agricultural and Biological Organisations in Scotland |
| CIP | International Potato Centre - Peru |
| COST | European Co-operation in the field of Scientific and Technical Research |
| DEFRA | Department for Environmnent, Food and Rural Affairs |
| DfID | Department for International Development |
| EAPR | European Association for Potato Research |
| ECRR | Edinburgh Centre for Rural Research |
| ECSA | European Chips and Snacks Association |
| EHF | Experimental Husbandry Farm |
| ELISA | Enzyme linked immunosorbent assay |
| EPPO | European Plant Protection Organisation |
| ESTs | Expressed Sequence Tagged Sites |
| FF | Flexible Funding (SEERAD) |
| FLAIR | Food-Linked Agro-Industrial Research |
| GILB | Global Initiative on Late Blight |
| GIUS | Glasshouse Investigational Unit for Scotland |
| H-GCA | Home-Grown Cereals Authority |
| HDC | Horticultural Development Council |
| HPLC | High Performance Liquid Chromatography |
| HRI | Hannah Research Institute |
| IACR | Institute of Arable Crops Research |
| ICTV | International Committee for the Taxonomy of Viruses |
| IOBC | International Organisation for Biological Control |
| IMP | Individual Merit Promotion |
| ISHS | International Society for Horticultural Science |
| ISPP | International Society for Plant Pathology |
| IVEM | Institute of Virology and Environmental Microbiology |
| MAFF | Ministry of Agriculture Fisheries and Food |
| MLURI | Macaulay Land Use Research Institute (now the Macaulay Institute) |
| MRI | Moredun Research Institute |
| NERC | National Environmental Research Council |
| NFT | National Fruit Trials |
| NFU | National Farmers Union |
| NIR | Near Infra-Red |
| NMR | Nuclear Magnetic Resonance |
| NPTC | National Proficiency Test Council |
| ORSTOM | Organisation for research in science and technology overseas |
| PCR | Polymerase Chain Reaction |
| PD | Post-doctorate |
| PVRO | Plant Variety Rights Office |
| RAPD | Randomly Amplified Polymorphic DNA |
| RFLP | Restriction Fragment Length Polymorphism |
| RNAi | RNA interference |
| RRI | Rowett Research Institute |
| SABRI | Scottish Agricultural and Biological Research Institutes |
| SAC | Scottish Agricultural College |
| SASA | Scottish Agricultural Science Agency |
| SCRI | Scottish Crop Research Institute |
| SEB | Society for Experimental Biology |
| SEERAD | Scottish Executive Environment and Rural Affairs Department |
| SET | Scottish Enterprise Tayside |
| SNSA | Scottish Nuclear Stocks Association |
| SPD | Senior Post-doctorate |
| SSCR | Scottish Society for Crop Research |
| STS | Sequence Tagged Sites |
| UNDP | United Nations Development Programme |
| WHO | World Health Organisation |

# The Scottish Crop Research Institute

## Site plan

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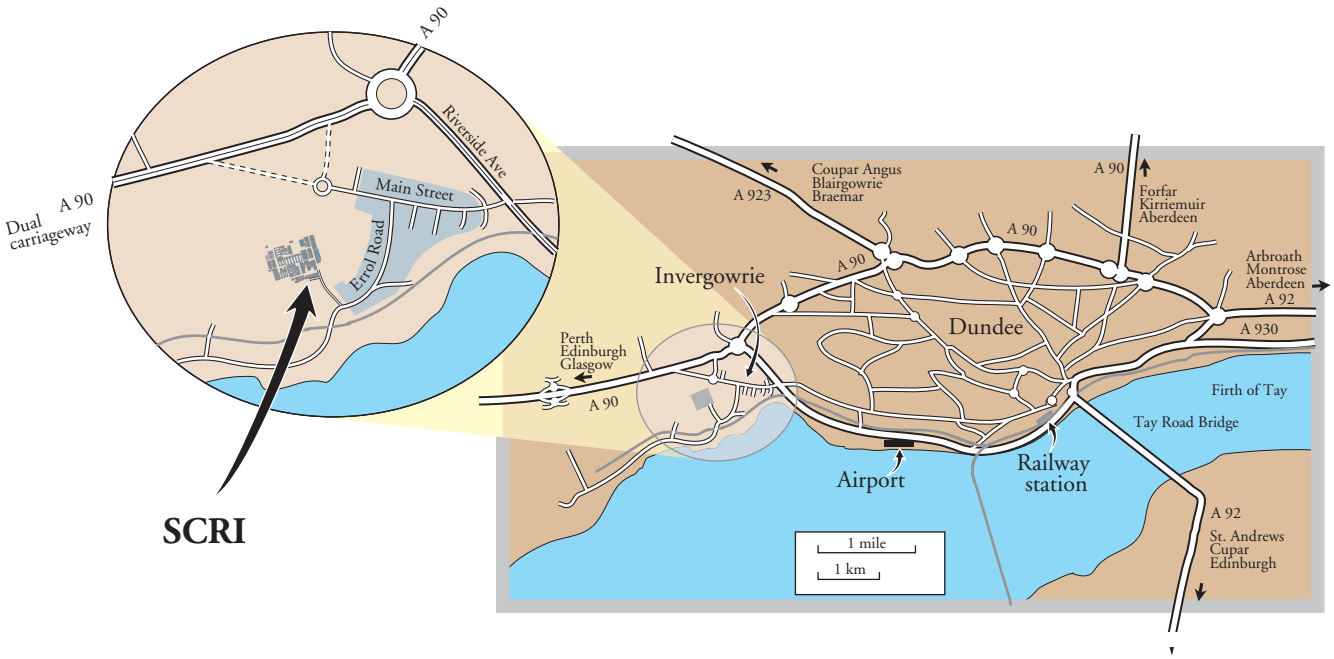


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It is located at Invergowrie 6km west of the centre of Dundee. Access is via Riverside Avenue, Main Street and Errol Road.

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