Scottish Crop Research Institute

Annual Report 1999/2000

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Governing Body

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J.E. Godfrey, B.Sc., A.R.Ag.S.

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Professor R.J. Cogdell, B.Sc., Ph.D., F.R.S.E.
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P. Whitworth
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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 1999/2000

J.E. Godfrey, Chairman of the Governing Body

I am pleased to report on another successful year at the Scottish Crop Research Institute (SCRI). The recommendations from our 1998 Visiting Group Report and the science strategy document published by the Scottish Executive Rural Affairs Department (SERAD) have helped reshape our own science strategy and these changes are reflected in our latest Corporate Plan. In addition, we have implemented additional corporate governance procedures to bring us in line with current best practice.

The pace of change is accelerating and it can be helpful to stand back and review the arena in which we operate. The reasons for research are varied and can include: 1) increasing output; 2) improved quality; 3) production and distribution efficiency; 4) increased market share; and 5) innovative products and presentations. The word 'Research' implies discovery and/or a desire for changing a process or creating a product but in itself is an incomplete concept and encompasses a spectrum of activities - Research, Development, Extension, Advice, Application and, last but not least, Commercialisation.

Today, we have a new research paradigm which demands closer affiliations between the public and private sectors. Genomics and biotechnology are providing new opportunities to add value to agriculture and the industries upstream and downstream of agriculture, but are providing new challenges for routes to commercialisation. Creativity and innovation are paramount in both the research and commercialisation arenas, where we have to move away from crop-specific to customer-targeted solutions. Identifying and operating at multiple points in the food chain and the utilisation of non-food products, arising from primary production where product benefits accrue, is critical to future profitability.

Mylnefield Research Services Limited is our commercial arm and has had its most successful year to date, but we recognise that there are limitations to its activities. During the year, the Mylnefield Trust was set up as a separate legal entity enabling us to be more flexible for potential investors and contractors, with the aim of attracting additional commercial research activity associated with the Institute.

Investment in research has to be recouped within varying product-marketing cycles. This can be difficult for commodity production with inelastic demand, but it is particularly important for developing economies to generate farm surpluses to release resources from agriculture to partake in the benefits of increasing world trade. This in turn helps the developed economies to trade their industrial products. In this increasing global economy, the timing of research and ownership of intellectual property with early exploitation is crucial. This will increase the pressure to shift from horizontal supplying organisations to vertically integrated supply chains.

Governments, with their wider economic and political objectives, invest in research so that as efficiency gains are achieved, resources can be transferred to other competing sectors for the benefit of the country. While not all research has an immediate commercial significance, there are the whole areas of research which lead to public benefit such as improvements in safety, health, and environment. SCRI is at the forefront of helping to evaluate environmental risk arising from GM technology. The commercial benefits of such research are only likely to be felt in the longer term.

At SCRI, we recognise this changing environment and adjust to it. We have a mix of Government and commercial funding which, coupled with our excellent research staff, keep us at the forefront of life science and environmental research. I welcome our new Deputy Director, Wayne Powell and the new Head of Finance and Administration, Douglas Watt. They will, I am sure, contribute to the efficient running and expansion of SCRI. I extend my thanks to the Governing Body, the Director Professor John Hillman, and the staff for all their enthusiasm and dedication to SCRI. This Annual Report is testament to our success.

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 classified as a Non-Departmental Public Body because over 50% of the total funding is received as grant-in-aid from the Scottish Executive Rural Affairs Department (SERAD), formerly the Scottish Office Agriculture, Environment and Fisheries Department. All members of the Governing Body are appointed by the First Minister, formerly the Secretary of State for Scotland. Staff are not formally civil servants, but are members of the Scottish Executive Rural Affairs Department Superannuation Scheme, 1999. SERAD 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 SERAD. The Pay and Grading System, and Staff and Manage-

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 for the benefit of industry and other end users".

To achieve this Mission, SCRI aims

- to be 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 improve 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 critical masses in key strategic areas that are 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 technology-transfer operation that promotes and facilitates collaboration with global industry;
- to meet end-user needs.

ment Codes are administered by the Biotechnology and Biological Sciences Research Council (BBSRC).

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 four divisions and nine interrelated research units. 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 SERAD.

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, formerly the Research, Education and Advisory Services Unit of SERAD. 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, SERAD commissions BBSRC to appoint a Visiting Group to review the work of the Institute. A Visiting Group took place in November 1998. The Visiting Group carried out a scientific audit of the quality and conduct of SCRI's core research programme and related work, and 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.

Remarks published in the Report of the Visiting Group included:

- the VG was impressed by the quality of research at SCRI;
- overall, SCRI's productivity was similarly impressive;
- the calibre of SCRI's staff was considered to be good and the VG identified several outstanding individuals.

The Report made a number of constructive recommendations to guide the continuing development of the Institute. A response to the Report was forwarded to SERAD along with an implementation programme. That programme has been initiated within the past year and most of the Visiting Group recommendations implemented.

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 which is wholly owned by the Mylnefield Trust, 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 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 only 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. In the past years, SCRI has contributed 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.

Details of the annual accounts, Corporate Plan, health and safety provisions, and the SCRI/MRS quality assurance arrangements are available on request.

On behalf of the staff and Governing Body, it is a pleasure once again for me to acknowledge with gratitude the staff of SERAD for their continuing support of, and demonstrable commitment to, our research programme and to our development. Regardless of the enormous pressures upon them in recent years, they function rigorously, openly and fairly, as always, to the highest professional standards of British public service. 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.

SCRI continues to remain buoyant in generally difficult times for science in the UK, justifying its existence in every respect. We have every confidence in meeting future challenges. Scientifically, technologically and commercially, our prospects are outstanding.

Report of the Director

John R. Hillman

Global perspectives of factors influencing agricultural, biological and environmental sciences, and their associated industries : 1999-2000*

Preamble

Food production and the use of products from living organisms for non-food purposes – timber, paper, rubber, starches, oils and fats, cotton, wool, medicines, cosmetics *etc*. – are subject to complex market, social, legal and political forces. In recent years, catastrophic food shortages caused by biotic and abiotic factors have not figured large in reports by the broadcast and publishing-based media, irrespective of severe problems for large numbers of subsistence farmers in less-developed countries (LDCs). For example, in India, following crop failures, around 1000 farmers committed suicide in Andhra, Karnataka, and Maharashtra in the last 3 years, according to P Chengal Reddy. At the same time, the scale of public-sector investments world-wide into research on improved cultivars and animal breeds, pests and diseases of crops and livestock, whole-organism physiology and biochemistry, and the associated agronomic and development work, declined further. By way of example, financial support for the institutes comprising the Consultative Group on International Agricultural Research (CGIAR) system has been sharply constrained. Parenthetically, the European Union's administrative funding failure in 1999 reduced the financial support of the CGIAR's International Potato Center (Centro Internacional de la Papa) by $1.9m, leading to a major deficit. Agriculture, horticulture, and forestry – the primary production industries – are especially vulnerable to adverse weather and the rapid surmounting of control measures by a vast array of pests, weeds and diseases. Yet primary production is no longer regarded internationally as a high-priority area of research spend. In other words, comforted by current levels of global production, there is a largely urban-based assumption that the existing scientific, engineering and technological effort is adequate to safeguard the growing global population. Over-production in certain areas, aided by the continuing decline in commodity prices, has placed further pres-

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

sure on investments in the primary industries and their associated research activities.

In the more-developed countries (MDCs), pressure groups and special-interest groups gained greater influence over democratically elected governments, and many non-governmental organisations (NGOs) engaged directly with international governmental-based bodies. Certain pressure groups were actively opposed to the application of modern scientific advancements which they regarded in a political context as manifestations of globalisation, multi-national corporations, undesirable social change and environmental harm, all reinforced by a legally complex patent protection system. Cynicism, suspicions, misconceptions and prejudices about 'profits' and the rôle of the profit motive and private-sector companies in modern socio-economic systems were frequently expressed. Some of the more physically active pressure groups continued to enjoy the taxation benefits of charitable status and uncritical support by large sections of the press and television.

One particular target in the UK for many anti-technology protestors, some of whom were willing to attack legally permissible field trials, was the agricultural biotechnology sector. Playing on the naïvety of a substantial proportion of the population and political representatives to understand basic principles of conventional plant breeding, ecology, genetics, scientific research, risk-benefit analyses, the difference between regulatory and so-called scientific failures, certain groups effectively destabilised agriculturally related European plant biotechnology. Agriculture in the UK was also influenced by the aftermath of the bovine spongiform encephalopathy (BSE) fiasco, animal-rights protestors, livestock and crop diseases, proponents of 'organic' manure-based systems, increased regulation and associated imposed costs, falling commodity prices, falling profits, raised standards and customer expectations, the growing discontinuity between scientists and the public, and entrenched attitudes to subsidies of various kinds. Mistrust of the food supply and the high levels of subsidy inputs meant that the industry increasingly assumed the mantle of a quasi-nationalised public service, more akin to urban transport, attending to the needs of a population that is becoming both divorced from, and unappreciative of, the challenges facing producers and processors, organic and conventional alike.

Almost all areas of human endeavour influence the agricultural, biological and environmental sciences, and their associated industries. Research and Development (R&D) programmes are directly and indirectly shaped by a multiplicity of factors, including economic performance, political decisions, trading blocs, employment and intellectual property (IP) laws, population pressures, defence-spending, wars, refugee pressures, poverty, taxation and consumer issues, media pressures, scandals, balance of payments, profitability and entrepreneuralism, international comparators, the quality of the labour force, perception of science and technology and, not least, by the stream of scientific discoveries and inventions.

Early in 2000, the 'first draft' of the human genome was completed, well in advance of the projected completion date. The genetic revolution has raised major bioethical issues in the use of human embryonic stem and germ cells, on ownership and exploitation of genetic information –particularly by medical insurance companies, cloning, transgenic organisms, and transgenic foodstuffs. Infectious diseases world-wide accounted for 13.3m of the 53.9m deaths world-wide in 1998, according to the World Health Organization (WHO); the main diseases were acute respiratory infections (including influenza and pneumonia, 3.5m), AIDS (2.3m), diarrhoeal diseases (2.2m), tuberculosis (1.5m), malaria (1.1m), and measles (0.9m). Infectious diseases accounted for 50% of deaths in LDCs, and took place against a background of growing drug resistance of microbes and poor nutrition. In 1999, a large number of outbreaks of infections in the UK were attributable to human consumption of contaminated food and water, caused principally by *Campylobacter; Salmonella; Rotavirus (Reovirus); Giardia lamblia; Cryptosporidium; Shigella; Clostridium difficile; Escherischia coli*, especially *O157; Entamoeba histolytica; Astrovirus; Aeromonas; Yersinia; Listeria; Calicivirus; Brucellosis; Staphylococcus aureus; Pleisomonas shigelloides;* and various *Bacillus* species. Provisional data for England and Wales revealed a total of 103 000 reported cases of food poisoning for 1999, with estimates of reported cases being only a small proportion of total cases, raising again the potential problems of manure-based agricultural and horticultural systems, problems compounded by the longevity of many bacteria and gut parasites, and poor food hygiene.

Fruit and vegetables (*e.g.* apples; tomatoes; citrus fruits; soft fruits such as raspberries and blueberries; cruciferous vegetables, such as broccoli, cabbage, turnip and swede *etc.*), and products derived from them, were associated with disease prevention,

notably of various kinds of cancer and ischaemic stroke. Uncontaminated foods derived from plants contain a wide range of compounds that may not have direct nutritional benefit, but which may affect the consumer. Those compounds may be exogenous (*e.g.* agrochemicals) or endogenous (*e.g.* potential toxins if consumed to certain levels, including alkaloids, cucurbitacins, cyanogenic glycosides, furocoumarins, glyco- and glucosinolates, hydrazine derivatives such as agaritine and gyromitrin, lathrogens, lectins, nitrates, phytoestrogens, protease inhibitors, psoralens, safrole, saponins, and vasoactive amines). Food preparation and processing can often add adventitious compounds, and offer opportunities for microbial contamination. A major target of plant breeding has been the reduction or elimination of undesirable, naturally occurring, endogenous compounds; new targets include the enhancement of factors regarded as beneficial to health.

In areas of science akin to the life sciences, there were exciting new discoveries and reports. Thus, B Conrad and R Taylor (Harvard), C Breuil (Université de Paris-Sud), and F Diamond (Rutgers) were able to prove the full Taniyama-Shimura Conjecture, that every elliptic curve is a projection of a modular curve. In nuclear chemistry, there was strong evidence for the existence of comparatively stable superheavy elements. This arose from joint research at the Joint Institute for Nuclear Research in Russia and the Lawrence Livermore National Laboratory in California, who announced the synthesis of element 114. Also, research at the Lawrence Berkeley National Laboratory, also in California, provided evidence for elements 116 and 188. These three super-heavy elements have a nuclear structure giving them half-lives much longer than their lighter short-lived neighbours on the periodic table of elements, and constitute members of the so-called 'island of stability', and are possibly stable enough to have commercial or industrial application. L Becker (University of Hawaii) provided evidence from extracts of the 4.6 bn-year-old Allende meteorite that the all-carbon, hollow, cage-like fullerines exist in nature, and were they to be present in asteroids and meteorites, could have provided some of the carbon essential to life on Earth. R H Baughman of AlliedSignal, New Jersey, reported the development of carbon nanotubes that flexed in response to changes in applied voltages, enhancing therefore their utility in microscopic and nanoscopic engineering applications. Developments by F Keilman and B Knoll (Max Planck, Martinsried), in atomic-force microscopy involving a tunable infrared (IR) beam focused on the tip, and an associated IR detector to detect scatter from the sample, have enabled high-resolution analyses of the chemical composition of thin films.

The most detailed composite images ever of electronic bonds were obtained by J C H Spence and J M Zuo (Arizona State University) using x-ray diffraction pattern analysis from a copper oxide compound. This research has particular relevance to the development of high-temperature superconductors. In applied chemistry, a team at the University of California, Los Angeles, and Hewlett-Packard Laboratories, Palo Alto, led by J R Heath, reported the use of the synthetic complexes, rotoxanes, as the first operational molecule-based logic gate, giving rise to the possibility of molecular-based 'chemical' computers within the next decade.

In atomic and optical physics, there was major progress in the USA, Japan and Germany in the development of atom lasers, whereby the output is a beam of atoms that exist in coherence *i.e.* have the same de Broglie wavelength. Atoms (sodium or rubidium) are trapped to form a new kind of matter – the Bose-Einstein Condensate (BEC) in which the atoms exist in the same quantum state. Thereafter, a portion of the trapped BEC is permitted to emerge as a beam, albeit short-lived. Nevertheless, the potential range of applications, including microscopy, lithography, and even atom holography to build tiny structures atom-by-atom, will create huge interest in life-science companies and academia.

One of the most apparent manifestations of globalisation is that of international jurisdiction, especially where it interferes with or overrides state sovereignty and local human rights laws, if any. This phenomenon was extended by the efforts made during the year to establish an International Criminal Court to try war-related crimes, crimes against humanity, and genocide; the initiative was brought into being by the Rome Statute which was signed by 89 states in 1998. Developments arising from the International Criminal Tribunal for the former Yugoslavia served to highlight current deficiencies in the jurisdiction of international tribunals, highlighting the potential for double standards, vexatious litigation, and the erratic incorporation of conventions such as the 1984 International Convention Against Torture, or the European Convention on Human Rights, into national legal systems.

Biological weapons, along with nuclear and chemical weapons, telecommunications and Internet-based attacks were regarded as potential threats from modern terrorism. The International Institute of Strategic Studies pointed out that modern terrorism involved individuals or small cells of activists driven, uncompromisingly, by fanatical beliefs or single issues. World-wide, according to a US State Department report, there were 273 terrorist attacks in 1998 and, although it was the lowest annual total since 1971, 741 people were killed and 5952 injured.

Plant-derived narcotics such as cocaine, heroin, and cannabis, exercised governments and international agencies such as the UN International Narcotics Control Board. Increasing well-publicised tolerance of recreational drugs and fumatories by politicians and the public, clandestine manufacture and distribution of synthetic drugs, along with poor importation and policing controls, would appear to reinforce the view that the so-called 'war on drugs' has not been as effective as originally hoped. Controls on the major growing areas in Latin America, Afghanistan and Pakistan could potentially involve biological control agents such as species-specific plant diseases, but their use would of necessity involve exceptionally carefully planned and monitored research and development work prior to reaching international agreement on the principles of such operations.

In the world of the media, the Internet exerted a major effect on television, radio, newspapers, magazines, journals and books, all of which are routes to transmit scientific, engineering and technological information. Deregulatory climates in many countries led to consolidation and conglomeration of media companies at the global level. Great emphasis was placed on entertainment and advertising and often involved telecommunication systems as a means to garner additional income. Cable and satellite television, as well as new radio stations, diminished the impact of the main traditional broadcaster, and the quality and spread of scientific- and fact-based broadcasting suffered. A similar pattern occurred in the competitive world of newspaper publishing; free newspapers, business news, lifestyle publishing, and sensationalism related to entertainers and the aristocracy, were primary areas of focus. Few newspapers were able to report scientific developments authoritatively, demonstrating to a large extent the inability of scientists properly to present their work to the public. Electronic publishing would appear to offer major opportunities for scientists, with the potential to replace conventional journals and books. On-line editing was becoming commonplace, as was the development of large-scale on-line relational databases required for bioinformatics. Questions of copyright and related intellectual property, and profitability, have yet to be properly addressed.

Prominent developments in higher education included the rapid development of information technology and social science, with traditional science courses in mathematics, physics and chemistry proving difficult recruiting grounds for outstanding students. Consortia of universities were initiated, such as Universitas 21 (a group of 21 universities in Australia, Canada, China, New Zealand, Singapore, UK and USA) aimed at attracting multinational business clients, and a European Consortium of Innovative Universities, comprising universities from Denmark, Finland, Germany, The Netherlands, Portugal, Spain, Sweden, and the UK, formed to benefit co-operatively from private-sector financial support. Universities in most countries actively sought non-state finance, and resisted attempts by governments to impose bureaucratic controls.

In the UK, the Millennium Dome was built to celebrate the year 2000. Standing on the prime meridian on a reclaimed site in Greenwich, near to the Thames Barrage, the Dome was noted for its roof of tensioned fibreglass membrane covering 8 hectares and suspended from 12 masts. Such is the standing of science that early hopes that the building would house British scientific, engineering and technological achievements, and exciting new R&D projects, did not materialise, despite an expenditure of over £600m derived for the most part from National Lottery funds.

Economics and Politics

Financial stability started to return to stock markets in developed countries by the second quarter of 1999, following the depressed period that followed in the wake of the Asian financial crisis starting in July 1997, and the default of debt in Russia in August 1998. The International Monetary Fund (IMF) revised up its projections for growth in 1999 from 2.3% to 3%, in tune with improving global economic conditions. According to the Organisation for Economic Co-operation and Development (OECD) *Economic Outlook*, December 1999, the percentage annual change in real gross domestic products (GDPs) of all developed countries was estimated to average 2.8%, compared with 2.4% in 1998, and 3.5% in 1997. The

highest levels were achieved by the USA (3.8%), and Canada (3.7%), demonstrating the dynamo effect of North America in the world economy. At 1.7%, the UK figure was less than the average of the European Union (EU) (2.1%). With an average of 3.5%, economic growth of the LDCs exceeded that of the MDCs, continuing a trend established over the past three decades.

As a result of massive price and cost rises during the 1970s, the almost universal application of fiscal disciplines by governments to fight inflation have now given rise to a situation whereby global inflation is at its lowest level for 40 years, with remarkably little variation in national inflation rates. In some countries, there were oft-expressed fears of deflation. Increased global competition impacted positively on supply-side price suppression. More detailed analysis of globalisation reveals the extent of economic integration and the expansion in foreign direct investment (FDI), particularly through mergers and acquisitions. About 70% of FDI flows was between multinational companies based in North America and Europe, but about 25% involved Asian LDCs. In 1999, there were about 60 000 transnational companies with about 500 000 affiliates. In 1998, sales by the affiliates were about $11 trillion, compared with world exports of slightly less than $7 trillion, and FDI rose by more than 40% to exceed $640bn with a total stock valuation of over $4 trillion. According to the UN Conference on Trade and Development (UNCTAD), FDI could have exceeded $700bn in 1999.

At the beginning of 1999, 11 of the 15 member states of the EU introduced a new single currency, the euro. This event coincided with a continuing deceleration in growth in the so-called 'euro zone'. The reasons for this included the effects of tight fiscal policies by those nation-states seeking to qualify for economic and monetary union in the preparatory phase for launching the euro, as well as a decline in demand for goods and services by LDCs. A combination of lack of international confidence by the money markets in the euro, and interest-rate amendments by the European Central Bank (ECB), led to substantial depreciation by at least 11% of the euro against all the major currencies. In the UK, this affected many R&D organisations that received substantial EU research grants. Euro depreciation both increased the competitive position of euro-zone exporters and stoked inflationary risks as demand in the domestic markets rose. The euro zone is protected from international free trade, and imports by the 11 members account for only 10% or less of GDP and inflation was therefore suppressed. By remaining outwith the euro zone, the more extrovert UK economy was able to retard inflationary tendencies to well below the government's 2.5% target, but special difficulties were posed for UK manufacturers and exporters irrespective of buoyant domestic demand. Nonetheless, there was ample evidence of rapid adaptation to the new export market scenario. A strong separation was evident between those leaders of industry and commerce in favour of entry to the euro zone, and those opposed to the imposition of economic and social 'harmonisation'.

In exceeding IMF growth projections, the UK economy showed unexpected resilience to the effects of a relatively strong appreciation of the pound-sterling against all the major currencies. With consumer demand growing by an annual rate of 5.2%, a low inflation rate regardless of oil-price rises and a housing boom in the south-east of England, a fall in unemployment, suppressed public expenditure, interest-rate cuts, and muted pay pressures despite the introduction of a minimum wage, the UK economy was relatively robust compared with the euro-zone. Price Waterhouse Cooper reported that using the Office for National Statistics definitions, the UK tax burden has risen from 35.5% of GDP in 1996-1997 to a projected 38.5% in 2001-2002. During this period, the percentage difference between the tax burden in the UK and the EU excluding the UK will have declined from six to less than two percentage points. S Nickell, N Batini and B Jackson noted that the labour share (proportion of wages, salaries and self-employment income in the gross domestic product) has risen to its highest level since the mid-1970s, indicating that wages have risen faster than productivity. A productivity gap between the UK economy and that of its international competitors could in the opinion of many commentators reflect a combination of a history of uncompetitive interest rates, a steadily depreciating currency and labour-management problems, not least the neglect of investment in innovative science, engineering and technology, and a lack of focus on design with quality. According to C Daykin, the Government Actuary, the number of people claiming incapacity benefits over the next two decades is projected to rise from 1.5m to 2.5m – more than 10% of the population. The current cost of sickness and incapacity benefits are already £7bn a year.

Further scrutiny of the euro-zone reveals that in addition to their languages, the economic structures, pension systems, development paths, political systems and

attitudes to wealth creation of the 11 founding countries were also diverse. Large budget deficits remained in France, Germany and Italy, limited as they were to a maximum of 3% under the terms of the Stability and Growth Pact. At 9.4%, the level of unemployment in the zone was high. GDP growth varied from 8.6% in Finland, 7.5% in Eire, 3.5% in Luxembourg, 3.4% in Spain, 3% in Portugal, 2.5% in The Netherlands, 2.5% in France, 2% in Austria, 1.4% in Belgium and Germany, and 1.2% in Italy (the UK figure was 1.7%).

The euro remained susceptible to comments by officials of the European Central Bank (ECB) and senior politicians in EU member states, and there was growing dependence on intervention aided by the Group of Seven industrialised nations G7 in the foreign exchange markets. One major factor influencing market sentiment was the persistent outflows of long-term capital, particularly to North America, where the market-place was deemed to be more liberal and less hidebound by regulation and labour-related social impositions. By the third quarter of 2000, inflation in all the euro-zone economies exceeded the ECB target of 2%. Another factor was the impact of rising oil prices. As an aside, there is the potential for non-oil-producing LDCs to be seriously affected by oil-price rises and oil shortages. For all MDCs, any disruption to supplies, possibly coinciding with seasonally related high demand, would amplify the effects of weakened equity markets. All oil-importing countries would inevitably suffer deteriorating terms of trade, not aided by the fact that many countries use oil-related taxation as a major component of their taxation base. The International Road Transport Union claimed that UK fuel taxes were the highest in the developed world, and according to the Finnish Technical Research Centre, the vehicle excise duty on a 40 tonne articulated vehicle in the UK was the highest in Europe. Modern agriculture, horticulture, forestry and retailing are heavily fuel-dependent.

In addition to the problems of the euro, were the constitutional crisis that followed a devastating report on the European Commission, delays to competition reform as well as to the Common Agricultural Policy, gross under-achievement of EU members in peacemaking operations in the Balkans, delays to enlargement of the Union, and disagreements between member states over BSE. A report in March 1999 by teams of former judges and auditors confirmed most of the allegations of a junior internal auditor, who had been arrogantly denounced previously by a complacent Commission. On the night that the report detailing mismanagement and nepotism, even in the area of research support, was published, J Santer and his 19 fellow commissioners resigned en bloc. A new Commission was appointed, led by R Prodi. In June, there was overall a poor turnout for elections to the European Parliament, with participation as low as 25% in some countries.

Globalisation is expressed through the operation of a large number of international organisations, many functioning at government level (Table 1), and many involve agriculture, horticulture and forestry at the level of primary production through to international trading and protection of rights. Complexity reigns within trading blocs; for example, the EU operates through a number of bodies that cut across the activities of the 15 nation states (*e.g.* The Council of the EU, the European Commission, the European Parliament, the Court of Justice of the European Communities, the Committee of the Regions, the Economic and Social Committee, the European Central Bank, the European Court of Auditors, the European Investment Bank, and the European Police Office). Such issues as enlargement and external relations, the legislative processes, the Community budget, the single market relating to non-tariff barriers and the European Economic Area, the European Monetary System and the associated single currency, common foreign and security policies, and, not least, the reform of the Common Agricultural Policy (CAP), are shaped by the major treaties (Treaty of Rome, 1957; the Maastricht Treaty, 1991; the Amsterdam Treaty, 1997, and the forthcoming Treaty of Nice).

Many believed that world trading conditions would deteriorate in 1999, but the projected rise in volume of world trade was 3.7%. Growth in global exports was restrained to 2.4%, the value of global exports were estimated to be $6.844 trillion. Recovery of East Asian economies coupled with the strength of the US economy contributed in large measure to drive international trade. MDCs increased their imports by 5.9% above the 1998 level, but imports by countries in economic transition were depressed.

According to the 2001 index of economic freedom, produced by the Heritage Foundation and the Wall Street Journal, Hong Kong has the freest economy followed by Singapore and then the Republic of Ireland; the USA is fifth and the UK seventh. In neat juxtaposition, Transparency International, the global anticorruption organization, stated in its *Bribe Payers*

Body	Founded	Member States
Andean Community http://www.comunidadandina.org	1997	Bolivia, Colombia, Ecuador, Peru, Venezuela
Arab Maghreb Union (AMW) http://www.maghrebarabe.org	1989	Algeria, Libya, Mauritania, Morocco, Tunisia
Asia-Pacific Economic Co-operation (APEC) http://www.apecsec.org.sg	1989	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, Vietnam
Association of South East Asian Nations (ASEAN) http://www.asean.or.id	1967	Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Singapore, Thailand, Vietnam
Caribbean Community and Common Market (CARICOM) http://www.caricom.org	1973	Antigua and Barbuda, the Bahamas (but not the Common Market), Barbados, Belize, Dominica, Grenada, Guyana, Jamaica, Montserrat, St. Christopher and Nevis, St Lucia, St Vincent and the Grenadines, Suriname, Trinidad and Tobago. Haiti due to be admitted, and other countries are associate members or observers.
The Commonwealth http://thecommonwealth.org	1926 (Imperial Conference) 1947, 1949 and 1991 (Harare Commonwealth Declaration)	54 member states together with their associated states and dependencies. With exception of Mozambique, all were formerly parts of the British Empire or Legion of Nations.
Commonwealth of Independent States (CIS) http://www.cis.minsk.by	1991	Armenia, Azerbiajan, Belarus, Georgia, Kazakhstan, Kyrgyzstan, Moldova, Russia, Tajikistan, Turkmenistan, Ukraine, Uzbekistan
Council of the Baltic Sea States (CBSS) http://www.baltinfo.org	1992	Denmark, Estonia, Finland, Germany, Iceland, Latvia, Lithuania, Norway, Poland, Russia, Sweden, EU
The Council of Europe http://www.coe.fr	1949	41 member states, with special guest status to Armenia, Azerbaijan, and Bosnia-Herzegovina
The Economic Community of West African States (ECOWAS) http://www.cedeao.org	1975	16 member states
The European Bank for Reconstruction and Development (EBRD) http://www.ebrd.com	1991	58 countries, the EU, and the European Investment Bank
European Free Trade Association (EFTA) http://www.efta.int	1960	Iceland, Lichtenstein, Norway, Switzerland, (Austria, Denmark, Finland, Portugal, Sweden and UK left to join the EU)
Food and Agriculture Organisation of the United Nations (FAO) http://www.fao.org	1945	180 states and the EU
International Fund for Agricultural Development (IFAD) http://www.ifad.org	1977	161 members
International Monetary Fund (IMF) http://www.imf.org	1944	182 members
League of Arab States	1945	Algeria, Bahrain, the Comoros, Djibouti, Egypt, Iraq, Jordan, Kuwait, Lebanon, Libya, Mauritania, Morocco, Oman, Palestine, Qatar, Suadi Arabia, Somalia, Sudan, Syria, Tunisia, UAE, Yemen
Mercosur http://www.mercosur.org	1988	Argentina, Bolivia, Brazil, Chile (Associate), Paraguay, Uruguay

Table 1 Examples of international, government-level organisations connected to economic, cultural and scientific development, health and defence.

Table 1 (cont.)

The Nordic Council http://www.norden.org	1952	Åland Islands, Denmark, Greenland, Iceland, Faroes, Finland, Norway, Sweden
North American Free Trade Agreement (NAFTA) http://www.nafta.net	1992	Canada, Mexico, USA
North Atlantic Treaty Organisation (NATO) http://www.nato.int	1949	Belgium, Canada, Czech Republic, Denmark, France, Germany, Greece, Hungary, Iceland, Italy, Luxembourg, The Netherlands, Norway, Poland, Portugal, Spain, Turkey, UK, USA
Organisation for Economic Co-operation and Development (OECD). http://www.oecd.org The OECD is associated with the Nuclear Energy Agency, International Energy Agency, Development Centre, Centre for Educational Research and Innovation, and European Conference of Ministers of Transport	1961	Australia, Austria, Belgium, Canada, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary,Iceland, Irish Republic, Italy, Japan,Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Spain, Sweden, Switzerland, Turkey, UK, USA
Organisation for Security and Co-operation in Europe (OSCE) http://www.osce.org	1975	55 participating states
Organisation of African Unity (OAU) http://www.oau-oua.org	1963	53 members
Organization of American States (OAS) http://www.oas.org	1890/1948	35 member states
Organisation of Arab Petroleum Exporting Countries (OAPEC) http://www.oapeccorg.org	1968	Algeria, Bahrain, Egypt, Iraq, Kuwait, Libya, Qatar, Saudi Arabia, Syria, Tunisia (membership dormant), UAE
Organisation of the Black Sea Economic Co-operation (BSEC) http://www.bsec.gov.tr	1992	Albania, Armenia, Azerbaijan, Bulgaria, Georgia, Greece, Moldova, Romania, Russia, Turkey, Ukraine
Organisation of the Islamic Conference (OIC)	1971	55 members
Organisation of the Petroleum Exporting Countries (OPEC) http://www.opec.org	1960	Algeria, Indonesia, Iran, Iraq, Kuwait, Libya, Nigeria, Qatar, Saudi Arabia, UAE, Venezuela. Ecuador and Gabon have withdrawn membership
The Pacific Community http://www.spc.org.nc	1947	26 member states and territories
South Asian Association for Regional Co-operation (SAARC) http://www.south-asia.com	1985	Bangladesh, Bhutan, India, the Maldives, Nepal, Pakistan, Sri Lanka
Southern African Development Community (SADC) http://www.saep.org/sadc/sacd.html	1992	Angola, Botswana, Democratic Republic of the Congo, Lesotho, Malawi, Mauritius, Mozambique, Namibia, South Africa, Seychelles, Switzerland, Zambia, Zimbabwe
The United Nations (UN) http://www.un.org	1942/1945	188 member states

Comprises six principal organisations:

1 The General Assembly, responsible for other specialised bodies:
a. The Conference on Disarmament (CD)
b. The United Nations Children's Fund (UNICEF)
c. The United Nations Development Programme (UNDP)
d. The United Nations High Commissioner for Refugees (UNHCR)
e. The UN Relief and Works Agency for Palestine Refugees in the Near East (UNRWA)
f. The United Nations High Commissioner for Human Rights
g. The UN Centre for Human Settlements (Habitat)
h. The UN Conference on Trade and Development (UNCTAD)
i. The Department of Humanitarian Affairs (DHA)
j. The International Seabed Authority
k. The UN Environment Programme (UNEP)
l. The UN Population Fund (UNFPA)
m. The UN Institute for the Advancement of Women (INSTRAW)
n. The UN University (UNU)

Table 1 (cont.)

o. The World Food Council (WFC)
p. The World Food Programme (WFP)

2 The Security Council

3 The Economic and Social Council

4 The Trusteeship Council

5 The Secretariat

6 The International Court of Justice

There are also independent Specialised Agencies linked to the UN:

• Food and Agriculture Organisation of the UN (FAO) qv.
• International Civil Aviation Organisation
• International Fund for Agricultural Development (IFAD) qv.
• International Labour Organisation
• International Maritime Organisation
• The International Monetary Fund (IMF) qv.
• International Telecommunications Union
• UN Educational, Scientific and Cultural Organisation (UNESCO) qv.
• UN Industrial Development Organisation (UNIDO) qv.
• Universal Postal Union
• World Bank (International Bank for Reconstruction and Development, International Development Agency, International Finance Corporation) qv.
• World Health Organisation (WHO) qv.
• World Meteorological Organisation (WMO) qv.

There are also independent Non-Specialised Agencies linked to the UN:

• International Atomic Energy Agency
• World Trade Organisation (WTO) qv.

United Nations Educational, Scientific and Cultural Organisation (UNESCO) http://www.unesco.org	1946	188 member states
United Nations Industrial Development Organisation (UNIDO) http://www.unido.org	1966	168 member states
Unrepresented Nations and Peoples Organisation (UNPO) http://www.unpo.org	1991	52 full members and 5 supporting members
Western European Union (WEU) http://www.weu.int	1948/1954	28 members, associated members and observers

The World Bank (International Bank for Reconstruction and Development, IBRD) http://www.worldbank.org — 1945 — 181 members

Two affiliates: International Finance Corporation (IFC), and International Development Association (IDA)

The Consultative Group on International Agricultural Research (CGIAR) was established in 1971 and is an informal association of 58 public- and private-sector members that supports a network of 16 international agricultural research centers. The CGIAR's budget for 1999 was US$330m and is co-sponsored by the World Bank, the Food and Agriculture Organisation of the United Nations, the United Nations Development Programme, and the United Nations Environment Programme, together with individual donations and contracts. The CGIAR's mission is to contribute to food security and poverty eradication in LDCs through research, partnership, capacity building, and policy support. It promotes sustainable agricultural development based on environmentally sound management of natural resources, focusing on five major thrusts:

(i) Increasing productivity in developing-country agriculture through genetic improvements in plants, livestock, fish, and trees, and through better management practices; (ii) protecting the environment through conservation of natural resources (especially soil and water) and reductions of the impact of agriculture; (iii) saving biodiversity, through one of the world's largest *ex situ* collections of plant genetic resources (over 500,000 accessions of more than 3,000 crop, forage, and agroforestry species), held in trust for the world community; (iv) improving policies that influence the spread of new technologies and the management and use of natural resources; and (v) strengthening national research in developing countries through partnerships with national programs and training in research techniques, administration, and management.

The 16 CGIAR Research Centers are:

• CIAT – Centro Internacional de Agricultura Tropical (International Centre for Tropical Agriculture – founded 1967) http://www.ciat.cgiar.org/
• CIFOR – Centre for International Forestry Research – founded 1993 http://cifor.cgiar.org/
• CIMMYT – Centro Internacional de Mejoramiento de Maiz y Trigo (International Center for the Improvement of Maize and Wheat – founded 1966) http://www.cimmyt.org/
• CIP – Centro Internacional de la Papa (International Potato Centre – founded 1971) http://cipotato.org/

Table 1 (cont.)

• ICARDA – International Center for Agricultural Research in the Dry Areas – founded 1977 http://icarda.cgiar.org/ • ICLARM – International Center for Living Aquatic Resources Management – founded 1977 http://www.cgiar.org/iclarm • ICRAF – International Centre for Research in Agroforestry – founded 1977 http://www.icraf.cgiar.org/ • ICRISAT – International Crops Research Institute for the Semi-Arid Tropics founded 1972 http://www.icrisat.org/ • IFPRI – International Food Policy Research Institute – founded 1975 http://ifpri.cgiar.org/index.htm • IITA – International Institute of Tropical Agriculture – founded 1967 http://www.cgiar.org/iita • ILRI – International Livestock Research Institute – founded 1995 http://www.cgiar.org/ilri/ • IPGRI – International Plant Genetic Resources Institute – founded 1974 http://www.ipgri.cgiar.org/ • IRRI – International Rice Research Institute – founded 1960 http://cgiar.org/irri/ • ISNAR – International Service for National Agricultural Research – founded 1979 http://www.cgiar.org/isnar/index.htm • IWMI – International Water Management Institute – founded 1983 http://www.cgiar.org/iwmi/ • WARDA – West Africa Rice Development Association – founded 1971 http://www.cgiar.org/warda/		
World Health Organisation (WHO) http://www.who.ch	1946	191 members
World Intellectual Property Organisation (WIPO) http://www.wipo.int The International Union for the Protection of New Varieties of Plants (UPOV) founded in 1961, is linked to WIPO, and has 40 members. The European Patent Office operates under the European Patent Convention, covering 20 signatory states and is not an instrument of the EU.	1967	173 members
World Meteorological Organisation (WMO) http://www.wmo./ch	1950	179 member states and 6 member territories
World Trade Organisation (WTO) http://www.wto.org	1995	134 members, with 31 further applicant states

Index, that China, South Korea, Taiwan, and Italy were among the 19 leading exporting countries most involved in paying bribes to public officials, contrasting sharply with such countries as Australia, Austria, Canada, and Sweden. In its Corruption Perception Index, Azerbaijan, Cameroon, Indonesia, and Nigeria, were listed as the world's most corrupt countries.

Although the WTO meeting in Seattle in late 1999 took place at a time of global economic slowdown, and was widely regarded as a failure (see Agriculture and Food Section), the seventh annual meeting of the 21 members of the Asian-Pacific Economic Co-operation (APEC), held beforehand in Auckland, New Zealand in September 1999, was considerably more positive. There was agreement by the member countries who account for two-thirds of world trade to resist protectionist measures and liberalise trade operating with free and fair competition. Nevertheless, progress by the member states to achieving the deadline of removing trade barriers, by 2010 for MDCs and 2020 for LDCs, has been limited. What is more, anti-capitalist demonstrations in Seattle led to the arrest of 500 people and damage of more than $2.5m. Similar demonstrations took place at the subsequent WTO meetings in Washington, the World Economic Forum in Melbourne, and the International Monetary Fund (IMF) and World Bank Group meeting in Prague. The protestors were diverse but in essence sought protectionist measures to counter lack of safeguards against the exploitation of labour and the environment. Through the Global Compact, the UN sought in July 2000 a partnership between business and the UN, involving 50 companies and 12 labour organisations and environmental groups in an attempt to promote free trade unions, core labour standards and protection of the environment. Rather than confront companies by issuing a legally binding code of conduct, the UN generated a declaration of nine principles drawn from the Declaration of Human Rights, the Earth Summit of Rio de Janeiro in 1992, and the Social Summit held in Geneva in 1995.

By the end of 1999, there were over 100 regional trade agreements in force, whereby trading barriers were removed between members of the groupings. Of all the trading blocs, however, that of the EU appeared to be the most self-centred such that intra-regional trade accounted for 62.5% of total trade compared with, for example, the Association of South-east Asian Nations (ASEAN) where intra-regional trade was around 20%. Bilateral free-trade agreements were beginning to be regarded as the best way to stimulate global liberalisation compared with

broader slow-moving multilateral schemes, with Singapore a prominent participant.

The overall current accounts of MDCs started to move into deficit after six consecutive years of surplus, much of the movement being accounted for by the growth in deficits of Australia, Germany, Spain, UK, and USA. Most euro-zone countries, however, had large surpluses reflecting the competitive boost given to their exports by depreciation of the euro.

In the LDCs, which most often are highly agriculture-dependent, the current account deficit fell from $77bn in 1998 to $56bn in 1999, as higher oil prices improved the lot of oil-exporting countries. Even so, the position for Africa remained challenging at $19bn, and the external debt of the LDCs rose to $2.3 trillion. In April 2000, 30 of the world's poorest nations formed the World Association of Debt Management Offices (WADMO), a self-help group designed to improve their debt management capacity and to inspire confidence in the donor countries. According to the IMF (*World Economic Outlook*, October 1999), output in 1999 was expected to increase by 3.5% in the LDCs, compared with 3.2% in 1998. Latin America, however, mainly suffered from a drop in output. Thus, trade in the Southern Core Common Market (Mercosur), comprising Brazil, Paraguay and Uruguay, fell dramatically during 1999, reflecting primarily the effects of recession and the devaluation of the Brazilian real. Contrasting with a failure to liberalise intraregional trade, the member countries considered the proposal from Argentina on the adoption of a single currency, perhaps based on a basket of currencies such as the dollar, yen and euro. In September, Brazil and Argentina opened talks on the problems threatening the unity of Mercosur, not least the effect of the real devaluation. Only the Asian LDCs were expected to show a marked increase in output with a 5.3% annual change in real GDP, up from 3.7% in 1998. Africa was projected to show a 3.1% increase, the Middle East and Europe 1.8%, the Western Hemisphere 0.1%, and those countries classified as 'in transition', 0.8%.

One undesirable symptom of the globalisation of trade was the 'banana' war launched by the USA with some Caribbean countries against the EU in respect of the EU banana import regime. This high-profile disagreement started in 1995, leading to a WTO panel finding in 1997 that, by giving preferential entry to a fixed tonnage of bananas from associated African, Caribbean and Pacific countries, the EU violated the rules of the WTO. The ruling was confirmed by an Appeals Body in late 1997. A revised EU banana regime was eventually adjudicated by a new WTO Panel to be inadequate, and arguments persisted over whether the USA could pursue a policy of imposing penalty rates of duty on a diverse range of European goods. Unresolved issues about the appropriate mechanisms to support agrarian-based LDCs, cultural links, and the range of options open to nations under the dispute settlement procedures of the WTO, were aired by groups and individuals dissatisfied with globalisation.

According to the *Financial Times*, nearly all stock markets continued their upward trends in 1999 and the first part of 2000. By the year-end close of 1999, only the Brussels BEL20 (-5%), the Irish ISEQ overall (0%), and Manila Composite (9%) posted less than 12% increases during the year. Various commentators pointed towards the inter-related trends of attractiveness of technology stocks, the impact of new technologies on increased international competition and greater price transparency, and a more permanent shift to higher growth with low, stable inflation. A similar era was noted by DeAnne Julius, the economist, in the period 50 years up to World War I, a time when new technologies drove global growth and eventual price stability. Round-the-clock electronic trading using electronic communication networks – computerised systems for matching the orders of buyers and sellers of securities without the intervention of specialists or market-makers – as well as increased access to the Internet, started to alter profoundly the investment scene as on-line brokerages began to threaten the hitherto closed operations of the world's traditional stock exchanges which had consistently impeded off-exchange trading. The open competition, however, created regulatory concerns, especially with regard to internal controls and risk-management systems designed to protect investors.

Consolidation of financial services in Europe and the USA, particularly leading to the integration of banking and insurance, was a pronounced feature of 1999-2000. Policy differences were expressed on the allocation of supervisory responsibilities for financial conglomerates incorporating banks, insurance companies and securities firms, but there was no international governmental consensus on how to oversee transnational organisations. Related supervisory issues concerned classification of assets, loan-loss provisions, the rôles of external and internal auditors and associated financial-reporting practices, risk-based capital

adequacy standards, the effective use of market discipline, and money laundering. An increasing level of importance was attached to the Basel Committee on Banking Supervision.

In *Productivity Developments Abroad*, C Gust and J Marquez, two Federal Reserve economists, reinforced the view that the high level of US productivity over the past quinquenium was a reality rather than a statistical mirage. Only two other countries in the OECD, Australia and Switzerland, recorded similar improvements in productivity growth. In Japan and most of the large EU countries, productivity growth declined over the period, even though there were substantial investments in information technology. The US improvement could be satisfactorily explained in terms of the business cycle. Likewise, there was no correlation between the use of hedonic price indexing which takes into account improvements in quality as well as changes in prices and measured productivity growth. There was no evidence to indicate improvements in the quality of the US workforce. Rather, they point to a combination of the growth of spending on information technology – which has raised its share of total US capital stock to 7.4% compared with 2% to 3% in the larger EU economies, and network efficiencies and other beneficial effects of utilising IT equipment effectively (*e.g.* number of Internet sites and secure Internet servers).

Leaked reports from the World Bank claimed that 223 of its funded projects in October 1999 would result in the involuntary (forced) resettlement of 681 000 households and more than 2.6m people, mainly in the East Asian and Pacific region. As a counter to such criticism, the Bank implemented a new operational directive and established a compliance operation. Large dam projects, in particular, frequently fail to deliver promised benefits and underestimate the social disruption and environmental damage they cause, according to the World Commission on Dams, a group set up in 1998 by the World Bank. China and India, however, pressed ahead with major schemes for generating hydroelectric power, controlling floods, and supplying irrigation schemes. The international committee of government ministers overseeing policy noted that the Bank should focus its efforts and work out an appropriate division of labour between itself and international agencies such as the UN Development Programme. International standards of fiscal and monetary policy are favoured by the (G7) industrialised nations, and it is envisaged that the IMF should have a central rôle in the surveillance of codes and standards, and promoting their implementation. The Financial Stability Forum – the international network of central bankers and financial market regulators - favours incentives for countries to adopt such standards. Transparency is seen as the key to avoid future financial crises, yet the Group of 24 (G24) countries, which includes influential emerging market countries, contested the trend towards compulsion, claiming that the application of codes and standards was highly asymmetric, and that industrialised countries should first demand transparency from their hedge funds.

In *The Least Developed Countries 2000 Report*, UNCTAD pressed for reconsideration of aid, debt and trade between MDCs and LDCs. Economic stagnation or decline has occurred over the past decade in 22 of the 48 countries classified by the UN as LDCs. UNCTAD seeks a substantial rise in aid, improved debt relief, greater access to foreign markets for LDC experts, and modification of domestic policies to enhance productive capacity and global competitiveness.

Of all UN agencies, the UN Development Programme (UNDP) has the broadest remit and the widest geographical spread of 136 countries. In relying on voluntary contributions, it has been squeezed during the aid squeeze of the 1990s to operate on a core 'no-strings' budget of $700m, down from $1.2bn in 1992, but non-core, project-related funding has reached $1.6bn. Unlike the World Bank, the UNDP is regarded as being much closer to recipient rather than donor countries. Much of its work is in governance and policy advice, laying the foundations of, but not monitoring, elections. It also facilitates self-help projects, internet projects, helping administer and co-ordinate governance in politically and economically fragile countries. It even helps improve co-ordination between UN agencies. There is an expectation by LDCs that the UNDP will take a high profile in debt relief, preferential trading arrangements and official development assistance, relieving LDCs of internationally imposed constraints.

A plethora of world-wide web-based e-commerce (.com) sites were launched during the year; few were profitable, but until technology stocks became unattractive in mid-2000, investors behaved as if e-commerce would provide substantial capital and income returns in the short term. Several more traditional service-based companies adjusted their marketing strategies to improve their allure to investors and to improve their efficiency of operation. Business-to-

business (B2B) trade began to have strong deflationary Internet effects. Stock-market activity throughout the world increased markedly.

Internationally, there were concerns about Internet transactions leading to the potential loss of sales-related taxes, abrogation of intellectual property rights, on-line and credit-card fraud, failure to provide pre-paid goods, data-privacy, computer virus infections, unsolicited e-mail messages, socially disruptive communications, and forms of intercommunications that diminished the representational rôle of conventional politicians. Science thrived in the Internet environment, however.

Exploitation of the capacity of the Internet was aided by sharp declines in the costs of computer hardware and software, as well as by high-speed Internet access *via* digital subscriber lines, cable modems, and satellite downloading. Antitrust suits were filed in the USA against Microsoft and the Intel Corporation. Mobile telephone access to the Internet stimulated rapid expansion of large sectors of the microelectronics market and telecommunications industry.

Research in 2000 by Goldman Sachs (*The IT Revolution – New Data on the Global Impact*) pointed out that the use of information and communications technology (ICT) in the UK merited an upward revision from 2.9% to 3.43% in business-sector growth over the 1996-1999 period, matching the US experience. Although there is debate about whether there is an ICT-derived 'new economy', ICT investment has been associated with growth in the USA and UK, if not in Japan. Further scrutiny of data for labour productivity growth in the UK, however, reveals a decline from an average of 3.35% *per annum* during 1990-1995 to 1.77% for the 1996-1999 period.

Conflicts and Populations

Defence expenditure during peacetime poses special difficulties for democratic governments, and the competition with civil expenditure can be fraught, not least in the area of research outlays. Historical precedence shows that the sheer magnitude of challenges that have to be addressed during and following periods of conflict, and the effects of conflicts on civilian populations and the economy, serve to demonstrate the wisdom of sustaining a healthy but balanced defence infrastructure that can offer assistance to afflicted countries. During 1999, conflicts occurred in Angola, the Democratic Republic of the Congo (Congo-Kinshasa), and Sierra Leone. Russia embarked on an offensive against Chechnya; the civil wars in Afghanistan and Sri Lanka continued to test the resolve of the people directly involved; border disputes occurred between India and Pakistan as well as between Ethiopia and Eritrea. The civil war in Colombia and offensive manoeuvres between North and South Korea were long-standing conflicts. A UN-organised peacekeeping force was commissioned to curb the ethnic violence, forced migration, and anarchy in East Timor. In Europe, a 78-day bombing campaign was launched by the North Atlantic Treaty Organization (NATO), under the leadership of the USA, against Serbia in an attempt to prevent maltreatment of ethnic Albanians in Kosovo.

Negotiations on nuclear-arms treaties did not progress satisfactorily during the year. The Comprehensive Test Ban Treaty was rejected by the US Senate in October, no further amendments were made to the 1972 Anti-Ballistic Missile Treaty, and the Russian legislature refused to ratify the 1993 Strategic Arms Reduction Talks II Treaty. In respect of non-nuclear arms, the 1997 Ottawa Landmines Convention came into force in March 1999, although landmines were used by ethnic Serbs and Albanians in Kosovo, and by Russia in Dagestan. A new Conventional Forces in Europe treaty was signed at the November meeting of the Organization for Security and Co-operation in Europe.

In April 1999, a summit was held in Washington, USA, to record officially the 50th anniversary of the formation of NATO, welcoming the formal membership of the Czech Republic, Hungary and Poland, and agreeing the new Strategic Concept. Concerns were expressed about the expansion of NATO into Eastern Europe engendering an anti-NATO position by non-participants. A Membership Action Plan for nine aspiring European democracies was also agreed at the meeting. The NATO bombing campaign against Serbia, however, created tension with Russia and China, as it was a conflict based on humanitarian or moral reasons rather than on security reasons, and disregarded the primacy of national sovereignty. By avoiding the use of ground forces and relying on US military capability, the cohesion of NATO was sustained. There was a view that NATO should have received specific UN authorisation, but that would have been unrealistic given the history of UN interventions. In April 1999, only six (Armenia, Belarus, Kazakstan, Kyrgyzstan, Russia and Tajikistan) of the nine signatories to the 1992 Commonwealth of Independent States (CIS) sought to sign the prolongation protocol, whereas Azerbaijan, Georgia, and Uzbekistan refused. Georgia, Ukraine, Uzbekistan, Azerbaijan, and Moldova formed

the loose GUUAM grouping as a counterbalance to the Russian-dominated CIS.

At the beginning of 1999, the number of refugees and people of concern to the Office of the United Nations High Commissioner for Refugees (UNHCR) was around 22m, over 11m of which were refugees as defined by the 1951 UN Refugee Convention and are legally entitled to international protection, and more than 5m were internally displaced persons, and the remainder returnees, asylum seekers, and stateless people. It is estimated that there are around 20-25m internally displaced persons for whom UNHCR does not have a mandate. Most were in Africa, Asia and Europe, half were women and 14% were children under 5 years of age. More than 80% of the 5 200 UNHCR staff were in the field, working in 120 countries. The main donors to the UNHCR were the USA, Japan and the EU.

Approximately 450 000 Sierra Leonians fled the country and sought refuge mainly in Guinea and Liberia. More than 330 000 other refugees returned to Mali and Niger. In the Horn of Africa, there were further displacements and mass expulsions arising from the conflict between Eritrea and Ethiopia. By the beginning of 1999, Sudan hosted 392 000 refugees from Chad, Eritrea, Ethiopia, Uganda, Democratic Republic of the Congo (Congo [Kinshasa] formerly Zaire) and Somalia. Complicated patterns of conflict in the Great Lakes region of Africa accounted for mass displacement of peoples in Congo [Kinshasa] and the Republic of the Congo (Congo [Brazzaville] formerly the French colony of Middle Congo), involving the movement of refugees into Tanzania and northern Zambia. There was also transhumance of refugees from Burundi to Tanzania, and from Angola to Congo [Kinshasa] and Zambia.

In Asia, about 100 000 Karen and Karenni refugees from Myanmar (Burma) remained in camps along the border with Thailand. It was estimated that as a result of the conflict between government forces and the Liberation Tigers of Tamil Eelam, there were 650 000 internally displaced persons in Sri Lanka. In South America, around 1.6m appear to have been displaced within Colombia, and many had fled to neighbouring countries.

Elsewhere, more than 250 000 people were displaced by the Russian forces from Chechnya, many fleeing into Ingushetia. Over 33 000 people were displaced in Dagestan. Well over 1m refugees and displaced people were to be found in the Transcaucasus, principally in Armenia, Azerbaijan, and Georgia. In Spring 1999, 1m people left their homes in Kosovo only to return within 11 weeks. Unsettled conditions in Afghanistan meant that by the end of 1999 there were more than 150 000 internally displaced persons, with thousands of refugees in neighbouring countries.

The USA accepted about 50% (78 000) of refugees resettled by UNHCR; by the end of 1998, Canada hosted 159 000 persons of concern to UNHCR, over 13 000 of whom were accepted as refugees.

With remarkable candour, Kofi Annan, the Secretary-General of the UN, acknowledged in September 1999 deficiencies in both the operations of the organisation and the actions of some of its member states. Much of the inability of the UN to act to prevent large-scale state- or rebel-induced violations of human rights reflected its problems of funding and inefficiencies in some of its associated organisations. It was only by the payment of $824m that the USA was able to save its vote in the General Assembly after it had been warned about its persistent failure to meet its UN Charter obligations. The estimated cumulative debt of the USA to the UN was between $1.2bn and $1.6bn. Japan was in arrears ($506m) as was Germany ($98m).

During the year, the UN abandoned its peacemaking rôle in Afghanistan and virtually terminated its Observor Mission rôle in Angola after the abandonment of the Lusaka Protocol. The UN unsuccessfully interacted with Iraq whilst France, UK and the USA enforced 'no-fly' zones. Likewise, it had difficulty in gaining unanimity for the support of NATO air strikes against Yugoslavia, but the UN High Commissioner for Refugees (UNHCR) tried to repatriate about 900 000 refugees and 600 000 displaced persons although by the end of the year it was not possible to restore the multi-ethnic composition of Kosovo. After the Libyan government surrendered for trial in The Netherlands two of its citizens suspected of involvement in the 1988 destruction over Scotland of Pan Am flight 103, the UN lifted sanctions on Libya. The Security Council created the UN Transitional Administration in October 1999 to run East Timor after the collapse of order in the Indonesian province. Two important failures of the UN were noted by the Secretary-General: (i) allowing in 1995 Serbs to overrun the Bosnian so-called 'safe area' of Srebenica; and (ii) neither preventing the 1994 genocide in Rwanda nor punishing the guilty parties.

Hitherto, in its peacekeeping rôle the UN would wait for the parties to fight to a standstill and then respond to their call to monitor the resulting truce, remaining resolutely neutral throughout. The August 2000 Brahimi Commission Report noted that the standing and credibility of the UN peacekeeping effort in the 1990s was damaged by its failure to distinguish victim from aggressor. In recommending faster deployment of peacekeepers into the field, and switching regular peacekeeping administrative costs to the main UN budget, it was realised that the UN Department of Peacekeeping Operations was trying to function on a budget in 2000-2001 of just $2.6bn, attempting to run 15 different peacekeeping operations, 27 000 soldiers and 9 000 police, with tiny administrative staffing and administration costs of less than 2% of total budget. Kofi Annan in his speech to the General Assembly championed intervention on humanitarian grounds regardless of whether it infringed sovereignty. Questions arose as to who will meet the costs or the responsibility of carrying out the new policy; non-democratic developing countries in turn feared they may come subject to UN intervention.

In view of the fact that many countries have not recently, or have never, taken a population census, global and national population figures are estimates. Accurate current data even for the populations of capital cities are scarce, and the definition of cities and their areas are variable. The UN report *The Sex and Age Distribution of the World Populations*, revised in 1994, provided the following medium variant data for the total population of the world: 1930, 2.07bn; 1960, 3.019bn; 1995, 5.176bn; 2000, 6.158bn; 2010, 7.032bn; 2020, 7.888bn; 2030, 8.671bn; 2040, 9.318bn; 2050, 9.833bn. The increase to date has been made possible by the increased availability of high-quality food, and improved healthcare. Further analysis of the data shows great divergence in the continental areas that are expected to bear the brunt of the population increases between 2000 and 2050. Africa will expand from 832m to 2.14bn; Asia 3.754bn to 5.741bn; Latin America, including Mexico and the remainder of the American south of the USA, 524m to 839m; North America 306m to 389m, and Oceania 31m to 46m. In contrast, the population of Europe is projected to decline from 730m to 678m.

Agriculture and Food

During 1999-2000, world agricultural markets were subject to pressures ranging from greater competition as a result of increases in food production, persistent but declining effects of the economic problems affecting many parts of Asia and Russia, decreasing food aid, the declining political and economic influence of agriculture in both MDCs and LDCs, the growing rôle of the World Trade Organization (WTO) in loosening regulation of agricultural trade, changes to the agricultural policy of the EU, continuing substantial support payment to US farmers, and highly publicised consumer-related trading issues such as BSE, GM crops, the USA-EU 'banana war', and American beef treated with hormones.

Analyses by the UN Food and Agriculture Organisation (FAO; http://apps.fao.org; FAO *Production Yearbook*; and the online FAOSTAT database http://apps.fao.org/default.htm) revealed increases in the crucial indices of total agricultural and food production (Table 2), although the increase in food output was less than the rise in global population so that *per capita* food production declined slightly.

Regarded by many as a debacle and failure, the Seattle meeting of ministers from the 135 member countries of the WTO, during 30 November to 3 December 1999, was disrupted by protestors unwilling to accept a new round of negotiations to liberalise international trade. Presaged by the Uruguay Round, agriculture was a focus for exporting nations seeking access to importation markets mainly by the elimination of subsidies that distort trading and ratcheting down the controls exerted by state trading agencies. There were opposing views as to whether agricultural products

	Total agricultural production					Total food production					*Per capita* food production				
	1995	1996	1997	1998	1999	1995	1996	1997	1998	1999	1995	1996	1997	1998	1999
World	109.0	113.2	116.0	117.4	118.7	109.8	114.2	116.9	118.8	120.0	102.0	104.6	105.7	106.0	105.7
MDCs	94.5	97.3	98.8	97.5	98.1	94.9	98.0	99.4	98.5	98.8	92.6	95.2	96.3	95.1	
LDCs	120.9	126.4	130.3	133.9	135.8	122.5	128.0	131.8	136.1	138.1	112.2	115.3	116.8	118.7	118.6

Table 2 Indices of World Agricultural and Food Production in More-Developed Countries (MDCs) and Less-Developed Countries (LDCs). (1989-1991=100).

should be treated the same as other traded products, and subject to the same rules. Both the EU and Japan overtly subsidise and protect their agricultural sectors, the USA less so, but members of the Cairns Group offer minimal-to-zero market support. The USA and EU represent the two largest agricultural trading blocs. At least, the WTO ministers agreed that existing tax moratoria on sales over the Internet should be extended for a further 2 years. The WTO talks resumed in Geneva in April 2000, where members again failed to agree on who should chair the agricultural liberalisation talks over the coming year.

As a result of the collapse of the Seattle WTO meeting, and deferral by the US Congress of the bilateral China-USA negotiations, the accession of China to the WTO was under threat, especially because of the tight regulatory control China imposed on agricultural products.

Estimates of production, utilisation and ending stocks of the major agricultural commodities usually require amendment as trading data are released. The following accounts must be considered in concert with my previous Annual Reports.

In 1999-2000, the US Department of Agriculture (USDA) revised previously released data and forecast that the production of wheat, coarse grains (barley, maize, millet, oats, rye, and sorghum) and milled rice was 1 856m metric tonnes (mmt), down from production levels of 1 871 mmt in 1998-1999. Utilisation of cereals for livestock feed use, for human food, and for non-food industrial feedstocks, rose from 1 854 mmt in 1998-1999 to an estimated 1 859 mmt in 1999-2000, with most of the increased utilisation attributable to the consumption of milled rice. Minimal declines in grain ending stocks were expected, from 347 mmt in 1998-1999 to 345 mmt in 1999-2000. Stocks remained at 19% of utilisation.

Wheat production declined from 589 mmt in 1998-1999 to 584 mmt in 1999-2000, irrespective of near-record harvests in Argentina, Australia and Canada. Production levels declined in the other major producing nations, and wheat ending stocks fell to 131 mmt. Global rice production increased from 392 mmt in 1998-1999 to 396 mmt in 1999-2000, but trading levels and prices in the commodity were expected to decline as various Asian countries reduced their imports, so that ending stocks rose from 57 mmt in 1998-1999 to 59 mmt in 1999-2000. World production of coarse grains was expected to fall from 890 mmt in 1998-1999 to 876 mmt in 1999-2000, matching utilisation levels, and with world trade expected to shrink, prices were expected to be lower than originally projected.

The global production of the seven major oilseeds (soybean, cottonseed, groundnut or peanut, sunflower seed, rapeseed, copra, and palm kernel) was forecast by the USDA to rise from 293.6 mmt in 1998-1999 to 296.9 mmt in 1999-2000. Declines in soybean and groundnut production were offset by an increase from 36.7 mmt in 1998-1999 to 42.7 mmt in 1999-2000 in rapeseed production, chiefly in Canada, China, India, and the EU. There were increases in the production of vegetable oils and high-protein meals, and record utilisation of oilseeds and their products meant that there was increased world trade and consequently lower ending stocks (27.6 mmt). Nonetheless, prices remained weak and subject to competitive pressures from cereal products.

Data from the World Health Organization (WHO) on the number of tobacco smokers provide the scale of the challenge to implement the organization's anti-smoking treaty. World-wide, there were 1.142bn smokers, the bulk of whom reside in the East Asia and Pacific zone (401m), Eastern Europe and Central Asia (148m), Latin America and Caribbean (95m), Middle East and North Africa (40m), South Asia (86m cigarettes and 96m biris). A proposed framework convention on tobacco control, due for adoption in 2003, will state objectives for reducing tobacco use, and will have legally binding protocols on specific measures such as advertising, sponsorship, health warnings, taxation, and reduced production subsidies – a special point in respect of CAP policies.

The Environment

Expectations that the Kyoto Protocol would become fully operational by early 2001, with functional compliance-monitoring regimes and commencement of technology-transfer mechanisms for the benefit of LDCs, were downgraded following the talks held in Bonn in June 1999, where it was agreed to submit a series of draft texts to the next Conference of Parties to the UN Convention on Climate Change in November 2000 covering the clean development mechanism, emission trading, joint implementation and apportionment of blame. At, and following the UN Conference on Environment and Development (the Rio Conference) in 1992, the UK alongside 180 other countries signed and ratified the Framework Convention on Climate Change, and intended to

reduce the risks of global warming by limiting the emissions of 'greenhouse' gases. The 1997 Kyoto Protocol to the Convention was signed by 84 parties, but only ratified by 22 to date. Under the Protocol, MDCs agreed to cut emissions of greenhouse gases (the six main gases are carbon dioxide, methane, nitrous oxide, hydrofluorocarbons, perfluorocarbons, and sulfur hexafluoride) by 5.2% below 1990 levels by 2008-2010. EU members agreed to an 8% reduction, but the target set for the UK is 12.5%. Phenomenal complications in weather patterns caused by variable solar activity, volcanic eruptions, local warming arising from growth of towns and cities, large-scale vegetational (habitat) modifications, El Niño and La Niña events *etc*., when viewed against known records of 'extreme' weather events, make it difficult properly to assess anthropogenic effects on modifying weather by altering the gaseous composition of the atmosphere. Predicting the impacts of climate change and attempts to curtail greenhouse-gas emissions are fraught with difficulty, especially in judging over time the buffering and reservoir capacity of the seas, oceans, and terrestrial vegetation, and the ability of ecological habitats to adjust to environmental perturbation. In the previous Annual Report, I referred to the breathtaking naïvety of attempts by the international community to stabilise climate change and consequential ecological change by adjusting a few variables, *i.e.* certain atmospheric emissions. Such an observation does not detract from the need for vigorous pursuit of energy efficiency, renewable energy sources and elimination of pollution, however, and it may well be possible to address the problem of 'holes' in the ozone layer.

Under the auspices of the UN Environment Programme, the Intergovernmental Negotiating Committee for an Internationally Legally Binding Instrument for Implementing International Action on Certain Persistent Organic Pollutants (POPs) agreed that production of eight of the 12 POPs should cease when the Treaty comes into force in 2002 or 2003. Thus, aldrin, chlordane, dieldrin, endrin, heptachlor, mirex, toxaphene and hexachlorobenzene would be banned. Against the wishes of the World Wide Fund for Nature, DDT would be permitted as an antimalarial insecticide. Polychlorinated biphenyls would continue to be allowed only in electrical equipment, but the position of dioxins and furans was unclear.

In September 1999, a multipollutant protocol to the UN Economic Commission for Europe Convention on Long-Range Transboundary Air Pollution was agreed, setting limits for emissions of ammonia, nitrogen oxides, sulfur, and volatile organic compounds, all of special relevance to agriculture. Initial indications were that 32 European countries would agree to the unmodified protocol, and Canada and the USA would agree to a modified version. Both the European Commission and several northern European states sought unsuccessfully for tougher action at a diplomatic meeting of the EU Council of Ministers later in that month.

A specific target of Greenpeace and environmental activists, the Brent Spar episode finally came to an end with its conversion to a quay rather than being dumped at sea. Contrary to impressions given by environmentalists to the public and politicians, the $68 million conversion involved a net energy cost slightly more than double that of the ocean-disposal option. Moreover, only 150 tonnes of oil were in its tanks, considerably less than the 5000 tonnes claimed by Greenpeace.

Pressure continued to be exerted on the nuclear industry, regardless of the claims that viable alternative sources of energy would lead to greater enhanced emissions of greenhouse gases.

Sea-surface temperatures that were colder than usual were detected in 1999 across the eastern and central Equatorial Pacific Ocean, triggering abnormally active weather patterns and various extreme events. This *La Niña* (the Little Girl) was associated with a pronounced hurricane period during June to November in the Caribbean and Atlantic basins. Evidence is accumulating to support the view that the frequency of *El Niño* events that are associated with *La Niña* events are increasing in frequency as a result of global climate change (see www.elnino.noqq.gov). Coral core records indicate that during the 19th Century, *El Niño* occurred every 10 to 15 years, but since the late 1970s, it has shifted to a four-year cycle. The year 2000 was predicted to be one of the hottest years on record. During the *El Niño* climate cycle, there is a weakened atmospheric circulation in the Pacific Ocean, triggered by higher-than-usual sea-surface temperatures, so that lighter, warmer waters flow eastward to the American coastline, replacing cooler, upwelling nutrient-rich waters that give rise to abundant fisheries such as those off the coast of Peru. Surface atmospheric pressure tends to be high over Australia and low over the southeastern Pacific, leading to weak trade wind circulation. The effects of *El Niño* are felt world-wide, wreaking havoc with weather patterns in the Americas, Africa, and Asia.

In September 1999, the UN General Assembly held a meeting called by the 42-member Alliance of Small Island States to discuss the danger posed by rising sea levels and increasingly violent and erratic weather associated with global-warming trends.

By acceding in 1999 to the Antarctic Treaty, Venezuela brought to 44 the number of nations that have agreed to use the region south of 60°S latitude for peaceful purposes only. The Treaty entered into force in 1961, and is essentially a managerial mechanism that encourages scientific research and cooperation, enforces and monitors environmental protection, and defers the contentious issue of sovereignty. Non-consultative, non-signatory nations are urged to adhere to the Treaty's new (1998) Protocol on Environmental Protection. Unregulated fishing was addressed in 1998 by member nations of the Antarctic Marine Living Resources Convention. Nearly 16 000 tourists were expected during the 1999-2000 season, many of whom were attributed to welcoming in the new millennium. There was intense scientific interest in Lake Vostok, an ancient and pristine body of water akin to Lake Ontario in area and depth, deep beneath the ice of East Antarctica. By stark contrast with the Antarctic, no treaty regime applies to the Arctic, nor agreed definition of a zone that may be described in terms of physical location (north of the Arctic Circle, latitude 66°30' north), botanic (above the northern limit of the tree line), climatic (above the 10°C July isotherm), or cultural (region encompassing the circumpolar cultures). Portions of seven nations (Canada, Finland, Greenland (part of Denmark), Norway, Russia, Sweden, and USA) are regarded as the Arctic zone, together with the Arctic Ocean. The land area consists of permanent ice cap, tundra, or tiaga, and the total population was estimated in 1999 to be 375 000.

In *Trade, Global Policy, and the Environment*, (World Bank Discussion Paper No 402, 1999, see also http://www.worldbank.org/) P.G. Fredriksson and associates attempted to analyse in detail the empirical links between trade and the environment, the 'pollution haven' hypothesis, and economic instruments for resolving global environmental problems. There appears to be a consensus that more open trade improves growth and economic welfare, and that increased trade and growth without appropriate environmental policies in place may have unwanted effects on the environment. Three 'effects' were considered. The 'scale' effect refers to the fact that more open trade creates greater economic activity, demanding greater inputs (*e.g.* raw materials, transportation, energy) and if existing technologies are deployed to increase outputs, then there is an increase in emissions along with depletion of resources. The 'composition' effect relates to changes in the relative size of the various economic sectors and sub-sectors following a reduction in trade barriers. Freer trade leads to countries specialising in sectors where they have a competitive advantage where they may have relatively abundant factors (minerals, labour *etc.*). Lax environmental regulations lead to polluting or resource-depleting industries. The 'technique' effect refers to changes in production methods that follow trade liberalisation. From a global perspective, free trade results in a more efficient use of resources. These three effects have both local and global implications.

In studies on trade, growth, and environmental and health effects, computerised general-equilibrium models were used to predict the environmental impacts of trade liberalisation. In the case of Indonesia, the damage caused by trade liberalisation was estimated to be only a fraction of the damage that normal projected economic growth and structural change would cause by the year 2020 if trade and environmental policies remained unchanged. In other studies, not surprisingly, there are widely differing trade and growth effects, and these environmental and health effects, of differing liberalisation programmes, *e.g.* linking with different trade blocs, or undergoing unilateral or complete liberalisation, select specific sectors for action, or restrict the extent of reforms. Such factors determine the scale, composition and technique effects.

In the debate as to whether economic growth leads to improved welfare, there are four key questions. Does pollution follow a 'Kuznets' curve, an inverted-U relationship, first rising and then falling as income increases? At what income level does the turn-around occur? Do all pollutants follow the same trajectory? Is pollution reduction in LDCs due primarily to structural change, or to regulation? H Hettige, M Mani and D Wheeler, in a fascinating article in the Discussion Paper, tested for a Kuznets effect by measuring the effect of income growth on three proximate determinates of pollution: the share of manufacturing in total output; the sectoral composition of manufacturing, and the intensity (per unit of output) of industrial pollution at the 'end-of-pipe'. Manufacturing share followed a Kuznets-type trajectory; the other two determinants did not. Sectoral composition became 'cleaner' through middle-income status and then stabilised. At the 'end-of-pipe', pollution intensity

declined strongly with income. On the basis of recent trends in water pollution in the OECD, the Newly Industrialised Countries, Asian LDCs and the ex-COMECON economies, the authors concluded that industrial water emissions level off in richer economies because pollution intensity has an elastic response to income growth. Unitary elasticity, however, implies that total emissions remain constant unless other factors intervene. Industry tends to deconcentrate or relocate over time as infrastructure and prosperity spreads, but the combined existence of seriously polluted waterways in prosperous MDCs would show that the Kuznets-type hypothesis does not always hold true.

Trade liberalisation strongly impacts on agriculture, horticulture and forestry, and in a case study in Kenya, preferential horticultural trade between Kenya and the EU had little effect on land use or Kenya's rural environment, but safeguarded marginal, survival-level producers. In the Sahel, distortions in paraffin (kerosene) and petroleum (gasoline) prices had almost no impact on the rate of woodland degradation.

Weak environmental regulations could be expected to lead to pollution-intensive industries ('pollution havens'), particularly in LDCs. Studies would indicate that factory locations and trade patterns do not appear to be strongly affected by environmental regulations, as LDC production is mainly focused on domestic markets. Sometimes, policy-makers compensate firms subjected to regulations (*e.g.* the 'Polluter Pays Principle' rarely hold true in agriculture), and stock markets, even in LDCs, are significantly affected by reports of environmental performance. The question of trade sanctions against 'free-riders' in international environmental agreements (*e.g.* the Montreal Protocol) is germane whilst there is no supranational enforcer. Political drag and regulatory chill in environmental policy-making invariably reflect on trade assessments by individual countries, but the existence of healthy democracies enforces the shouldering of global responsibilities. Environmental policies that involve taxation could well amplify existing tax distortions, just as subsidies to pollutors (such as intensive livestock agriculture and energy producers) distort the speed of addressing environmental pollution. There were also detrimental effects of weak environmental regulations on public finances, market prices, and competition. Fortunately, capital markets enforce environmental performance, where there are concerns over legal liability and damage to reputations. Not all polluters are stock-market listed, however: sobering as it is, publicly owned organisations and governments, especially in the centrally controlled economies, have been some of the worst-ever global polluters.

Nominally, the UN Environment Programme (UNEP) is the world's focal point for environmental concerns, the first UN body to be based in the developing world, in Nairobi. It was designed to act as a repository and disseminator of information, and co-ordinator of international responses. After a period of decline in the 1990s, the *Global Environment Outlook 2000* UNEP document offers a return to a central rôle, but this is now in jeopardy with proposals for the creation of a World Environment Organisation, and also with confusion over UNEP's interface with the Commission for Sustainable Development, based in New York. At the Montreal Biodiversity Conference in January 2000, the draft Cartegena Protocol on Biosafety was passed, and finalised at a meeting in Nairobi in May 2000. The protocol will take effect within 90 days after having been signed by 50 nations. In that it has the purpose to ensure public safety, maintain biodiversity, and protect and utilise organisms by providing guidelines for international trade and their use in foods, feed and processed goods, it will have a dramatic effect on biotechnology R&D, and the biotechnology industry. It is the first international treaty to incorporate the 'precautionary principle', rather than manage hazards by requiring hard scientific proof and qualification of risks; the precautionary principle insists that potential environmental risks should be dealt with even in the absence of any scientific certainty. Some regard the protocol as unworkable because it is too vague, legally and scientifically, leading to arbitrariness and vulnerability to industrial or environmental lobbyists. It is also regarded as an impedance to progress (see Plant Biotechnology section).

UK Perspectives

Reform of the House of Lords continued with the loss of right of the 750 hereditary peers to sit in the upper house. Interim arrangements were put into place whereby 92 hereditary peers were elected by their fellow hereditary peers to sit alongside the 580 life peers, bishops, and law lords. The future and constitutional rôle of the House of Lords remained unclear.

Under a system of proportional representation, elections were held in May 1999 for a new Parliament for Scotland, and a National Assembly for Wales. In Scotland, a Labour-Liberal Democrat coalition administration was formed; in Wales, a Labour minority administration was established. Elections in June to the European Parliament also involved the proportional representation voting system, which favours minority and marginal parties. A turnout of only 23% was taken by many to be a symptom of large-scale euroscepticism.

In December 1999, the Northern Ireland Assembly assumed wide powers of self-government, some nine months later than presented in the 1998 Good Friday Agreement. The failure of paramilitary groups to decommission arms essentially destabilised the executive, a position exacerbated by various actions which were regarded as offensive to the unionist and nationalist traditions.

Within the UK, environmental protection is sustained by over 50 international conventions and over 300 European Directives, plus the implementation of UK legislation through the Environment Agency, the Scottish Environment Protection Agency, and the Environment and Heritage Service for Northern Ireland. The EU-based effort is based on the Fifth Environmental Action Programme, *Towards Sustainability*, which was adopted in 1992 and is due for replacement by the Sixth Environmental Action Programme in 2001 if agreement can be reached. Sustainable development is an elastic term, but is widely defined to incorporate social and economic development as well as environmental development, *i.e.* development that meets the needs of the present generation without compromising the ability of future generations to meet their own needs. The first UK national sustainable development was published by Government in 1994, and the first set of indicators in 1996. The latest strategy, *A Better Quality of Life*, was published in May 1999, and contained 15 headline indicators backed by a further 150 indicators, against which progress will be measured in both the public and private sectors. Under Local Agenda 21, which was derived from the Rio Conference in 1992, Local Authorities will be expected to adopt sustainable development strategies by the end of 2000. A draft UK climate-change programme was published in March 2000, proposing a 20% cut in carbon dioxide emissions by 2010; a climate change levy to be introduced in April 2001; carbon trading, energy efficiency and management measures; reducing pollution and vehicular congestion, better countryside management and reduction in the use of fertilisers. A separate climate-change programme is envisaged for Scotland. Conservation of the natural environment through legislative enforcement was considered through both UK and EU routes. The UK was deemed to have been deficient in designating sites of international interest for birds – Special Protection Areas, and for other species – Special Areas for Conservation. Atlantic oakwoods, bogs and salmon rivers were omitted from the UK listing which is intended to form part of a Europe-wide network of conservation sites, termed Natura 2000. Moreover, Greenpeace won a legal ruling to have marine Special Areas for Conservation included within the UK's exclusive 200-mile economic zone and not only within the 12-mile territorial waters. Extra protection of Sites of Special Scientific Interest (SSSI) was one of the main features of the Countryside and Rights of Way Bill wending its way through Parliament, but the proposals to give public rights of access to moorland, downland, and registered common land caused disquiet in rural communities and wildlife bodies. Government-backed development of the tidal mudflats in Cardiff Bay to produce a marina was completed in November 1999. In so doing, the SSSI-designated mudflats were destroyed, removing a major over-wintering site for migratory birds, and incurring criticism from the EU. A nearby nature reserve on the Gwent Levels was thought to be inadequate as a replacement.

Although research and development (R&D) are regarded as subsets of science, engineering and technology (SET), R&D in the UK accounts for the overwhelming proportion of public expenditure on SET. Much of the remaining SET expenditure is directed towards scientific and technical postgraduate education and training, and technology transfer. Civil R&D spend is apportioned either in the funding directly from Government departments – as in the

case of SERAD's support for the Scottish Agricultural and Biological Research Institutes including SCRI – or through the Higher Education Funding Councils (HEFCs) and the Research Councils (RCs) such as the Biotechnology and Biological Sciences Research Council (BBSRC). The HEFCs support education, training and research at universities, whereas the RCs sponsor R&D both at academic institutions and other research bodies, although institutions are not eligible to apply for virtually all of the HEFC and RC spends. HEFCs funding is a component in the Vote of the Department for Education and Employment in England and Wales, and its equivalent north of the border, whereas the RCs are financed by the Office of Science and Technology through the Science Budget or Science Vote. These two funding streams constitute the Dual Support System.

Using the base year 1997-1998, government-funded R&D declined in real terms from £6.32bn in 1995-1996 to £6.12bn in 1998-1999, but is set to increase to £6.79bn in 2001-2002. Further analysis of the figures shows that the R&D spend in the civil departments, as opposed to the universities and RCs in support of the 'science base', is set to decline from £1.28bn in 1997-1998 to £1.17bn in 2001-2002, continuing a trend that has been evident since the late 1980s.

In the *Forward Look* (Cm4363, July 1999) issued by the UK Government, the three main thrusts of public expenditure were to (i) ensure that British science is and continues to be world class; (ii) support the exploitation and development of existing and new technologies, particularly through encouraging partnerships and the flow of people between universities and business; and (iii) support Government departments in meeting their policy commitments – hence the phrase "evidence-based policy". In their *Fifth Report*, the House of Commons Select Committee on Science and Technology recommended that in future editions of *Forward Look*, there should be another objective – to generate useful knowledge and inventions, both as a contribution to enhancing the competitiveness of the UK economy, and as a stimulus through innovation to wealth creation and improved quality of life. The *Fifth Report* was hard-hitting, noting deficiencies in the latest Foresight programme, expressing anxiety over the decline in civil department's R&D investment, recommending the creation by the Government of a sustainable environment to encourage investment by industry in R&D, and concluding that departmental arrangements be amended to improve the status and impact of SET.

The Government's science White Paper produced by the Department of Trade and Industry (DTI; http://www.gov.uk/ost/aboutost/dtiwhite/.html) recognised that innovation is the motor of the modern economy, requiring a combination of scientific excellence, the right climate, competitive markets, public confidence and incentives to flourish.

Bibliometric analysis during the period 1981-1998 demonstrated that the UK's record for scientific analysis for scientific research and excellence is second only to the USA, and the UK leads the world in the life sciences. According to the Office of Science and Technology (a sub-set of the DTI), with only 1% of the world's population, the UK is responsible for 4.5% of the world's spend on science, yet produced 8.2% of the world's scientific papers, received 9.2% of world citations, and claimed around 10% of internationally recognised prizes steadily throughout the last century. In 1998, exports *per capita* of high-technology goods and services were the highest of the G7 countries. The key proposals of the White Paper were (i) a £1bn programme in partnership with the Wellcome Trust to renew the infrastructure for science; (ii) a £250m spend in key new areas - genomics, e-science, and basic technology such as bioengineering, nanotechnology, and quantum computing; (iii) improved remuneration for research students; (iv) launch in partnership with the Wolfson Foundation and the Royal Society an initial fund of £4m to assist in the recruitment of up to 50 top researchers; (v) raising the profile of science to young people; (vi) facilitating the creation of networks linking public and private-sector bodies, and researchers and industries; (vii) provide the best framework for scientists and businesses to make international links; (viii) establish a Higher Education Innovation Fund of £140m over 3 years; (ix) launch a new Foresight fund initially up to £15m; (x) run a further round of the University Challenge Competition; (xi) create new Regional Innovation Funds of £50m a year, support 50 Business Fellows and, inspired by the US Small Business Innovation Research Fund, introduce a Small Business Research Initiative; (xii) change the rules for Government-funded research so that all research bodies own the Intellectual Property Rights, encourage incentives and risk-taking by staff, and provide £10m to commercialise public-sector research; (xiii) Government to act to the highest standards as a regulator, implementing stronger guidelines on how scientific advice should be used in drawing up Government policies and committing scientific advisers to Government to

adopting high levels of openness and transparency in their work.

With regard to the future of its excellence in science, the UK is witnessing the fall in numbers of graduates in physics, chemistry, chemical engineering, and technology.

To ensure that applied work and interdisciplinary collaboration are not constrained by the Research Assessment Exercise (RAE) carried out by the Funding Councils, there will be a combination of monitoring the outcome of the next RAE in 2001 and the launch of the Higher Education Innovation Fund mentioned above. Science and technology are central to gaining competitive advantage, but many UK industries invest less in R&D than those in competing countries. The pharmaceutical sector is the most important innovative, high-technology industry in the UK, accounting for nearly 25% of high-technology R&D investment compared with an OECD average of 8%. This performance, however, could be imperilled by animal rights activists and legislative and regulatory changes that impede the progress of research.

In a much-welcomed and constructive response to the Baker Report on Public Sector Research Establishments (PSREs; see *Creating Knowledge-Creating Wealth: Realising the Economic Potential of Public Sector Research Establishments* Published 1999 – available at www.hm-treasury.gov.uk/docs/1999; and *The Government's Response to the Baker Report* – available at www.hm-treasury.gov.uk/pubs/html/docs/main.html), the Government accepted the thrust of the recommendations, and specifically implemented changes, including (i) prompt changes to the civil service conduct rules to allow government scientists new incentives and rewards, subject to safeguards, for participating fully in exploitation and entrepreneurialism *e.g.* through equity in spin-out companies; (ii) tackling the well-known risk-avoidance culture in PSREs, encouraging well-managed risk-taking; (iii) addressing the need for advice to help commercialise their discoveries and inventions; (iv) all relevant departments and RCs, in partnership with PSREs, to produce timetabled action plans for ensuring that PSREs can effectively pursue knowledge-transfer activities; (v) ensure that PSREs are able to participate fully in governmental schemes which incentivise the transfer of knowledge to industry; (vi) ensure that the next Prior Options Reviews of PSREs fully address the recommendation that departmental PSREs are put at greater arm's length from government.

Despite frequent reports in the media and accounts of anti-science and anti-technology activists, the White Paper noted that over two-thirds of people in Britain agreed that science and technology are making our lives healthier, easier and more comfortable, over three-quarters agreed that scientists and engineers make a valuable contribution to society, and 84% agreed that they were amazed by the achievements of science. That around 14 to 27% disagreed or neither agreed nor disagreed with any of the three propositions might be deemed to be an expression of ignorance or an inability to accept the pace of change.

Legislation and regulation rapidly introduced by forceful demands in the media (*e.g.* the statistically unjustifiable ban on beef-on-the-bone, the unenforceable requirement to label genetically modified food in restaurants, excessive spending and accountability controls in the public sector *etc.)* essentially pander to a risk-averse society, ironically as symptoms of greater open government, and lack of trust in regulators and scientists. Simply to remove the bulk of legislation, however, let alone retard wholesale the introduction of new legislation, would expose ministers, civil and public servants, to the vagaries of bad publicity as well as adversely affect vulnerable consumers and weaker members of society. One challenge for government regulation comes in its interface with a single dominant company or organisation in a prominent area of activity, to ensure 'public interest', however that is defined. Government wishes neither to be the manager nor adversary of the business, if the confidence or private-sector investors is to be maintained. There is a large body of informed opinion that the establishment of a robust competition framework provides a suitable mechanism for dealing with dominant or monopolistic organisations, and where absolutely necessary, assets can be retained in the public sector but their operations can be transferred to competing private companies. This argument can be turned and extended to research provision where the public sector has until recently become a virtual monopoly addressing public-sector interests and industry-sector, but nowadays most advances in computing, telecommunications and biotechnology are being made in the private sector, and new forms of interlinking are required.

One of the most important and succinctly written documents on UK agriculture is the annual account *Agriculture in the United Kingdom* produced by the Ministry of Agriculture, Fisheries and Food, the Scottish Executive Rural Affairs Department, the Depart-

ment of Agriculture and Rural Development (Northern Ireland), and the National Assembly for Wales Agriculture Department. (See also www.maff.gsi.gov.uk/esg and *Basic Horticultural Statistics for the UK*.) Provisional Data in the calendar year 1999 edition indicated that the contribution of agriculture to the total economy gross value added (GVA), at current prices £6.991bn), declined from 1.0% in 1998 to 0.9% in 1999, following a long-term trend of decline; in the period 1988-1990, the average contribution was 1.5%. About 2.1% (593 000) of the UK workforce was employed in agriculture, a figure that omits many groups whose employment is dependent on primary production, such as many employees in the public sector, food-processing, and industrial-feedstock industries. Importation of food, feed, and drink amounted to £17.269bn, compared with £17.198bn in 1998, and amounted to 8.8% of total UK imports. Imports of alcoholic drinks amounted to £1.787bn for the EU and £0.757bn for the rest of the world. Statistical factors have been introduced to devalue processed imports to the value of their unprocessed food content. This has reduced the estimated value of food imports, which in turn reduced the estimated value of food consumption, and thus UK food production as a percentage of UK food consumption has increased. Exports of food, feed, and drink declined from £9.246bn in 1998 to £8.715bn in 1999, of which alcoholic drink contributed £1.081bn to the EU and £1.658bn to the rest of the world. These agriculturally related exports amounted to 5.3% of total UK exports, down from 5.6% in 1998, and an average of 6.1% in the period 1988-1990. The UK was 68.4% self-sufficient in all food types in 1999, compared with 67.3% the year before, and an average of 73% in the period 1988-1990. For indigenous-type food, however, the UK was 81.6% self-sufficient; the level for 1998 was downgraded from 82.3% to 81.4%. Household final consumption expenditure on household food and alcoholic drink at current prices was up from a revised £84.196bn in 1998 to £85.3bn in 1999; yet again, there was an astoundingly high proportion of that spend – over 36% - devoted to alcoholic drinks (£31.2bn)! Household food and alcoholic drinks accounted for only 15.2% of total household final consumption expenditure. Domestic food expenditure alone was only 9.7% of total household expenditure compared with 5.6% for alcoholic drinks.

In June 1999, the total area of agricultural land, including common rough grazing, was 18 579 000 hectares, of which 4 709 000 hectares were devoted to crops, and 33 000 hectares were left fallow. In the period 1988-1999, an average of 18 932 000 hectares, were committed to agriculture, 5 135 000 hectares of which were harvested for crops. More detailed analysis of the cropping data reveals that the area devoted to cereals declined from a revised figure of 3 420 000 hectares in 1998 to 3 141 000 hectares in 1999, mainly attributable to increased compulsory set-aside (raised from 5% to 10%) leading to declines in the wheat area from 2 045 000 hectares to 1 847 000 hectares, and in the barley area from 1 255 000 hectares to 1 179 000 hectares. The potato area increased to the area grown in 1996 of 178 000 hectares. Other arable crops, excluding potatoes, were grown on an increased area of land, up from 1 192 000 hectares in 1998 to 1 211 000 hectares in 1998. This increase was accounted for by the dramatic increase in the area devoted to linseed, up from 99 000 hectares in 1998 to 209 000 hectares in 1999. In contrast, there were declines forecast for oilseed rape, down from 506 000 hectares to 417 000 hectares; sugar beet not for stock feeding, down from 189 000 hectares to 183 000 hectares; and peas for harvesting dry and field beans, down from 213 000 hectares to 202 000 hectares. The area of land for horticulture was 179 000 hectares, closely similar to the 1998 value of 178 000 hectares. Of this area, the bulk was attributable to vegetables grown in the open (126 000 hectares, up from 123 000 hectares in 1998); with 28 000 hectares committed to commercial and non-commercial orchards; 13 000 hectares for ornamentals; 9 000 hectares for soft fruit including wine grapes; and 2 000 hectares for glasshouse crops.

Without taking account of direct subsidy payments, the average price of agricultural products fell by 4% between 1998 and 1999, and inputs fell by 1.8%. The average price of agricultural products has fallen by 25% over the last 4 years from a peak in 1995. Illustrating the pressure on producers, the average price of agricultural products is 11% lower than 10 years ago, whereas the average price of inputs has increased by 13%.

In terms of production, despite the 8% decline in cereal growing area, overall production of cereals fell by 1.3% to 22.5mmt. The value of production fell by 6.1% to £2 349m. Provisional cereal yields in 1990 were 8.12 tonnes per hectare for wheat, 5.74 for barley, and 6.17 for oats. Wheat production declined from 15.47 mmt in 1998 to 15.11 mmt in 1999, valued at £1,545m. Barley, one of SCRI's mandate

crops, recorded a small increase in production from 6.63 mmt in 1998 to 6.67 mmt, but the value of production declined over the same period from £397m to £330m. Oat production declined to 0.575 mmt with a value of £57m.

Potato production in 1999 sharply increased from the 1998 trough of 6.417 mmt to 7.1 mmt, valued at £755m. In 1995, the crop which is a key mandate crop for SCRI, was valued at £1 088m. Potato seed production, including farm-saved seed, increased from 404 800 tonnes to 427 600 tonnes. Oilseed rape production was forecast to have increased from 1.57 mmt to 1.667 mmt with a production value of just £356m, compared with £407m the previous year's crop. Sugar beet production in 1999 was adjudged to have been 10.328 mmt, adjusted at standard 16% sugar content, and was valued at £291m. Linseed was clearly seen as a financially safer planting option than crops such as oilseed rape, and production rose dramatically in line with the doubling in planting area from 143 000 tonnes to a provisional 295 000 tonnes with a value of £129m of which 79% was accounted for by subsidy payments.

In horticulture, vegetables grown in the open in 1999 on an estimated total area of 148 500 hectares were valued at £646m, and £317m for protected crops grown on an area of 1 200 hectares. The highest valued horticultural commodities were mushrooms (£173m), lettuces (£102m), carrots (£94m), tomatoes (£67m) and cabbages (£65m), followed by peas (£50m) and cauliflowers (£38m). Orchard (top) fruit production on an area of 25 300 hectares was valued at £108m, and soft fruit at £132m on an area of 8 900 hectares, mainly attributable to two crops of importance to SCRI, strawberries (£84m) and raspberries (£36m). Raspberry production in Scotland continued with its long-term decline to around 2 400 tonnes. In the UK, the planted hectares of raspberries has declined to 2 355 with a yield of 5.6 tonnes *per* hectare; in Scotland, the yield is even lower at 3.9 tonnes *per* hectare. Ornamental production on 18 700 hectares was valued at £682m, compared with figures of production on 19 200 hectares valued at £659m in 1998. The value of production in 1999 was attributed to £371m for hardy ornamental nursery stock, £273m for protected crops, and £37m for flowers and bulbs in the open.

MAFF estimated that the measure 'Total Income From Farming' (TIFF), which is acutely sensitive to relatively small percentage changes in the values of outputs and inputs, was broadly unchanged from the 1998 value, at £2 339m, though considerably less than the 1997 figure of £3 193m or of £5 279m in 1995. Paid labour costs in 1999 were estimated to be £1 963m, slightly less than in 1998. Net Value Added at Factor Cost is one of the best measures of value added by the industry because it includes all subsidies on production (some are not included in output *e.g.* set-aside and agri-environment payments), but makes no allowance for interest, rent or labour costs. In 1999, it was estimated to be £5 110m, a fall of 1.9% from the previous year. TIFF is derived by deducting interest, rent and paid labour costs from Net Value Added at Factor Cost. It was because interest payments were 13% lower than the previous year, and both rent and labour costs were slightly lower, that TIFF was largely unaffected. According to the accountants Deloitte and Touche, farmers have experienced an effective 90% drop in income over the past 5 years and can expect to see heavy losses in 2001. Thus, the average farmer would register a profit of £8 000 in 2000, but a loss of £4 000 in 2001, equivalent to a loss of £22 *per* hectare. The main factors were adjudged to be rising fuel costs, the strength of sterling relative to the euro, and the failure of retailers to pass on profits to the producers. Across the EU, there was a 4% drop in income in 1998-1999 from agriculture, but the most pronounced falls occurred in Ireland (-13%), Denmark (-11%), Spain (-8%), Belgium (-7%), The Netherlands (-6%), and Germany (-5%), whereas increases were posted in Luxembourg (+5%), Sweden (+6%) and Portugal (+14%). Within the UK, the TIFF figure of £2 339m comprised £1 989m for England, £239m for Scotland, £71m for Northern Ireland, and £40m for Wales. Agriculture's share of total regional gross value added at basic prices was 0.9% for the UK, 0.9% for England, 1.3% for Scotland, 2.4% for Northern Ireland, and 1.1% for Wales. In terms of the share of total regional employment, agriculture accounted for 2.1% in the UK, 1.8% in England, 3.1% in Scotland, 8.5% in Northern Ireland, and 5.2% in Wales.

The total UK public expenditure on agriculture in 1999-2000 was forecast to decrease by about £310m to £3.1722bn. Of this, spending under the CAP heading was forecast to decrease from £3.193bn in 1998-1999 to £2.924bn in 1999-2000, of which 42% was apportioned to the arable area payments scheme, 16% to beef and veal (non-BSE measures), 13% to sheep meat, 11% to beef and veal (BSE measures), 5%

to milk, 5% to sugar, and 2% to cereals.

Among the numerous agricultural policy developments in 1999 were attempts to reform the CAP throughout the Agenda 2000 negotiations. The limited changes should deliver annual net savings to the UK economy of approximately £200m in 2001 rising to £500m in 2008. Under the EU Rural Development Regulation (RDR), the 'second pillar' of the CAP, plans were submitted to the European Commission for England, Scotland and Wales. The RDR integrated framework supports agri-environment schemes, less-favoured areas, woodland planting and management, and structural adjustments in the farming industry. Complex economic, environmental and social factors surround the implementation of schemes relating to modulation; cross-compliance; set-aside; area aid compensation; intervention prices; commodity payments for cereals, oilseeds, fibre, flax, hemp and sugar; payment windows; structural funds; the wine regime; the agrimonetary system; marketing schemes; environmentally sensitive area schemes; countryside and rural stewardship schemes; organic farming scheme; habitat scheme; farm woodland premium scheme; countryside premium scheme; Tir Gofal (Land in Care); nitrate sensitive areas scheme; nitrate vulnerable zones; farm waste grants; producer organisation funds; short-rotation coppice establishment grants *etc*. This is further complicated by a tranche of livestock-related schemes.

During 1999-2000, the first statutory review of the British Potato Council (BPC) took place, involving a consultation exercise, an economic evaluation, and a poll of levy payers. Established to commission or undertake R&D, collect and disseminate statistical information, promote potatoes on the home market, and develop export opportunities, the BPC occupies a pivotal position connecting industry and academia. Confirmation of its continuation was widely welcomed by the R&D community. Likewise, following statutory review, the continuation of the Horticultural Development Council (HDC) was announced for another term of 5 years. The HDC commissions near-market R&D on behalf of the horticulture industry, but excluding the producers of apples, hops and pears. For both levy boards, meeting the needs and perceptions of the growers is a tough challenge during a period of low profitability.

In November 1999, the Bill to establish the Food Standards Agency (FSA) received Royal Assent as the Food Standards Act 1999. The FSA is a non-Ministerial government department accountable to Parliament through Health Ministers and to their devolved equivalents. It provides the primary source in Government of advice on food safety and standards, taking a strategic view of policy across the whole food chain and operating at arm's length from Ministers and the devolved administrations. Regional executives were appointed in Scotland and Wales. Formed largely as a response to the BSE and *E.coli* O157 debacles, the FSA is designed to operate with a presumption of openness, addressing issues of food safety, public confidence and risk. Its four main functions will be to (i) develop policy and provide advice and information to Ministers, devolved administrations and other public bodies; (ii) provide advice and information to the public and other interested parties; (iii) commission research and surveillance and keep itself informed of relevant developments; (iv) set standards for and monitoring of food law enforcement. Sir John Krebs, the eminent scientist, was appointed Chairman. At some time in the future, the relationships between the various food-related agencies in the EU, and their rôles in international trade will come under close scrutiny, especially in the development of policies that are proportionate to the real risks involved.

Plant Biotechnology

Biologists are now entering an exciting phase of research, the post-genomic era of investigating the functional analysis of genes and their direct and indirect products. Functional genomics employs a battery of approaches including (i) the creation of stable and transient-expression transgenic organisms; (ii) phage display systems, for example, searching domain repertoires; (iii) procedures for studying protein-protein interactions, particularly *via* transcriptional activation of one or several reporter genes; (iv) high-throughput gene expression profiling at the transcript level (DNA microarrays); (v) differential display; (vi) serial analysis of gene expression; (vii) protein composition, configuration and levels proteomics; (viii) gene trap methodology; and (ix) bioinformatics and computational genomics. DNA microarray analysis enables the survey of thousands of genes in parallel, and is deployed in expression monitoring, polymorphism analysis, and, to a limited extent, sequencing. Proteomics is a term that encompasses all the methods that analyse patterns of gene expression at the protein level, *i.e.* the proteome – the complete set of proteins encoded by

the genome, including the set of proteins expressed both in time (*e.g.* development and disease status) and space (location). Two approaches tend to be adopted: the expression model and the cell-map model.

Regardless of the rapid advances in structural and functional genomics, proteomics and 'metabolic profiling' (the latter is a term A M M Berrie, B A Knights and I employed in 1972 at Glasgow University as we embarked on extensive analyses of plant extracts using combined gas chromatography – mass spectrometry; the modern jargon is 'metabolomics'), genetically modified (GM) crops and GM foods agitated politicians, pressure groups and the media, "organic" growers, and beekeepers. By the end of 1999, most supermarket chains withdrew foods containing GM ingredients. In October, the Government announced that no new GM foods would be allowed on sale in the UK before 2002. Even though biotechnology clearly encompasses R&D and products that transcend transgenic organisms and their products, the issue of GM crops and GM foods dominated biotechnology-related industrial, commercial and public-sector research agendas in Europe generally, and the UK in particular.

"In this book, I have tried to show that the scientific attitude has a well-defined rôle in the dialogue between the possible and the actual. The seventeenth century had the wisdom to introduce reason as a useful and even necessary tool for handling human affairs. The Enlightenment and the nineteenth century had the folly to consider it to be not merely necessary but even sufficient for the solution of all problems. Today, it would be still more foolish to decide, as some would like, that because reason is not sufficient, it is not necessary either. Yet, while science attempts to describe nature and to distinguish between dream and reality, it should not be forgotten that human beings probably call as much for dream as for reality. It is hope that gives life a meaning. And hope is based on the prospect of being able one day to turn the actual world into a possible one that looks better." François Jacob, The Possible and the Actual. 1982

In the authoritative *Brief Global Status of Commercialized Transgenic Crops: 1999*, by C. James, from the International Service for the Acquisition of Agri-Biotech Applications (see www.isaaa.org and also www.agbiotechnet.com), the following points were made that illustrate the expanding influence of transgenic technologies in agriculture: (i) Despite mainly European-based resistance to GM crops, the global area of transgenic crops increased in 1999 to 39.9m hectares, a 44% increase over the 1998 level. (ii) Seven transgenic crops (soybean 59% of area; maize 23%; cotton 10%; oilseed rape/canola 8%; potato; squash and papaya) were grown in 12 countries (USA, 28% of total; Argentina 6.7%; Canada 4.0%; China 0.3%; and smaller areas in Australia, South Africa, Mexico, Spain, France, Portugal, Romania and Ukraine). By 1999, 30% of the global area devoted to soybean was down to transgenic soybean, 14% of the oilseed rape/canola area was transgenic, 10% of the cotton area, and 8% of the maize area. (iii) The dominant transgenically introduced traits were herbicide tolerance, insect resistance, and stacked genes of insect resistance and herbicide tolerance. (iv) The transgenic seed market was estimated to reach a value of $2.7-3.0bn in 1999. (v) Expansion of transgenic crops is anticipated, particularly in LDCs, but public acceptance in MDCs (especially the EU) will be influential in shaping market demand, introducing regulatory processes such as food labelling and farming procedures, and thereby affecting commodity prices. (vi) Phenomenal advances in genomics-based R&D, plus a wide range of new transgenic products in the R&D pipeline, emphasise the huge potential of transgenic crops (especially wheat, rice and maize), to address the major challenges facing mankind in such countries as China and India, as well as generate new wealth-creating markets and address quality of life issues. (vii) Governments have a crucial rôle both to implement regulatory programmes that inspire public confidence and to exert leadership in communicating information on transgenic biology.

Throughout the debate on GM crops, the production of improved or superior cultivars/varieties that have desirable input and output traits continue to present the greatest challenge to global agriculture, and figure as the prime target in international foresight programmes on agriculture and horticulture. In countries such as China, where a fifth of the world's population is dependent on just 7% of the world's cultivated land area, as well as in other LDCs, there is no current viable alternative to investigate the potential of transgenic crops and trust that conventional agriculture continues to perform as well as it does. Nonetheless, the theoretical risks of transgenic technology have been aired to such an extent that the EU invoked the 'precautionary principle'. On the basis that there is insufficient scientific evidence to conclude that there is no risk to consumers from trans-

genic crops or products derived from them, imports of certain GM crops have been prohibited, and commercial transgenic crops plantings largely brought to a halt. The fact that transgenic crops and their products pose no greater a risk to health and the environment than conventional or 'organic' agriculture has not persuaded the EU and most of the member states to adopt a more sophisticated approach to the GM public-perception issue, and put in place regulatory and monitoring systems that are trusted by the public. At the end of February 2000 in Edinburgh, Sir John Krebs closed the OECD GM-food Conference in Edinburgh, noting that current GM releases had so far shown no ill-effects on human health. Nonetheless, as new and more sophisticated GM foods come on stream, the mechanisms needed to approve these foods must be enhanced, modified and improved. The concept of 'substantial equivalence', coined by the OECD in 1993, would have to be reviewed. The conference was attended by about 400 scientists, regulators, NGOs and representatives of consumer organisations, the food industry, and pressure groups.

In Hunger Site 2000 (http://www.thehungersite.com) it is reckoned that in LDCs, 24 000 people die each day from chronic malnutrition. Livestock and crop performance at a global level are grossly inadequate. With regard to food crops, the scientific targets are numerous: increased yield; better water use efficiency; better nutrient-use efficiency; improved resistance to biotic stresses (viruses, bacteria, fungi, nematodes, insects, weeds *etc.*); improved tolerance of or resistance to abiotic stresses (temperature, salinity, aluminium toxicity, drought *etc.*); delayed senescence/ripening; enhanced quality (proteins, lipids, carbohydrates, minerals, vitamins *etc.*); improved harvestability; diminished allergens or toxins; better taste, texture and appearance; greater uniformity; and improved storage and processing qualities. Similar targets apply to non-food and dual-purpose crops, such as cotton (see Table 3). Many such non-food crops tend to be low-yielding and place heavy demands on inputs that have deleterious environmental effects. Access to freshwater, just 0.007% of all the water on earth, is the major limiting factor to crop production. In fact, agriculture accounts for 93% of global consumption of water through rainfall or irrigation. According to the World Meteorological Organization (*Comprehensive Assessment of the Freshwater Resources of the World*, 1977), two-thirds of the world's population could be facing water shortages by 2025.

Mention should be made of such advances as the work of I Potrykus and P Beyer who demonstrated in rice the incorporation of several genes simultaneously that code for β-carotene, the precursor of vitamin A, and the joint work between the University of Florida and Monsanto Inc. in incorporating the gene for glutamate dehydrogenase derived from *Chlorella sorokiniana* into wheat, leading to greatly increased soil nitrogen use efficiency. In fact, the recent achievements of the maize, rice and wheat breeding programmes have been prodigious. This gives optimism for overcoming the current annual genetic gain in cereal productivity of less than 1% *per annum*, subject to overcoming impediments such as market-distorting subsidies and regulations, underfunded agricultural R&D projects, malfunctioning public-private partnerships *etc*.

A convoluted set of inter-company relationships and overlapping technologies in the agricultural genomics industry coupled with mergers, acquisitions, alliances, spin-out companies as well as intense legal activity over intellectual property ownership and rights, have collectively presented an awkward face to investors, to NGOs, and to those opposed to biotechnology. Consequently, the year was one of crop biotechnology facing shrinking investments, an irony when the scientific potential has never been so great, and future food-security needs so pressing.

For the poor, and those that represent them and act on their behalf, the benefits of agricultural biotechnology require a measure of 'freedom to operate'. Given population pressures, the steady loss of cultivated land, diminishing access to freshwater, falling commodity prices, and reduced support for the publicly funded network of research bodies in MDCs and LDCs, the poor farmers in LDCs can only but contrast their lot in life with the technologically dependent rich in their own countries and elsewhere. Such is the impact of modern communications technology in raising expectations. M S Swaminathan has made the point that India has over 16% of the world's population, and 15% of its farm animals, but occupies only 2% of the land area and receives only 1% of its rainfall. Despite being largely illiterate, India's farmers have harnessed new technologies, saving India from mass starvation, but the scale of the demand for food is increasing remorselessly, and India needs to double food production in the next 10 years to ensure food security.

Of the various concerns – biological, social, economic, ethical, political – about agricultural biotechnology, the issue of risk and benefit comes to the fore. Do the

benefits apply only to the few? What are the risks of not applying the technology? What are the known and theoretical risks of applying the technology to health and to the environment? What acceptable alternatives are there? What are the risks and benefits when the issue is driven by the publishing and broadcast media? Is it possible to insure against risks to people, property and the environment ('common goods')? What are the risks and benefits of a patenting system or UPOV system and can they be combined? What are the risks of having monopolistic suppliers of seed – public or private?

These questions must be placed alongside the analyses of the International Food Policy Research Institute (IFPRI) in *The World Food Situation : Recent Developments, Emerging Issues, and Long Term Prospects*, 1997. Increases so far in world food production have actually kept in advance of the increases in global population. Even so, the growth rate of world agriculture has declined from 3% in the 1960s to just 2% in the last decade; aggregated projections with reasonable initial and modestly optimistic assumptions, indicate that the world food supply will continue to outpace world population growth at least to 2020. The *per capita* availability of food is projected to increase by about 7% between 1993 and 2020. As G J Persley in *Agricultural Biotechnology and the Poor* (CGIAR and the US National Academy of Sciences, 2000) points out, therein lies a paradox. Firstly, despite the increasing availability of food, currently around 0.8 billion of the global population are food-insecure, with children and women the most vulnerable to dietary deficiencies. Secondly, food insecurity is remarkably prevalent at a time when, for various reasons, global food prices are in decline. A F McCalla pointed out in 1998 that, in the period 1960-1990, world cereal production doubled, *per capita* food production increased by 37%, calories supplied increased by a similar amount, yet real food prices fell by almost 50%. Persley noted that the basic cause of the paradox is the linkage between poverty and food security, *i.e.* access to food depends on income, at a time when according to the 1997 report of the World Bank, *World Development Indicators*, more than 1.3bn people in LDCs are classified as absolutely poor, with incomes per person of $1 a day or less, with another 2bn people only marginally better off. In the LDCs, most of the population depend on agriculture and devote their energies and income on food.

K M Leisinger, in the 1999 IFPRI report *Biotechnology for Developing Country Agriculture*, and in *Agricultural Biotechnology and the Poor* mentioned above, considered ethical changes of agricultural biotechnology for LDCs, and attempted to distinguish between technology-inherent and technology-transcending risks. The former are essentially biosafety risks where they relate to health and the environment; the latter emanate from the political and social context in which the technology is used *i.e.* not risks specific to the technology but where its deployment may carry certain risks (*e.g.* reducing biodiversity, increasing poverty gaps between and within societies, adversely affecting trade).

Possible areas of concern over GM crop technology in respect of human health cover potential toxicity, carcinogenicity, food intolerances, use of antibiotic-resistance gene markers, potential allergies, and unintentional modification of nutritional value. There are no clear cases of harmful effects of authorised and released GM crops and food products derived from them, on human or livestock health. Nonetheless, as with any technology, including long-established plant breeding, any potential risk is addressed by considering any potential release on a case-by-case basis.

Food allergens have exercised many in the GM debate, oftentimes quoting the well-publicised study of J A Nordlee, S L Taylor, J A Townsend, L A Thomas and R K Bush in 1996 (*New England Journal of Medicine* 334, 688-692) in which a gene encoding a Brazil nut methionine-rich seed-storage protein was introduced into soybean. It was because the protein was derived from a well-known allergenic source, and that serum and skin tests confirmed the presence of the allergen, that the development of the modified soybean was discontinued and not allowed into the marketplace. Food allergies, *i.e.* adverse immunologically mediated reactions to antigen molecules in foodstuffs, affect about 2% of adults and 4% to 6% of children, and are mainly attributed to exposure to four animal foods (eggs, fish, shellfish, milk) and four plant foods (peanuts/groundnuts, soybeans, wheat, tree nuts), but other major foodstuffs such as chicken, oriental and yellow mustard, tree and grass pollens, latex, apples *etc*. contain known allergens. The allergens are proteins or glycoproteins with an acidic isoelectric point and molecular masses in the range 10,000 to 80,000 daltons, and tend to be resistant to food processing and digestive enzymes. The concern that GM crops might specifically cause allergies is not supported by evidence. In fact, GM technology (antisense RNA) has been used by T Matsuda, A M

Alvarez, Y Tadce, T Adachi and R Nakamura in 1993 to reduce the expression of a major allergen found in rice. Also, very detailed studies by S B Lehrer and C Reese in 1997 (*International Archives of Allergy and Immunology* 113, 122-124) demonstrated in modified soybeans that, qualitatively and quantitatively, the transgenic high-oleate strain appeared to be allergenically the same as the parental wild-type despite the fact that the levels of several proteins were elevated. To date, there is no cause for concern about the allergenic potential for proteins introduced into foods from sources with no history of allergenicity. There is no room for complacency, however, and from the work of H A Simpson, S L Taylor, and R L Fuchs in 1996 (*Critical Reviews in Food Science and Nutrition, IFBC/ILSI* 36(s), 165-186), there is now a safety-assessment multipartite decision-tree for assessing the allergenic potential of foods derived from GM crops, beginning with the characterisation of the source of the introduced gene, and assuming that genes transferred from sources known to be allergenic, encode for one or more allergens unless proven otherwise. This framework for risk assessment involves assessment of introduced proteins in the context of their known history of allergenicity, similarity of their amino acid sequences to known allergens, the ability of the proteins to be digested, and their level of expression. In assessing and attempting to minimise risks, at this juncture there are no specific peculiarities of transgenic technology that do not also apply to conventional breeding. Moreover, allergy is a disease more frequent among the middle- and upper- income classes in MDCs than in the LDCs, where it is not a major factor in health and nutrition of the population.

Another contentious issue is the extent to which GM crops introduce ecological and environmental risks. R J Cook of Washington State University, in *Agricultural Biotechnology and the Poor*, mentioned above, observed that there is no evidence of any crop species having become invasive weeds because of plant breeding. He reinforced the fact that there are no new unique issues in the testing of GM crops, emphasising that the same protocols used to assess the environmental effects of GM plants equally apply to plants derived from conventional plant breeding – both in the past and currently. It is the product rather than the process that should be evaluated. Among the new risks envisaged and imagined outwith the normal range of sexual compatibility include the potential for spread of traits through outcrossing (gene transfer – the resultant hybrids may alter the population dynamics of species in natural habitats giving rise to 'genetic pollution'); induction of difficult-to-control weediness; the inadvertent selection of pesticide resistance in insects and nematodes populations (super-pests); a reduction in biodiversity caused by weed-free monocultural systems; the creation of new pathogens through recombination of viruses or virus components (in plant viruses, genomic variation caused by remarkable levels of mutation is amplified by recombination events of great complexity, pseudo-recombination [genome segment reassortment in multipartite genomes], and acquisition of extra nucleic acid components); pleiotropic effects including formation of allergens and toxins, the inadvertent expression of genetic material from pathogens will cause uncontrolled hypersensitive responses in susceptible species, tantamount to a 'genetic disease'; and human pathogenic microorganisms incorporating antibiotic resistance from antibiotic-resistance marker genes in the first generation of GM crop releases.

Not only are there strange concepts over 'ownership' or 'belonging' of genes to certain species, or even over what is considered to be 'conventional' breeding, there would also appear to be the erroneous view that species and ecosystems are genetically static. Gene flow occurs at various rates in all ecosystems. Virtually all agriculture and horticulture – including domestic gardening – involve the use of alien species, sometimes leading to the introduction of new pests and diseases, but most often the introduced species reaps the benefits of growing in environments lacking the depredation of the pests and diseases from their centres of origin. During the past century, thousands of new cultivars have been introduced into global agriculture and horticulture. Conventional plant breeding has reduced rather than increased (i) the tendency for crops to become weeds, (ii) the level of anti-nutritional and toxic compounds, (iii) erratic dormancy, (iv) traits harmful to non-target organisms, frequently to the point of decreasing the competitive ability and increasing the vulnerability of crops to pests and diseases, (v) variability or pleiotropy, through rigorous screening and statutory testing. In those cases where theoretical or actual gene flow occurs, the fundamental questions distil down to 'so what?' and 'how can it be controlled?' Conventional agronomic practices provide environmental risks in respect of the effects of replacing native flora and fauna with monocultures, modifying the water and nutrient states of the soil, tillage disturbance, modification of the soil microflora

and microfauna by crop rotations, use of pesticides that disrupt flora and fauna, and large-scale disturbance to natural gene flow patterns, including migratory pathways. Erosion and loss of *circa* 0.3% of global cultivated land (approximately 1.5bn hectares), per annum caused by a combination of urban encroachment and poor agronomic practices, mean that the efficiency of production on the restricted land area must be raised without further imperilling natural and semi-natural habitats, ecological refugia and dispersal corridors for flora and fauna. The very essence of good agronomic practice, with both conventional and GM crops, is to reduce risks, *e.g.* prevention of gene flow from outcrossing with wild or crop relatives by the use of herbicides, crop rotations, establishing minimum distances between crops, harvesting before flowering, or not growing the crop. Crop pests and diseases are controlled by the cultivation of resistant varieties and/or pesticides. Such measures ensure sustainability of the cropping system, and may require monitoring by independent authorities, not least in the European context where there is a sensitised public and body politic, concerns over agricultural sustainability, and realisation that a higher proportion of land is farmed in Europe than in the USA, meaning that what is deemed to be 'biodiversity' must be allowed to thrive in the farmland area, alongside and within crops. In many instances, advances in modern agricultural engineering advances mean that weed-infested crops can be cleaned and separated post-harvest, opening up the possibility of using diverse crop mixtures rather than monocultures. Biotechnological approaches also allow biodiversity to be monitored and quantified.

As far as technology-inherent risks are concerned, there are no demonstrable adverse effects from the cultivation and consumption of current GM crops, but precautionary biosafety guidelines and protocols for assessing risks on a case-by-case (crop-by-crop, gene-by-gene) basis are available from the OECD, UN Environment Programme, UN Industrial Development Organisation, the World Bank, and the EU, offering science-based hazard identification and risk assessments. Straightforward, non-pejorative regulatory frameworks will need to be devised with each new generation of transgenic material, with sunset clauses operating in these cases where crops and products have been demonstrated to be of very low risk.

Scientists have difficulty in addressing technology-transcending risks and ethical issues in the deployment of transgenic crops, regardless of using such terms as 'genetically improved' crops. There is no doubt that potential food-safety and environment risks have been grossly and sometimes obscenely over-publicised as a vehicle to draw attention to technology-transcending and/or 'ethical' and 'moral' issues of concern, and it is interesting to compare the public treatment of mobile telephones with that given to GM crops, in respect of perceived risks, acceptability, and benefits. As in information technology and computing, the major advances in biotechnology have been driven by the private sector which requires various degrees of exclusivity, sometimes confidentiality, in order to function competitively in the marketplace. This alone raises particular challenges for public-sector bodies in MDCs and LDCs to establish constructive relationships with the private sector.

Oppressive intellectual property rights; restricted access to foodstuffs; the neglect of medicines, dietary nutrients and crops crucial for LDCs (the so-called low-profitability 'orphan' products and crops); focus on just one essential technology to the exclusion of other, more conventionally but less financially attractive technologies; pressure to downgrade regulatory processes; market policies which are overtly not pro-poor even to the extent of not boosting the economies of LDCs; and the perceived lack of consumer benefits have all been raised as reasons to retard the advance of biotechnology. The CGIAR system (see Table 1) agreed in 1998 to a statement of ethical principles underlying the use of biotechnology by its various Centers. Emphasis was placed on its objectives, transparency of operation, commitment to fairness, honesty, integrity, intellectual rigour, accountability, and precautionary approaches to genetic resources. Interestingly, the statement made reference to the fact that the CGIAR is guided by its particular humanitarian and equity-based concerns, and not to the pursuit of knowledge for its own sake. Fundamental concerns about the sacred and inviolate nature of life forms and their ownership ('core values') are more problematical for scientists knowledgeable about genes, functioning in a pluralistic society, and cognisant of perpetual human intervention in food production, environmental modification, and the domestication of pets, livestock and crops. Blatant disregard for such perceptions and sensitivities would be wholly unjustified, however, given the exquisite sophistication and unquantifiable value of all life forms, and the fascination they hold for all biologists. For some members of society, biotechnology represents an example of the unacceptable rapid rate of progress and societal pres-

sure, with alien vocabulary and techniques. Hitherto, society has evolved almost imperceptibly in tune with its foodstuffs and cultivars – but this is no longer the case, nor can it be. Whereas it is justifiable to argue that the unfettered pursuit of knowledge and understanding is intrinsically good for humanity and the progress of scholarship, it is my firm view that scientists must be interested in and concerned about moral, ethical and social issues that result from their studies, and be willing to comment publicly on them. As science, engineering and technology are increasingly shaping the development of civilisation, human interrelationships and the environment, there is now unprecedented analysis of science, the attitudes of scientists, and their responsibilities for the applications of their work, often without the participation of scientists themselves. Elements of originality, professionalism, integrity, pragmatism, rationality, responsibility, erudition, openness, independence, strength of character, and determination to function for the betterment of humanity, are all required by scientists, overarching any short-term functions or bending to political or popularity pressures, regardless of discomfiture caused.

Related to ethical aspects of ownership is market-place ownership and the concept of intellectual property (IP) as categorised by plant variety rights, patents, trademarks, copyright, designs, licences, and trade/commercial secrets. All categories of IP offer market advantages and certain disadvantages, some more than others. Patents, above all, exercise consumer groups, anti-capitalists, and those representing the poor. On the one hand, patents provide the owner of that patent with monopoly commercial rights to the invention for a limited period, usually not exceeding 20 years, and only in the country that grants that patent. This offers the owner an opportunity to recoup R&D costs, legal, manufacturing and marketing costs, plus the opportunity to make a profit. Without the prospects of monopoly rights and the incentive to make profits, expensive-to-develop technologies would never enter the marketplace. It is the special feature of industrialised economies that they have rigorous IP regulations, which in turn provide a main driver to the operation of capital markets, safeguarding the rights of patent owners and incentivising technological developments. The monopoly position achieved by the patent holder is at the price of public disclosure of the invention to enable other scientists, engineers and technologists to use the invention in their research, and for the public and society to benefit from the acquired knowledge. Membership of the WTO enables a minimum-level measure of harmonisation of legal frameworks across national borders under the Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPs), which should start to apply to relevant LDCs by 2005 and, in this regard, the World Intellectual Property Organisation will provide technical assistance and training. In the area of genomics-based biotechnology, there are certain developments that will be the subject of a large period of legal debate, exacerbated by a complex of overlapping patents. Much hinges on Article 27 of TRIPs which provides for the availability of patents for any inventions (products, processes, systems *etc.*) in all fields of technology provided that they are new and not obvious, involve a demonstrable inventive step, and not least, are capable of industrial application. The legal costs of sustaining a dormant IP portfolio cannot be justified in public-sector bodies, other than to ensure the exploitable availability of the technology and to prevent a monopoly position from being taken in vulnerable LDCs. Certain provisional patents in biotechnology would appear to be discoveries rather than inventions, and some are obvious and may be formulated to inhibit the patent positions of others: such may be the basis of future patent challenges.

Human ingenuity and considerable expenditure can be involved in isolating and patenting naturally occurring genes, but if specific uses/functions cannot be ascribed for those genes, especially in broad-sweeping claims that overlap with other patents (a feature of the US Patent Office), then there must be doubt as to the validity or longevity of such patents in the international arena. There have been claims that the agricultural biotechnology industry is transforming into a small number of multinational companies that could monopolise world food supplies. At present, though, around 70% of the world seed trade is controlled through the public sector. Nonetheless, for three of the major staple crops (maize, wheat, potato), more than 3000 out of a total of 3500 patent applications for gene sequences have been filed by nine companies (Affymetrix, AstraZeneca, Aventis, Danisco, Dow, Du Pont, Monsanto, Novartis and National Starch). There is obviously a fundamental difference between a patent application and a patent awarded, and even after being awarded, expensive defence of the patent may be needed. To this uncertainty must be added the effects of delaying market releases, reducing the opportunity even to recoup costs.

One of the potential downsides of IPR enforcement

for transgenic organisms and biological materials containing genetic information is their ability to self-reproduce, but molecular fingerprinting techniques for monitoring and policing purposes provides a valuable tool for detecting both transgenic and conventionally-bred organisms and their transgenic-gene-linked products. For LDCs, concerns have been expressed about the patent system at levels *viz*: (i) increased costs to cover royalties, alone considered to be a factor in prompting the tendency to avoid IPR strictures; (ii) jeopardised existing indigenous industries, products and services; (iii) hidden constraints on local R&D efforts regardless of the fact that the patent system should not infringe the freedom to use patent information and products for research purposes, indeed IPR enforcement will give confidence to donors that IP will be safeguarded; (iv) misgivings and misconceptions over the loss or theft of indigenous natural resources including know-how by MDCs (see *The Commercial Use of Biodiversity. Access to Genetic Resources and Benefit-Sharing* by K ten Kate and S A Laird, a report for the EC published by Earthscan 1999) contravening the provisions of the Convention on Biological Diversity; (v) lack of information about patents being issued, and the costs and complexities of challenging patents internationally – concerns shared by MDCs; (vi) specific technologies such as genetic use restriction technologies or even biotechnology itself could be regarded as unethical or immoral absolutely, or just unethical and immoral when used in a capitalist context. Fortunately or unfortunately, depending on the philosophical standpoint taken, the market-place will decide. Knowledge comes at a price.

As discussed in previous SCRI reviews, the UPOV convention has been widely regarded as a reasonably efficient mechanism for dealing with the entry and use of conventionally bred cultivars in the marketplace. The rights of breeders, researchers and farmers are protected. Transgenic modification of existing cultivars to address certain market niches needs a fresh appraisal, and review of the interrelationship with patent law and rewards to the IP owner. Of greater potential concern, but necessary for orderly development of most capital markets, is the existence of commercial secrecy arrangements which could lead to cartels and suppression of essential knowledge. Balancing the rights and needs of individuals, companies, the state, and trading blocs, is awkward, but the increasing tendency for scientists of all affiliations to be more conscious of the wealth-creating possibilities of their work, places special responsibilities on them to ensure that high professional standards are applied.

A particularly useful report by H Ross and D Tennant *A Definitive Guide to GMOs, Genetically Modified and Novel Foods in the EU. The Law and Technology of GMOs in Europe*, Monitor Press, 2000, highlights ill-conceived, piecemeal EU legislation regulating GMO release, GM food and GM additions; it also considers claims for functional foods and the relationship of UK producers with EU legislation. The current legal situation is complex, trying to cope with 'concerns', 'fears', risks and hazards, often taken to extreme. The furore over the accidental sowing in the UK during the Spring of 2000 of Canadian-sourced GM oilseed rape seeds served to emphasise the gulf that separates North American and European perceptions of GM technology. In May 2000, the Tokyo Grain Exchange became the first exchange in the world to launch a futures index specifically for non-GM soy bean futures as a reaction to Japanese processors refusing to use GM products.

In the UK, the *Food Safety Act 1990* implements the EU-wide obligation to maintain acceptable food safety. As given in previous Annual Reports, the deliberate release of live GM organisms into the environment *e.g.* by cultivating a GM crop, either for experimentation or for releasing GM products into the marketplace, is governed by *Council Directive 90/220/EEC*, implemented in the UK through the *Environmental Protection Act 1990* and the *Genetically Modified Organisms (Deliberate Release) Regulations 1992*. As is well-known at SCRI, a protracted process is involved in notifying the Competent Authority (in the UK, the Department of the Environment, Transport and the Regions, other departments and agencies, and the Advisory Committee for Releases into the Environment) of the intention to release a GMO into the environment, by submitting a detailed technical dossier specifying the GMO, its physiological, pathological and ecological characteristics and the potential for gene flow, descriptions of the receiving environment, monitoring and emergency controls, and the personnel involved. Accompanying the technical dossier is a statement regarding background information and potential impacts and risks of the release on human health and the environment. Summaries of GM-release notification are forwarded to other EC Member States. Often, further information is sought and enquiries may be held, leading to difficulties in scheduling seasonally dependent activities. A notice of the intention to release a live GMO into the environment must be published in the relevant local news-

papers, and notified to owners of the site, Scottish Natural Heritage (or the Nature Conservancy Council in England, or the Countryside Council in Wales) and other agencies if the proposed release might affect controlled waters. The conditions of release, when permitted, are inviolate. Criminal proceedings can be initiated by the Competent Authority if the terms of consent are breached. There is a continuing duty of notification if any new relevant information comes to hand. Usually, there are conditions placed on land use and monitoring post-harvest. It is hoped that simplified procedures will be introduced for those GMOs which are of known low risk and/or hazard, and/or are repeat releases.

Consent at EU level is required to market any product containing GMOs. For products that will not be eaten, an extensive dossier is needed, including constraints on the conditions of use, handling, labelling and packaging. *Directive 97/35/EEC* introduced compulsory labelling for all new agricultural products containing GMOs notified under *Directive 90/220/EEC*, but the amount of information needed for mixtures of GMOs and non-GMOs is limited and there is no enforcement of segregating GM crops at source. Unlike the environmental-release phase, other Member States can impede the market-release phase until qualified majority agreement can be reached, and EC Commissioners or national Ministers can in any case delay placing their signatures on documents. The concept of a continuing duty to notify Competent Authorities of new information on health and environmental matters still applies. Blocking action by Austria, Italy, Luxembourg, and others, on the marketing of GM products should be placed before the European Court of Justice, otherwise the WTO may be needed to resolve a potential trade war with those countries exporting GM goods. In principle, current processes fulfil the cautious species-by-species, gene-by-gene approach advocated by SCRI, but the mechanisms are a bureaucratic contortion.

Should GM crops or products be sold as foodstuffs or be used as an ingredient in other foodstuffs, then a further application must be made in the UK under the *Novel Food and Novel Food Ingredients Regulations 1997* to address any potential food safety issues. This UK statutory instrument relates to the hastily introduced *EU Novel Foods Regulations : Regulations (EC) No 258/97* which also applies to products (foods and food ingredients) other than GMOs. It encompasses products that have not been used to any significant (but not quantified) degree for human consumption in the EU; foods containing GMOs as defined in *Directive 90/220/EEC*; food and ingredients produced from but not containing evidence of GMOs; food and ingredients with new primary or molecular structures; food and ingredients consisting of or isolated from microorganisms including fungi and microalgae; food and ingredients from plants and animals other than those with a history of safe use and produced by traditional or conventional methods; food and ingredients involving novel processes and where significant changes to that food may occur. There are various exclusions, *e.g.* flavourings, processing aids such as enzymes and various additives, and extraction solvents. *Recommendation 97/618/EC* details the scientific requirements needed to support an application which will be dealt with by the Competent Authority, other Member States and the European Commission, and the Standing Committee on Foodstuffs. Lack of confidentiality, anti-competitive trade practices, overburdening bureaucracy, and wanton lack of velocity have been levelled at the EU processes. In the UK, applications are considered by the Advisory Committee on Novel Foods and Processes (ACNFP) which may consult other bodies. New legislation has been drafted (the *Novel Foods and Novel Food Ingredients (Amendment) (England) Regulation 1999)*, with parallel proposals for Scotland and Wales, opening up further the deliberation of ACNFP to public scrutiny and comment.

The existence of GM products already in the marketplace (*e.g.* Monsanto's Round-up Ready soybean, *Commission Decision 96/281/EEC*; and Novartis' Bt Maize, *Commission Decision 97/98/EEC*) before the introduction of *Regulation (EC) No 258/97* led to the introduction of *Regulation No 1813/97* and *Regulation 1139/98*, specifying certain contradictory and anomalous labelling requirements. Negative lists; threshold levels; the type of wording; exclusions; options available to Member States; the incompatibilities that exist between legislation, enforcement and demands by pressure groups and retailers; and the difficulties facing the food producing and processing industries in interpreting rapidly evolving law collectively need to be resolved.

GM additives have attracted special attention. *Regulation (EC) No 50/2000* came into force in April 2000, specifying labelling requirements for foods containing additives and flavourings that have been genetically modified or produced from GMOs. The concept of 'substantial equivalence' is crucial, for labelling is not

required if the additives or flavourings are equivalent to their traditional counterparts in such aspects as composition, nutritional values, metabolic and physiological effects, and intended uses of the product. Clearly, the presence of transgenic DNA or protein would rule out equivalence, and due allowance must be made for natural variation. New and powerful analytical technologies arising from developments in sequence analysis, proteomics and metabolomics will assist in assessing compositional variation.

'Nutraceuticals', 'functional foods', health foods and drinks, food supplements, and medicines interdigitate to some extent scientifically, socially and legally, a situation which is likely to become commercially and legally active with the widespread introduction of GM technology. Central to European deliberations in this sector is *Directive 65/65/EEC* which defines a medicinal product as any substance or combination of substances presented for treating or preventing disease in human beings or animals. A medicinal product is also defined by the Directive as any substance or combination of substances which may be administered to human beings or animals with a view to making a medical diagnosis or to restoring, correcting or modifying physiological functions in human beings or in animals. Such broad-ranging definitions sit uneasily with the UK's *Medicines Act 1968*, a position that is being adjusted by judgements from the European Court of Justice. Claims in the EU about the health benefits of functional foods and the like have to face the challenge of *Directive 79/112/EEC* which prohibits claims for any food preventing, treating or curing human disease, as well as *Directive 65/65/EEC*, in addition of course, to national law. Further convolution arises from 'organic' foodstuffs and the operation of *Regulation 2092/91*, implemented in the UK by the *Organic Products Regulations 1992*, which designates the UK Register of Organic Food Standards as the national Competent Authority. It issued 'standards' requiring organic food and farming to remain free of all aspects of genetic engineering and its products, this presents special challenges for practitioners, regulators and consumers to agree thresholds, definitions and monitoring processes. Such 'standards' are promulgated regardless of the fact that on current evidence, GM foods are amongst the safest food products on the market, subject as they are to unusually high levels of testing.

Driven by the 'precautionary principle', the development, release and marketing of GM crops, GM products, and probably all biotechnology, could be brought to a halt where there are possible risks to health or the environment, even where the scientific evidence is insufficient, inconclusive, or even uncertain. As S Holm and J Harris of the University of Manchester pointed out in *Chemistry & Industry* p 913, December 1999, normally it is rational to weigh each piece of evidence according to its epistemic warrant *i.e.* the reasons for believing it. Good epistemic warrant supported by a robust theory and many experiments should hold sway over evidence with a lesser epistemic warrant. The precautionary principle, for which there is no agreed definition, distorts judgement, favouring precautionary measures to prevent the possibility of harm, even if the causal link between the activity and the possible harm has not been proven, or the causal link is weak and the harm is unlikely to occur. It would not be possible even to generate empirical data to assess if the theoretical risks were real. Such irrationality would stifle all scientific and technological progress at a stroke, and is also a weapon for restraining free trade.

Substantial equivalence, equivalence and wholesomeness (see *Commission Recommendation 97/618/EC*, in tandem with *Council Directives 90/219/EC, 90/220/EC, 94/15/EC*, and *Regulation 258/97/EC* on labelling) are the subject of powerful, even daunting, regulatory controls. Possible 'feed-through' effects in respect of feeding GM products to livestock were considered by L Donaldson and R May in their 1999 report *Health Implications of Genetically Modified Foods*, which made five recommendations strengthening monitoring and regulatory controls, and research, and encouraging the phasing out of antibiotic-resistant marker genes.

Government has expressed a desire to support the success of the UK biotechnology industry and ensure that the lead is sustained in Europe. The sheer range of products and processes arising from biotechnology, its phenomenal potential, and its integral rôle in the knowledge economy, mean that all nations and trading blocs need to embrace and foster biotechnology. In promoting a suitable environment for biotechnology, the concept of industry clusters as an important component of success has gained acceptance after analysis of the position in the USA. Clusters can be defined as geographic concentrations of interconnected companies, specialist suppliers, service providers, firms in related industries, and associated institutions. In *Biotechnology Clusters*, the August 1999 report from the Department of Trade and Industry, ten factors are seen to be critical for success:

(i) A strong science base with a critical mass of leading-edge science and academic entrepreneurs, operating with clear intellectual property policies. (ii) An entrepreneurial culture. (iii) A growing company base with support for the early development of research-driven companies. (iv) The ability to attract and retain the best management and scientific staff from overseas and larger companies: quality of life, employment opportunities for partners, career development and share options were regarded as important incentives. (v) Availability of finance during a period of a growing shortfall in the amount of equity finance available for biotechnology companies. Changes in capital gains taxation could help. (vi) Specialist premises with flexible leasing arrangements and planning-system modifications are required. (vii) Proximity to business support services including patent agents, appropriate lawyers, and recruitment and property advisers are helpful, as is proximity to large-scale companies in industries related to biotechnology such as pharmaceutical, agri-food and chemical companies. (viii) To date, biotechnol-ogy companies have

Carbohydrates
- Fabric stiffeners
- Detergents
- Fermentation substrates
- Cosmetics and toiletries
- Paint additives
- Pharmaceuticals and nutraceuticals
- Water-purification treatments

Oils, fats and waxes
- Biodegradable polymers, plastics and plastic foams
- Biosolvents
- Fuels (e.g. biodiesel)
- Linoleum
- Lubricants and anti-binding treatments
- Paints and surface coatings (e.g. varnishes, alkyl resins)
- Printing inks
- Surfactants, soaps and detergents
- Emulsifiers
- Oilcloth
- Rubber additives
- Plasticisers
- Hydraulic fluids
- Non-drying, semi-drying and drying oils
- Polishes
- Cosmetics

Proteins
- Adhesives and glues
- Controlled release of pharmaceuticals and other chemicals
- Cosmetics
- Packaging
- Pharmaceuticals and nutraceuticals
- Plant-protection and pest-control agents

Fibres
- Composites, including laminates, particle and ply boarding
- Geotextiles
- Growth media
- Insulation, fillings and stuffings
- Ion-exchange
- Matting and non-woven products for filtration
- Woven textiles, cordage and twine
- Pulp and paper
- Extenders for plastics
- Absorbents

Whole plants
- Timber, leaves and shoots for construction, furniture, fencing, packaging and protection, shelter, vessels, pilings, cooperage
- Energy by combustion of whole plants and their derivatives (e.g. fuel wood and charcoal)
- Cork for seals, gaskets, flooring, insulation and floats
- Fumatories
- Pollution control of land, air and water, including control of particulate matter, noxious and toxic chemicals, noise, sewage
- Hydrological management
- Ground stabilisation and reclamation, shelterbelts
- Soil treatment, including composts, green manures and mulches
- Carbon dioxide management (Kyoto Protocol)
- Visual amenity – the living landscape
- Habitat structure, including recreational habitats (domestic, urban, parks)
- Decoration

Speciality extracts and preparations
- Colourants and dyes
- Disinfectants
- Antibiotics
- Dentifices
- Preservatives
- Essential oils
- Insect attractants and repellants
- Masticatories
- Odours and perfumes
- Personal care and beauty products
- Plant-protection compounds and mixtures
- Polishes
- Resins and varnishes
- Rubber and balata products
- Astringents
- Sweeteners
- Medicinals (depressants including sedatives, narcotics and tranquilisers, psychodelics and hallucinogens, stimulants, analgesics, emetics, laxatives, cathartics, birth-control agents, purgatives, ointments, liniments, anthelmintics, etc.)
- Sizings
- Rayon
- Leather manufacture
- Insulators
- Acid-resistant receptacles
- Plant growth regulators
- Popular health-care products

Many species can have dual-purpose food and beverage, and non-food uses. Primary production sources from agriculture, horticulture, forestry, and from natural and semi-natural habitats. See IENICA http://www.csl.gov.uk/IENICA/index.htm

Table 3 Uses of Non-Food Annual and Perennial Angiosperms and Gymnosperms

been able to recruit scientists and technicians to meet their needs, and in many areas there are innovative training programmes. Even so, entrepreneurial abilities are still lacking, to build the skills needed for commercialising R&D. (ix) Effective networks are developing rapidly through regional development bodies; the BioIndustry Association provides the national focus. (x) Finally, a supportive policy environment, recognising that clusters must be business-driven, sets the macro-economic conditions which support innovation and ensure that regulations are both necessary and proportionate. Regional economic development agencies can play a leading catalytic rôle.

In fact, proteomics replaced genomics as the buzzword in the investment portfolios in biotechnology. The enormous range of protein compositions and configurations require massive computing power, analytical capabilities and laboratory skills to discover their modes of action and potential wealth-creating utility.

My previous reviews have concentrated on food-related matters, but non-food primary products are of critical importance to humanity, and will increasingly be the focus of biotechnology. The actual and potential benefits of plant-derived non-food products are based not only on their intrinsic utility but also on their renewability or sustainability, especially compared with oil-based products. With few exceptions, they can be biodegradable and are not considered toxic. These desirable characteristics were emphasised by the House of Lords Select Committee on Science and Technology in their report of December 1999 into non-food crops, and by M Askew in the IENICA project. Botanists are well aware of the extraordinary number of uses of plant-derived products (Table 3), as well as the actual and potential higher-plant species available for exploitation. Application of modern sciences can and will dramatically shift the balance of industry towards utilising the full panoply of benefits of renewable resources, and agricultural and forestry biotechnology will inevitably start to play a major rôle in optimising the sourcing of customised plant products.

Concluding comment

Once again, I am pleased to state unequivocally that 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 UK and international science, launching major scientific initiatives, and we participate extensively in higher educational science, launching major scientific initiatives, and we participate extensively in higher educational activities. I thank SERAD and all our sponsors, and congratulate and offer my gratitude to my colleagues for their loyalty, forbearance and outstanding efforts. I thank especially Mr J E Godfrey and the Governing Body for their commitment and contribution to our development.

The Opening of the New Research Glasshouse

T.D. Heilbronn & S. Millam

Following a major, £1.6 m investment, SCRI now has the most up-to-date facilities available for controlled, contained studies. This new building, officially opened on 9th June 2000 by Mr John Home Robertson, Deputy Minister for Rural Affairs, comprises two main areas: a headerhouse area, housing laboratories, offices and associated facilities; and a large, fully computer-controlled, glasshouse area. The building was designed to provide modern, integrated facilities for plant science research, and will house a number of projects of both fundamental and applied interest.

The glasshouse (approximately 1000m^2) consists of three main designated areas. The West wing comprises four large (80m^2) 'low level' containment areas. Eleven medium sized (25m^2) and 11 small (12.5m^2) sections make up the East wing. This wing is divided into two areas, a 'medium-level' containment area, and a 'high-level' containment area. Each area is separated by 'air-locks'.

The multi-component design of the glasshouse area will facilitate a great flexibility of usage, and the design of the complex conforms to existing and likely future containment requirements. The flexibility of design also can satisfy the differing requirements for containment or exclusion of plant viruses, microbial plant pathogens, and pollen.

Features The Glasshouse area has been designed with a number of efficiency features. These include mobile benching, which allows up to 80% of the floor space to be used, increasing the usable space by more than 20%, compared with fixed-benching systems. A 'Van Vliet' computer system controls heating, cooling and supplementary lighting. Internal thermal/shading screens also have been fitted to conserve energy loss, and enable potential energy savings of up to 40%. Ventilation is by filtered, forced-air, which can be arranged to provide positive or negative pressure, as appropriate, in individual cubicles.

The growth room area features nine 'Snijder' environmental growth cabinets with a wide range of utilities, including the ability to simulate sunrise and sunset, and to adjust light wavelength and intensity. There are also two vernalisation rooms capable of operating at -10^oC, and two large 'walk-in' controlled environment rooms.

The air-conditioned laboratories are designed for maximum flexibility of use. The two smaller areas are designed for plant tissue culture based applications and are equipped with laminar flow cabinets. The main lab has adjoining plant tissue culture growth rooms, each with fully adjustable lighting per shelf and a wide range of temperature options.

The new Research Facility was funded by the European Regional Developmental Fund, Scottish Executive Rural Affairs Department and Scottish Enterprise Tayside, with contributions from Mylnefield Research Services Ltd, The University of Dundee, and University of Abertay, Dundee.

The Opening Ceremony More than 130 specially invited guests attended the official opening on 9th June, and almost 1600 members of the public took the unique opportunity to visit the facility on 10th June, during the Public Open Day. The newly refurbished facilities for the Institute's research programme on Soil-Plant Dynamics, and for Spectrochemistry and Analytical Chemistry also were officially opened.

Edited extracts from the Director's welcoming address and the Minister's speech are shown below.

Address by Professor John Hillman, Director
On behalf of the staff and the Governing Body, Professor Hillman welcomed the Minister and distinguished guests to SCRI on the occasion of the opening of three key experimental buildings, 50 years after the initiation of research at Mylnefield, by the original Scottish Horticultural Research Institute. He said "SCRI has continued to thrive through some lean years of tight funding generally for science. We have achieved this by a combination of dedicated and able staff, the strong and unstinting support of the Governing Body and the Scottish Executive Rural Affairs Department (SERAD), and the support of a wide range of other public-sector and private-sector bodies."

He outlined the Institute's policy, which includes making a point of taking onto the staff outstanding young scientists to reinforce our growing international rôles, and described how the science has widened to encompass new technologies, new scientific disciplines, new concepts and new challenges. "Our commercial arm, Mylnefield Research Services Ltd, has grown and is particularly successful in a highly competitive commercial market. In so doing, its profits and activities bring us additional, unique resources, and we now have marvellous links with a whole range of industries from agriculture to food and drink, from biotechnology to forestry, from local industry to multinationals and through to healthcare. Over 100 organisations sponsor our work."

He spoke of his view that science has never been so exciting as it is now, and the commercial opportunities have never been so great. "SCRI embodies the very best of the UK Foresight Programmes. We are dedicated to improving wealth creation and the quality of life for mankind, and enhancing the competitiveness of UK industry. It is clear to the Governing Body and my staff that, regardless of our previous achievements, all of the developments currently in the SCRI and MRS Ltd pipelines mean that the best has yet to come. Scotland can be proud of SCRI."

"Today, you will inspect our latest development, a high-level research glasshouse facility to protect and cultivate our precious research material. My colleagues and I are grateful for all the initial efforts of the former Deputy Director, Professor Michael Wilson, now CEO of Horticulture Research International, and the commitment of Kevin Bazley and Michael Gale of Scottish Enterprise Tayside; the SERAD team, led by Dr Andrew Rushworth; Nigel Kerby of MRS Ltd; and contributions from Dundee University, and the University of Abertay, Dundee; and not least the officials connected to the European Regional Development Funding - Crawford MacCalman and Pat Muldownie of Professional Project Management provided excellent co-ordination with the builders, Muirfield Contracts Ltd and Bridge Greenhouses Ltd, and together with the suppliers, are to be congratulated on their efficiency and superb value-for-money. I should also like to thank the architect, Mike Rogers."

"The two refurbished buildings, one for Plants, Soils & Environment, and the other for Chemistry, demonstrate the special efforts of our Engineering & Maintenance team led by Steve Petrie. I also highlight the key rôles of Professor Iain Young, now at UAD, and Dr Bill Christie, recently retired Head of Chemistry. The Estate team led by Dr Graham Wood are to be congratulated for their efforts, too."

Professor Hillman commented on the importance that SCRI places on its Public Open Day, which is just one of the many ways in which SCRI contributes to the public understanding of science, before concluding "It is appropriate that the Minister visits us at this time – the various investments in us give us every confidence in our future."

Address by Mr John Home Robertson, Deputy Minister for Rural Affairs

The Minister thanked the Chairman of the Governing Body and Professor Hillman for the invitation, saying, "I welcome this opportunity to endorse the excellent science which is being worked on here at this Institute. This Institute's work is about the security of our food supply. It is as fundamental as that, and it is as important as that."

He spoke of the constant battle against pests, against weeds and against diseases, and how the economics of farming in Scotland today demand optimum yield from minimum inputs, in a changing climate. "Consumers rightly expect better quality, healthier produce and environmentally-friendly production methods. The farming industry does not always have the necessary expertise or the resources to undertake the complex procedures necessary in basic scientific research work, so we continue to rely on the research establishments, such as SCRI, to provide cost-effective research programmes. This Institute is a key component of the UK's capability in plant science, and is unique in its ability to undertake multidisciplinary research on this subject."

"The Rural Affairs Department's research programme seeks to address issues of particular interest in the context of Scotland's characteristic climate and geography. And it will obviously be relevant to crop research much further afield as well. This research will increase our basic understanding of key biological processes,

and how plants work. It will also identify features for development in plants, either through conventional molecular biology, or by using traditional techniques such as breeding and selection, or, indeed, through genetic modification. I know that is controversial nowadays, but it is important to understand not only the risks of that science, but also please let us understand the tremendous potential that there can be in that science."

He described how the Department had developed a new strategy for research which was published in the Spring of 1999. "The review highlighted the strength of the plant science programme, and identified an opportunity for SCRI to expand its research on cereals, focusing especially on spring barley - very important for the malting and whisky industry. With new equipment in its laboratories, and now this new glasshouse, the Institute is well placed to develop its position as a key centre for high quality research here in Scotland. This new glasshouse should provide the flexibility needed for the Institute's core work, and also for externally-funded research."

He stressed the safety considerations that had been paramount in the design of the complex, saying, "The controlled environment in the new glasshouse will enable the Institute to study a variety of organisms, ranging from viruses to plants, in accordance with national and international safety regulations, and that is very important. It is important to realise that the glasshouse is designed not only for containment but also for exclusion: it will keep bugs out as well as keeping them in!"

"The Institute's research into genetic modification forms part of a wider programme on plant science, crop improvement, and disease resistance. This in turn has the potential to improve the quality or quantity of food production, and to reduce environmental damage often associated with conventional agricultural practices. Ultimately, this should help to develop a more sustainable agriculture industry, and that must be in all our interests. The ability of the Institute to study biological processes at different scales, from individual cells, right through to whole plants, and to the wider environment, puts the Institute at the forefront in this important field."

The Minister then addressed the importance placed on the relevance of SERAD-funded research to the end user. "The rapid developments in plant science which have occurred over the last 20 years have meant that modern molecular techniques are now used to give far more precise and detailed information, on a much greater scale, than was ever previously possible. Fundamental scientific knowledge of this kind can have immediate commercial value in an industrial setting. This will produce many new scientific opportunities, and we want to take advantage of those opportunities. Recent inward investment to the Institute, and alliances that the Institute has forged with relevant companies, clearly demonstrate the Institute's high status in this sector. These developments at SCRI should help to enhance Scotland's reputation as one of the most dynamic locations for biological research in the whole of Europe."

He then turned his attention to the "vexed" question of GM science, "Well researched, and rigorously controlled GM science pioneered at Roslin, is producing drugs to help people suffering from cystic fibrosis. Work on crop science, here, can be equally beneficial. Scotland has a very fine reputation for quality science. I think that people are quite right to ask searching questions about GM science, but if we can be satisfied that the developments are good for mankind, and safe for the environment, it would be phenomenally silly to turn our backs on such beneficial and valuable ideas. There is a long, long history, of quality science in Scotland, and subject to rigorous precautionary controls, the Scottish Executive intends to support that good quality Scottish science."

Mr Home Robertson joined the Director in thanking all the various bodies (see above) who had jointly funded the costs of building the new facility, saying, "The project is a very successful example of partnership funding in support of new capital resources for economic development, and I welcome that. I am grateful to the other funders, for their vision and their commitment in supporting the project, and indeed to the project managers, engineers and contractors and their staff, for constructing such impressive facilities. Professor Hillman has already mentioned the very large number of people who have been involved in taking this project through from inception to completion, and I would like to add my personal thanks."

The Minister unveiled a plaque, and formally declared the new glasshouse and the refurbished ancillary accommodation open.

New technologies for the detection and identification of pathogens, pests and environmental pollutants

L. Torrance, A. Ziegler, M.A. Mayo, I. Toth, V. Blok & J.M. Duncan

The need for new technologies Plants have many enemies and these can cause a variety of diseases. However, plants can respond to attack in only a limited number of ways, which can make disease diagnosis – identifying the pathogen responsible – difficult and confusing. For example, yellowing of leaves, wilting or die-back are symptoms that can be induced by a variety of pathogens or pests and even by stresses such as herbicide damage or a lack of nutrients or water. Added to this is the propensity of pathogens to respond to plant defences by producing novel variants that can then induce novel diseases. On the other hand, plants can display a variety of symptoms when affected by closely related pathogens; sometimes a pathogen can cause no obvious disease symptoms in a tolerant cultivar but can have devastating effects in a susceptible one.

Figure 1 Unprotected potato plants infected by *Phytophthora infestans*, photographs were taken 10 days apart.

These problems are serious because plant pathogens and pests can cause large economic losses in yield and quality of food. For example, damage caused by potato cyst nematode alone costs the UK an estimated £50million annually[1].

Increased world trade in plants and plant propagation materials, and the rapid movement of plants between countries, have increased the risk of spread of harmful organisms. New diseases are emerging that are caused by the appearance of resistance-breaking strains, or by the introduction of pathogens to new hosts in other countries or continents. For example, new strains of potato blight (*Phytophthora infestans*), *Potato virus Y* and *Raspberry bushy dwarf virus* and the spread of the Columbia root knot nematode (*Meloidogyne chitwoodi*) are all posing serious threats to growers world-wide. Therefore, it is vital to ensure that only disease-free plants and materials are traded.

To facilitate trade whilst preventing the spread of disease, it is essential to have rapid, simple and accurate methods by which to detect and identify plant pathogens. In the European Union, plant passports are issued to guarantee plant health, but must be validated to be worthwhile. Tests and test protocols vary between countries, and in order to harmonise testing and build confidence, there is a need to devise standardised tests and methodologies that can be used to detect and identify economically important pathogens in different countries. These tests must be specific, robust and simple.

Rapidity of diagnosis is also an important aim as farmers and growers need to identify the cause of disease symptoms quickly and accurately so that the appropriate remedial action, such as spraying with anti-fungal agrochemicals, can be taken (Fig. 1).

Early detection by means of rapid and sensitive tests would assist control measures. They would also help to minimize chemical inputs and thereby be an important feature of attempts to develop sustainable crop production.

Basic and strategic research is done in the Pathology Division to understand the biodiversity and ecology of pathogens and pests. The results have both produced the background underpinning knowledge, and contributed to the development of the new technologies (antibodies, nucleotide sequences, novel methodology) needed to identify harmful plant pathogens. This article presents a brief overview of the technologies and applications.

Recombinant antibodies Antibodies can be highly specific and highly sensitive probes for the structure of virus proteins. They have been used in virus research to study the roles of surface features of virus coat proteins in interactions with host plants and vector organisms (e.g. aphid, nematode, protozoan). This combination of the specificity and sensitivity of antibodies has made them valuable reagents for the diagnosis of virus diseases. It is possible to express genes encoding the binding portions of antibody molecules by phage display. We have used this technology for antibody production to obtain valuable reagents (recombinant antibodies) (see Ann. Rep. 1995, 125-127). There are many advantages in using antibodies derived by phage display techniques. For example, the DNA encoding the recombinant antibody gene can be stored indefinitely, and it can also be manipulated so as to encode fusion proteins of antibody + tag (AFP) for use in a variety of different test formats. AFP can be produced cheaply in bacteria or in other systems (Ann. Rep. for 1997/98, 111-113 and 1998/99, 139-141), and this method therefore guarantees continuity of supply. These qualities make AFP ideally suited for standardised test methods.

AFP preparations that bind to particles of *Potato leafroll virus* (PLRV) readily detect PLRV in potato (Fig. 2). Currently, we are collaborating with four European partner laboratories, to devise and evaluate standard test kits that incorporate AFP that bind to particles of PLRV, *Tomato spotted wilt virus* or *Beet necrotic yellow vein virus.* The ultimate aim of this project is to produce prototype test kits for commercial exploitation.

An unexpected benefit from these developments has been the extension of this detection technology into the field of environmental pollution. We have successfully isolated recombinant antibody fragments that bind to microcystin-LR, a toxin produced by cyanobacteria, such as those in the genus *Microcystis.* These algae are common in reservoirs and lakes and are usually harmless. But in certain environmental

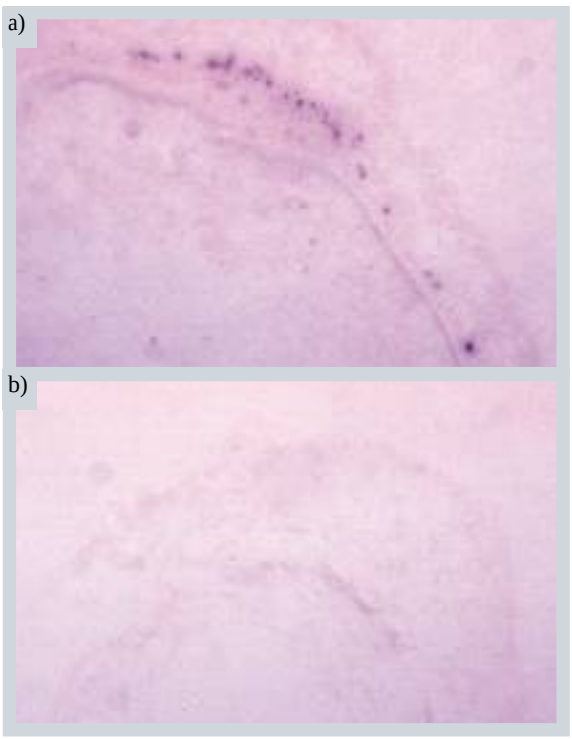

Figure 2 Detection of PLRV in potato stems by AFP stem print test a) the black spots indicate presence of PLRV in the vascular tissue b) non-infected stem.

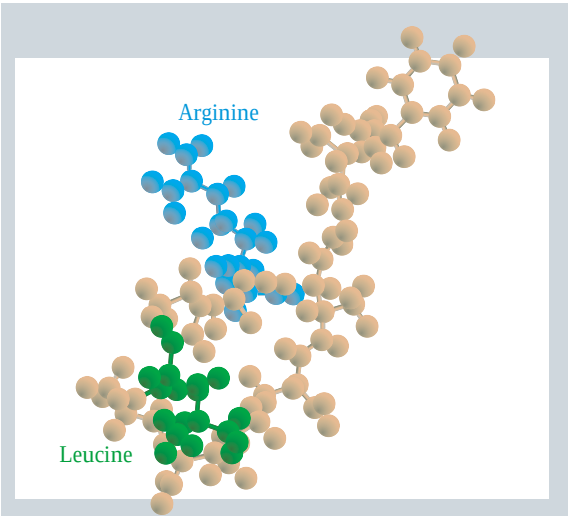

Figure 3 Model of the structure of microcystin-LR derived by NMR spectroscopy (Protein Database ID number 1LCM[2]).

conditions, they grow rapidly and the populations expand to produce floating masses of cells on the surface of water. These blooms can be public health hazards because they can contain toxins. For example, blooms of *Microcystis aeruginosa* release the toxin microcystin-LR into the water when the cells die and disintegrate. Microcystins are cyclic heptapeptide molecules (Fig. 3) that can persist in water for long periods. They are potent inhibitors of eukaryotic protein phosphatases, and can cause a range of health problems particularly in the liver, nerves and skin of humans and animals.

The next experimental steps are to optimise the AFP test and evaluate it against other methods used currently to detect microcystins. If AFP tests are shown to be effective, it should be possible to produce rapid simple tests for environmental pollutants based on our novel antibody technology. Such tests would provide a rapid way to identify the toxins in water.

Mimotopes Mimotopes are structures that bind strongly to antibodies in the same way as, and with similar affinities to, those of the homologous antigens. By using libraries of random composition peptides displayed on phage particles, it has been possible to select mimotopes that bind specifically to monoclonal antibodies that recognise particles of *African cassava mosaic virus*[3], Tomato yellow leaf curl viruses and PLRV. (Fig. 4). These mimotopes are robust and cheap to produce and provide a convenient and precisely defined positive control. The inclusion of mimotopes in test kits will help to validate the tests wherever the kits are used. This 'field' application should help to avoid the risk of spread of pathogens.

The use of peptide libraries as sources of controls for immunoassays could be extended to other pathogens, especially those difficult to obtain (such as some fungal and bacterial pathogens). The technique is being explored further in the EC project 'Standardisation of the immunodiagnosis and quantification of plant viruses by development of synthetic antigens.'

High affinity peptides, obtained by selection from phage-displayed libraries, could also be used in new ways of interfering with plant diseases and pests. For example, the appropriate peptide can be expected to inhibit the activity of enzymes coded for, or induced by, pathogens or pests, or inhibit the interactions between plants and either pathogens or pest-encoded proteins. For example, work is in progress to analyse peptides that bind to nematode surface proteins.

Nucleic acid technologies Techniques to study the genetic structure and function of pests and pathogens have made great strides in recent years. Over the past 15 years a major new technique, the polymerase chain reaction (PCR), has revolutionised the way we study the biology of pathogens. PCR, which allows specific amplification of a target DNA sequence, has made it possible to distinguish certain pathogens from their closest relatives, to quantify accurately the amounts of these pathogens present in diseased tissues and to detect them specifically even when they are present at vanishingly low concentrations.

At SCRI, PCR-based diagnostics have been designed for the assay of a number of fungal, bacterial and nematode pathogens. For example, quantitative PCR tests have been developed for *Erwinia carotovora* (blackleg) and *Spongospora subterranea* (powdery scab). These, and other, diagnostics have been made available to growers either directly, where growers are performing the tests in-house, or via a testing service provided by SCRI and others, including the British Potato Council. These tests are providing valuable information to growers to help them manage their stocks effectively. We will continue to improve these diagnostics as new technologies become available.

Tracking plant pathogens in the environment is an important area of research as this information allows

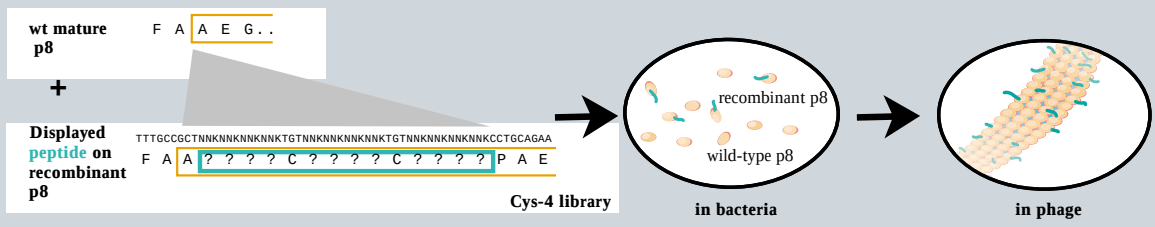

Figure 4 Phage display peptide library. Filamentous phage in the libraries have 2 copies of gene 8 (that encodes the major capsid protein). One is modified to have an N-terminal extension with randomised amino acid composition except for a pair of cysteine residues that constrains a peptide loop.

scientists to understand better how a pathogen adapts to new environments, how it spreads from one plant, field or even country to the next, and how to track and eliminate the source of a disease. Perhaps the main advance to this area in recent years has been molecular fingerprinting. Molecular fingerprinting of a pathogen provides a unique profile for a particular genus, species or even strain of a pathogen. The sensitivity of the fingerprint is determined by the particular technique chosen. For example, studying the intergenic transcribed region (ITS) region of the rDNA has allowed great strides to be made in the identification of fungal pathogens, including *P. infestans*, the causal agent of late blight, and other *Phytophthora* species. This technique has ensured that SCRI is a world-leader in the identification of these pathogens. Another technique called amplified fragment length polymorphism (AFLP) has allowed scientists to track individual strains within the environment, thus providing detailed information on disease spread and development.

Similarly, the intergenic spacer (IGS) region of the rDNA has been used at SCRI to develop a diagnostic for distinguishing individual root-knot nematodes (*Meloidogyne chitwoodi*, *M. fallax* and *M. hapla*). These nematodes infest potato and were recently designated as quarantine organisms by the EU (Ann. Rep. 1996/7, 191-3). Other PCR-based techniques such as random amplified polymorphic DNA (RAPD) and AFLP have allowed scientists to distinguish different introductions of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida* into Europe (Ann. Rep. 1995, 151-4). Also, microsatellites have been used to track individuals within the environment, and to follow population changes following selection pressures, thus providing detailed information on disease spread and development.

Phage typing Even high resolution methods like those described above are sometimes insufficient to discriminate between a harmless organism and a harmful one. A good example is the discrimination of *E. coli* O157 from other strains of *E. coli*. For this, we have turned to phage typing. This method was developed in the 1920s to fingerprint bacterial pathogens based on their susceptibilities to infection by bacteriophages. It has been recently adopted by scientists at SCRI for the study of plant pathogens and is now being applied to the detection of *E. coli* O157. The annual costs of infections by *E. coli* O157 in the USA alone from lost productivity and health care are currently estimated at $913 million. Effective investigation of the serious outbreaks caused by *E. coli* O157, and the saving of lives and money, depends on rapid, accurate fingerprinting of many isolates from different sources. The method, developed in association with Grampian Health Trust, has been patented and attempts are being made to commercialise it in kit format.

Conclusions and future perspectives The expertise and diagnostic tools produced at SCRI have been transferred to farmers and processors to assist the commercial production of high quality food crops for Scottish consumers and beyond. Furthermore, some spin-out research has been of value in the fields of medical and environmental diagnostics.

It seems clear that the need for such state-of-the-art diagnostics is unlikely to diminish. Pathogens evolve all the time and agricultural practices seldom remain static for long. A skilled research base has been, and looks certain to continue to be, the essential resource upon which pathologists can rely so as to rise to the diagnostic challenges posed by ever-resourceful plant pathogens. With the continuing support of SERAD, the European Commission, the British Potato Council and other sponsors, research at SCRI will continue to improve and devise new methodologies to meet these needs.

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A Dundee contribution to science: high-throughput mass spectrometry and new biological insights

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Recalcitrant problems in biology, agriculture, conservation, and environmental management need a continual input of the best new interpretations and technologies. A relatively recent innovation, which has produced much useful new information, is continuous-flow isotope ratio mass spectrometry (CF-IRMS). The CF-IRMS instrument provides a fast, reliable way of measuring the isotope ratios of elements (e.g. C, N, O, H, and S) in different kinds of samples. Much has already been learned and new phenomena have been identified by observing deviations from the expected values of isotope ratios. Much of this new science began, and is still being led, by research in Tayside.

As UK government policy turns increasingly to environmental concerns, and to agricultural conservation and sustainability of farming and biodiversity issues, this powerful new ability to study the processes of nature requires pride-of-place in research planning, environmental assessment and conservation. This is especially so in Scotland where the science was born, and where it continues to evolve new ways of resolving old and new problems.

Innovation of the technology and the chemistry

CF-IRMS was invented between 1977 and 1984 by Dr Tom Preston and Professor Nick Owens, two former graduate students of the then Professor W.D.P. Stewart of the Department of Biological Sciences, Dundee University. Dr Preston realised, while laboriously analysing hundreds of samples of lake sediments and plants for their nitrogen isotope abundances, that the analyses could be automated if an instrument called an elemental analyser was coupled to an isotope ratio mass spectrometer which had multiple signal collectors. In 1981, Dr Preston, by then a researcher at the Scottish Universities Research and Reactor Centre, was finally able to develop this concept in collaboration with Dr Owens, by then at Plymouth Marine Laboratory. The new instrument[1,2,3] so impressed two employees of a major mass spectrometer manufacturer that they quit their jobs and formed a new company (Europa Scientific Ltd, now PDZ Europa), which has twice won the Queen's Award for Industry.

Designed originally to measure ^{15}N enrichments for tracer studies, CF-IRMS was rapidly developed to measure isotope compositions at natural abundance levels: $\delta^{13}C$, $\delta^{18}O$, and $\delta^{15}N$. Over the last decade, the CF-IRMS approach has been extended to other elements (^{2}H and ^{34}S) and other sample types (^{18}O in organics and nitrate). Dundee has continued to be a major centre of activity in CF-IRMS development, with collaborative projects between SCRI and Europa Scientific Ltd on ^{2}H and ^{34}S and ^{18}O analysis. All of the wide range of CF-IRMS instruments in use around the world are descendants of this fundamentally Scottish invention. CF-IRMS was thus conceived in Dundee, proven in Plymouth and is now, globally, an essential research tool.

The simple (in hindsight) coupling of an elemental analyser to an IRMS to produce the CF-IRMS opened up a whole new range of applications and possibilities. For $\delta^{15}N$ and $\delta^{13}C$, the chief impact was sample throughput. Handling large sample sets for statistically sound interpretation of ecological and genetic problems became a realistic possibility. Once the potential for ^{13}C and ^{15}N was demonstrated, there was widespread interest in extending this productive analytical system to other elements, particularly H, O and S. The elemental analyser sample converter was ideal for bulk plant and soil samples, but there was also growing interest in the isotopic composition of individual compounds. This drove a further level of instrument integration, where a gas-chromatograph, separating complex mixtures, precedes a micro-combustion interface to the IRMS.

SCRI has continued the development of novel systems for both bulk sample and compound-specific stable isotope analysis of H, O and S, working closely with instrument manufacturers and international collaborators. Along with other developments in the field, the result is that instruments are now available to tackle a wide range of samples and elements. The pace of instrument development has been rapid and has sometimes run ahead of the production of adequate calibration materials. Models for interpreting the natural variations in isotope composition have still to catch up with the instrument developments. There is consequently a unique opportunity for timely studies of isotope natural history, not possible until now, which promise powerful tools to complement $\delta^{13}C$ and $\delta^{15}N$ in environmental and other fields.

Our design of new analytical systems has centred on two parts of the CF-IRMS, the sample converter and the mass-spectrometer. The conventional mass-spectrometer design is satisfactory for C, N, O and S analysis, but not for H; the helium carrier, which sweeps the analyte gas from the sample converter to the IRMS, cannot be separated fully from hydrogen in the conventional analyser. The helium is present in vast excess and is only one mass unit heavier than the very low intensity Hydrogen-Deuterium (HD) ion. Precise quantification of the HD ion is essential for natural abundance measurements, and this was achieved by using a specially designed analyser which produced a much greater separation of the ion beams[4]. Once measurement of hydrogen gas was possible, sample conversion of water and organic compounds was developed using pyrolysis and reduction on carbon[5,6]. Pyrolysis not only converts sample hydrogen to hydrogen gas, but also converts any oxygen to carbon monoxide which is suitable for measuring oxygen isotopes. Measurements of hydrogen and oxygen isotopes can now be made on very small volumes of water and organic compounds, bulk samples and individual components of complex mixtures. Appropriate standards, however, do not exist for organic compounds; we see a research opportunity for SCRI in such development and subsequent use of appropriate standards.

The CF-IRMS system for sulfur analysis is substantially the same as for C and N, but uses modified elemental analyser chemistry to achieve complete conversion of sample sulfur to sulfur dioxide. Using conventional off-line methods, sulfur has always been a difficult element to handle for isotope analysis; in a dedicated CF-IRMS, high throughput of plant samples of low sulfur content is possible. Enriched sulfur isotopes are not readily available and this has restricted the application of stable isotope methods to plant metabolism. However, using naturally distinct sulfate sources, we have studied uptake and re-location of sulfur in wheat over a growing season[7].

Biological problem solving

Since 1991, Dr Linda Handley (SCRI) has specialised in understanding the biological bases for the patterns of $\delta^{15}N$ values found in nature. These patterns are important because they lead to an understanding of natural processes which cannot be studied by any other means. After a substantial, critical review of the existing literature and a 3-year study of $\delta^{15}N$ patterns in complex vegetation and soils[8], Dr Handley called for a complete revision of this area of study and pointed out to an international audience[9] that $\delta^{15}N$ could not be used to trace, directly, sources of nitrogen, as almost all previously reports assumed, but chiefly revealed the occurrence and extent of biological and physical processes which transform nitrogen chemically and physically (e.g. nitrification, assimilation and loss by organisms and gaseous nitrogen loss).

The $\delta^{15}N$ research at SCRI has developed into two main areas, both of which are directly useful for UK environmental studies, conservation, sustainable farming and for providing the scientific bases for policy and regulation. The first main area concerns the chemical transformations and fates of nitrogen in soils and waters, including the role of plants. The second major area concerns functional plant biodiversity: its protection in nature, the assessment of biodiversity in crop plants for potential breeding, and whole plant performance of genetically modified crop plants. Both

of these lines of research have commercial potential.

Transformations and Fates of Nitrogen Soil nitrogen is changed naturally into nitrate-nitrogen, which leaks into drinking water, streams and ultimately into nearshore marine waters[10]; nitrogen arising from fish farm waste is suspected to have a role in the recently reported amnesiac shellfish poisoning[11] found in Scottish west coast waters, and atmospheric nitrogen pollution has been identified, globally, as a threat to the biodiversity of naturally nutrient-poor ecosystems[12], such as native moorlands and grasslands. It has been asserted for several decades that excess nitrate in drinking water caused the so-called blue baby disease in infants and has been suspected, on theoretical grounds, of causing stomach cancer. Today, the evidence is mixed and being revised[13], with some positive benefits apparently accruing to human health and some new concerns arising as well. It is established that nitrogen from land sources causes major changes in the nearshore marine environment[10,14] and nitrates from agriculture and urban sources are suspected of playing a role in causing nuisance and toxic algal blooms in estuaries and freshwaters, to the extent that this presumption forms one of the main bases for an EU Directive requiring designation of Nitrate Vulnerable Zones.

There has been no way to study the chemical transformations and fates of nitrogen on a long-term, large-scale basis such as whole fields or catchments. Some progress has been made in the last decade in studying the variations of nitrogen forms and amounts in receiving waters (surface and ground waters). However, the majority of nitrogen cycling events, including the generation of nitrate, occur in soils, and the lack of appropriate methods has prevented studying these processes in soils until recently. Most of the information that we have about the transformations and fates of nitrogen was determined by adding isotopically heavy (^{15}N-enriched) nitrogen tracers to either small-scale field plots or to laboratory experiments. This approach has limited use because nitrogen transformations are highly variable in space and time, and unpredictable on the larger scales which are relevant to environmental stewardship. To the extent that such methods are limited to point measurements in space and time, they are also unreliable. Additionally, most research conclusions are based on site-dependent case studies, which cannot be generalised to other sites. Hence, scientific underpinning is weak or absent for much of the management and regulation of nitrogen in the environment, and a substantial niche exists for basic and strategic research, were more suitable methods and technologies available.

Research at SCRI, using the natural background levels of the nitrogen isotopes (δ^{15}N) of soil and water, are changing this situation by innovating new methods and new interpretations. Early results show that mathematical modelling approaches can be used with direct sampling and analyses of δ^{15}N of soils or waters can be used to describe the chemical transformations and fates of environmental nitrogen. When fully developed, this approach will allow us to identify the polluter. It will be possible to assess whether nitrogen pollution regulations are working properly, long before the ultimate results can be detected in receiving waters, to identify trouble spots quickly and suggest improvements. It will also provide a rapid assay of soil nitrogen nutritional status by quickly assessing the relative importances of nitrification and denitrification in releasing and/or depleting the soil nitrogen bound in organic matter. This will enable increased precision of timing for fertilisation and provide an index to optimum fertilisation amounts by filling in the long-missing part of the puzzle - how much is lost as nitrogen gases.

Others have recently attempted to model soil nitrogen cycling using δ^{15}N of nitrogen pools, but have been forced to gloss over the lack of reliable chemical preparation methods for the isotopic analysis of these pools. Hence, the real work remains to be done. At SCRI, we have innovated the only method, so far, which is capable of measuring the δ^{15}N of nitrate-nitrogen in complex samples such as soil solutions and highly eutrophic waters[15]. A new and reliable method for determining the δ^{15}N of ammonium-nitrogen from soil solutions and eutrophic waters will be announced shortly from our laboratory.

Meanwhile, we have completed one full calendar year of measuring, fortnightly, the concentrations and δ^{15}N values of nitrate-nitrogen in the soil solution of a barley field in the Ythan River catchment, Aberdeenshire, and in its field drains. We have also measured the concentrations and δ^{15}N of nitrate in the Ythan River and its tributary burns. This is the first, accurate description of the spatial and seasonal, farming-related changes of the δ^{15}N of nitrate in parallel with nitrate concentrations in soils and surface waters and hence of the transformations of nitrogen in the soil which lead to the formation of nitrate. The δ^{15}N of the nitrate in soil solution also reveals the extent to which the sample comprises newly nitrified nitrogen available to the crop or for leaching with per-

colating waters *versus* the extent to which the analysed nitrogen is that remaining after excess nitrogen has been lost gaseously.

In the current climate of nitrate regulation and related environmental concerns, such as ozone depletion and atmospheric nitrogen pollution, it is increasingly important to be able to assess the transformations and losses of nitrogen from soil and waters. Researchers using conventional water chemistry[e.g.16,17] and now-acknowledged, inadequate methodologies for $\delta^{15}N$ of nitrate[18,19], have produced data suggesting that some wetlands routinely achieve very high (up to 60%) removal of nitrate as denitrification, mostly as N_2 gas, but that the process from soil may incur denitrification *via* a larger proportion of ozone-depleting N_2O gas[17]. Martin[20] reported wetlands reducing incoming dissolved nitrate to as low as 2 mg l^{-1}, while Sidle *et al.*[21] found that a constructed and managed wetland in Indiana, USA was less efficient at nitrate removal than an adjacent natural riparian marsh. Using existing expertise, experience and unique methodologies, SCRI is poised to further our understanding and ability to assess important aspects of nitrogen cycling, particularly in relation to nitrogen pollution and denitrification losses. We are especially well prepared to deliver innovative research on the role of wetlands in attenuating nitrogen loads.

Recently, hydrologists in Germany, Canada and the US have been able to distinguish empirically, in some cases, between nitrate arising from agriculture and nitrate in waters arising from animal sources, including human waste[22]. This has been done by simultaneously analysing the $\delta^{15}N$ and $\delta^{18}O$ of nitrate in waters. The underlying biological mechanisms are not understood, and we see this as a fruitful line of inquiry for the expertise existing at SCRI, building on, *inter alia*, the innovative work and accumulated experience in ^{18}O analysis at SCRI.

The basic technologies and concepts, largely initiated in Scotland, have started a cascade of isotope research centred on nitrate and on the $\delta^{15}N$ and $\delta^{18}O$ of nitrate and the $\delta^{13}C$ and $\delta^{18}O$ of dissolved organic carbon compounds in ground and surface waters, tracking pollution and studying water quality. For use with ground and surface waters (of relatively low organic nitrogen content and therefore susceptible to methods which are unsuitable for soil solutions analyses), scientists at the United States Geological Survey have developed new methods[23,24] for measuring the $\delta^{15}N$ and $\delta^{18}O$ of dissolved nitrate, a technology which found immediate acceptance among hydrologists and geochemists[22,25]; this same group is funded by the US Government to examine how nitrate concentrations increase under forest soils in a large catchment. The intensity of interest in the $\delta^{15}N$ and $\delta^{18}O$ of nitrate for use in hydrological studies is such that new methods are beginning to appear from several laboratories[e.g.23,24,26], although most of these laboratories still use older manual preparation methods instead of relying on the newer continuous-flow technology for determining $\delta^{18}O$. As the United States carries out a large programme of assessing water quality, several major research projects, on the scale of major continental river catchments, are using $\delta^{15}N$ and $\delta^{18}O$ to study the age and source of nitrates in surface and ground waters[27] . The US Environmental Protection Agency is beginning new research using $\delta^{15}N$ and $\delta^{18}O$, together with conventional water chemistry and hydrological modelling, to study the impacts of landforms and land use, interacting with vegetation, on surface and ground water quality[28] , an approach we have long argued is necessary for Scotland.

The method developed at SCRI by Johnston *et al.*[15] is the only suitable one to date for measuring the $\delta^{15}N$ of nitrate in soil solutions. This method, and other expertise at SCRI, could fill a widely recognised need[20,29] in soil nutrient cycling research, for systems of measuring nitrification, denitrification and soil respiration at depth in the soil profile.

SCRI researchers published the first evidence that previously unsuspected processes in the top layer of the soil caused sufficient changes in total soil nitrogen to change the $\delta^{15}N$ of whole soil substantially in the course of a growing season. The initial work was done on an abandoned agricultural field in primary succession[8], then in Scottish pastures in collaboration with Dr Carol Marriott of the Macaulay Land Use Research Institute[30,31]. Similar findings have now been reported by Butler *et al.*[32] for arable (maize) and grassland in Spain and in southern England. Our work was done for the upper 10 cm of the soil profile. Butler *et al.*[32] showed the same patterns occurring to 30 cm depth. We attempted to explain these new observations in terms of known processes and rates for nitrogen mineralisation and loss; such calculations did not account for the observations, demonstrating serious deficiencies in our information base (*inter alia* interpretations underpinning regulations) and highlighting the need for new research using new isotope techniques.

Current laboratory and field experiments at SCRI examine the nature of the nitrogen transformations which cause such isotopic patterns in soils and percolate waters. In an early study of Kenyan savannah vegetation[33] (done collaboratively with Professor Janet Sprent (Dundee University) and Dr David Odee (KEFRI, Kenya), then a student of Dundee University), an important link was demonstrated between plant $\delta^{15}N$ and soil moisture status. Soil moisture, intermittent or consistent, dominated the $\delta^{15}N$ patterns of the local vegetation, regardless of whether the potential source of plant nitrogen was atmospheric nitrogen, fixation by legumes, or soil nitrogen, and regardless of type of plants examined. Later work[34] with another student at Dundee University (Paul Hill) on the functional diversity of native *Juniperus communis* trees in Scotland, established similar relationships for foliar $\delta^{15}N$ and soil water relations. Most recently, a global survey of $\delta^{15}N$ values of plants and soils[35] documented that rainfall and temperature (as surrogate measures of soil moisture) so dominated ecosystem nitrogen cycling that it was reflected consistently in the nitrogen isotope values of plants and soils. The findings of this study were consistent with those of Austin and Vitousek[36], who found a trend for foliar $\delta^{15}N$ along a rainfall gradient in Hawaii.

Collaborative work in Spain[37] uses $\delta^{15}N$ and ^{15}N-enriched tracers to reveal the processes of nitrogen cycling in soils and plants following periodic fires in that region. In a collaborative study with a Canadian researcher[38], $\delta^{15}N$ values enabled the solution of a problem which years of research had been unable to resolve otherwise: how prevalent management of western red cedar forestry (including post-harvest burning) leads to a serious weed infestation which debilitates newly planted replacement forests.

In collaboration with Dr Chris Wheeler of Glasgow University[39], we were able to demonstrate, via $\delta^{15}N$ measurements, that most of the plant-available soil nitrogen in a west coast Scottish mire originated from atmospheric nitrogen fixation by free-living soil microorganisms, and was not contributed by the more conspicuous leguminous plants. A separate study provided new information about the nitrogen status of important forestry trees which fix atmospheric N_2 in association with the actinomycete, *Frankia*[40].

Functional Plant Biodiversity The UK is bound by international treaties and by EU Directives[41] to address the problems of conserving biodiversity, and there is a special biodiversity group for Scotland[42]. However, little is known about the existing whole-organism, functional biodiversity of plants. Additionally, it has been impossible to assess, within a reasonable length of time, whether environmental factors, such as air pollution, are affecting the functional biodiversity of plants. Research at SCRI, relying heavily on the stable isotopes of carbon and nitrogen, is providing a novel way to assess whole-plant functional biodiversity in interaction with environmental conditions.

The publication of a model explaining the central mechanism determining whole leaf[43] $\delta^{13}C$, opened a large new area of research on the environmental and population-based components of photosynthesis and water loss in plants using the C_3 pathway of photosynthesis[e.g.44,45]. A significant few of these papers related $\delta^{13}C$ to inter- and intra population genetic variations[e.g.44,46], especially in wild plants. As far as we are aware, SCRI's isotope group is the only laboratory, so far, to follow through extensively on the genetic component of plant $\delta^{13}C$ and relate $\delta^{13}C$ to molecular markers of identified genotypes; we were the first laboratory[47] to establish a genetic component for plant $\delta^{15}N$. This lead is now being taken up by others, such as Professor Richard Guy at the University of British Columbia[48]. Our approach has been unique from the beginning in taking advantage of the newly emerging techniques of molecular biology to establish at the outset that experimental material for physiological studies consists of molecularly-identified different genotypes. The next step is to understand the mechanisms behind the observed isotopic differences among genotypes and life forms. We continue to study the functional biodiversity of native British plants as expressed in the whole organism by $\delta^{13}C$ and $\delta^{15}N$ [49,50,51].

From the first field studies of foliar $\delta^{13}C$ and $\delta^{15}N$, it was recognised that there were inter-species, and intra-population variations around mean values, which could not be explained easily by a central mechanism. For $\delta^{13}C$, it was widely assumed that these variations represented small differences in water availability or leaf temperature, until Comstock and Ehleringer pointed out the genetic component to these variations[46]. For plant $\delta^{15}N$, the common, but untested, assumption was that variations of plant $\delta^{15}N$ represented small differences in the type and soil depth of the nitrogen which these plants used, e.g.[52]. In a series of papers[53,54,55,56,57,58,59,60,61,62,63,64,65,66],

researchers at SCRI described highly controlled hydroponics experiments, and one soil-based glasshouse experiment, which showed that genotypes of wild and cultivated barley varied in their shoot and whole plant $\delta^{15}N$ and $\delta^{13}C$ against a common isotope background for nitrogen and carbon. Further data[51] have now shown the same genotype-dependent variations of these isotopes for wild plants (a sedge, grass and dicotyledon) growing in a nutrient-poor soil, for native Scots pine in a common garden and in sites-of-origin[49], and for the important upland pasture grass, *Agrostis capillaris*, grown in a controlled environment chamber in sand culture[50].

Analyses of plant $\delta^{13}C$ and $\delta^{15}N$ are fast and reliable, and the results allow us to make some reasonable hypotheses about their causes, i.e. what is functionally different about genotypes having different isotopic values and, therefore, what genetic variations in a population of plants allow that population to survive the vicissitudes of competition and environmental changes. The hypotheses which arise from initial isotope screening can then be tested on individual genotypes which have shown extreme isotopic values, thus reducing the number of test organisms to a manageable level and increasing the certainty with which experimentation is designed.

Variations among plant genotypes of each isotope pair direct further investigations toward specific areas of research. Variations in $\delta^{13}C$ tell us that we should investigate genotypic differences in plant anatomy[67,68,69] and physiology[34,70,71] related to photosynthesis and post-photosynthetic carbon metabolism as well as differences in water use. $\delta^{15}N$ variations tell us that we should examine variations in nitrogen assimilation, internal allocation and losses of nitrogen for describing the biodiversity of plant strategies.

Much less is known about plant $\delta^{15}N$ than is known about $\delta^{13}C$; there is no central mechanism described which chiefly determines plant $\delta^{15}N$. However, progress is being made. Handley and Scrimgeour[8,9] have been major forces in causing a complete re-assessment of the way this isotope is regarded. $\delta^{15}N$ was initially regarded as a tracer, a naturally occurring form of ^{15}N-enriched compounds which had been used successfully to trace the fates of added nitrogen and estimate the amounts of nitrogen in systems. Especially after CF-IRMS instruments became widely available, a host of papers appeared purporting to use foliar $\delta^{15}N$ to trace the 'source of plant nitrogen.' Shearer and Kohl[72] claimed for many years (pre-CF-IRMS, when few could analyse $\delta^{15}N$) that they could quantify the amount of plant nitrogen which was derived from biological N_2-fixation and do it under field conditions. Although the educated isotope community never accepted this claim, Handley and Scrimgeour[8] were the first to argue strongly and overtly, with supporting data, that plant $\delta^{15}N$ is chiefly determined by isotopically fractionating processes and not by the isotopic value of the source nitrogen; they argued, further, that plants have an active and dynamic role in their own nitrogen metabolism and are not passive acceptors of an external nitrogen source. Handley and Scrimgeour's rejection of $\delta^{15}N$ as a direct measure of amounts of nitrogen fixed from the atmosphere (or from any other source) was based on theory, field observation and the results of a rigorously controlled glasshouse study[73]. Theirs was also the first clear rejection of $\delta^{15}N$ as a tracer of nitrogen in soils since the early 1970's[e.g. 74]. The view that $\delta^{15}N$ integrates processes, rather than traces sources, is now widely accepted.

In one of a series of experiments with genotypes of wild barley[63], we were able to show that the $\delta^{15}N$ of the plants was related to how much nitrogen was retained in the plants *versus* that lost through the roots, under environmental stress. This series of experiments[e.g. 63,47] documented that $\delta^{15}N$ of plants was variable according to the kind of environmental stress to which the plants were subjected (salinity, drought and nitrogen deficiency). These were the first reports that plant $\delta^{15}N$ was related to genotype and could be modified by stress. These experiments, and subsequent work, are unravelling the relationship between this easily measured variable and the biodiversity of plant responses to stress insofar as the nutritionally important element, nitrogen, is concerned. SCRI researchers described this new approach in a series of papers presented to a conference on linking plant physiology with molecular genetic approaches[58,59]. In another molecular based study[75], $\delta^{15}N$ helped to describe genetically based variations in the nitrogen and carbon metabolism of pea.

Most plants form fungal associations known as mycorrhizas, the most common type of mycorrhiza are, arguably, arbuscular mycorrhizas (AM). To understand plant $\delta^{15}N$, and how it varies in response to environmental stresses, the role of mycorrhizas must be understood. It is known that AM fungi assist their

plant associates in obtaining soil phosphorus[76]; no special role in nitrogen nutrition was known. In collaboration with Dr Melvin Daft of Dundee University and later with Dr Charo Azcón of Granada, Spain, Dr Chris Wheeler of Glasgow University and Dr Henrique Fonseca of Portugal, we showed that the presence or absence of AM[77,78,79,40], the type of AM and the interaction of these factors with drought or nitrogen deficiency, have a large effect on the nitrogen relations of herbaceous plants. In a study of Canadian western red cedar forests, the use of $\delta^{15}N$ enabled Chang and Handley[38] to link type of mycorrhizal association with changes in post-fire nitrogen and phosphorus cycling and determine the cause of a serious weed infestation problem. This study also demonstrated how the biodiversity of organisms in the plant-soil partnership can respond differently to contrasting climates under physical stress.

Our research group has made a unique contribution to the knowledge of biological nitrogen-fixation: it has been undisputed, general wisdom for decades that biological nitrogen-fixation does not discriminate between the heavy and light isotopes of atmospheric nitrogen. We have shown, in collaboration with Dr Peter Rowell, Dundee University, that biological N_2-fixation can incur substantial fractionations relative to source nitrogen, especially in organisms which are able to live independently of symbioses with higher plants[80]. We have also shown that the amount of this fractionation is correlated with the chemical type of nitrogen-fixing enzyme, nitrogenase, which is present. Exploitation of these results could lead to new and useful information about microbial nitrogen-fixation.

Soil Invertebrate Studies and Food Webs Food webs (who eats whom and what) have been notoriously difficult to study. Many organisms, because of their migrations, their small size, or because they live in soil or other largely inaccessible locations, cannot be observed directly, and their eating habits must be inferred from indirect evidence. Environmentalists have always been interested in the feeding habits of animals, but it is also crucial to agriculture to know what soil-dwelling organisms eat and what effect plant-eating pathogens have on agricultural crops.

Deniro and Epstein[81] showed that the $\delta^{13}C$ of a variety of organisms could be used to determine what they actually assimilated (as opposed to ingested) and that, on average[82], there was a 3.3 ‰ increase in whole animal $\delta^{15}N$ with each increase in trophic level (i.e., this work predicts that tigers would be 3.3 ‰ more isotopically enriched than the cows they ate, and cows would be 3.3 ‰ more enriched than the plants they ate). The relationship breaks down between primary producers (plants and algae) and their nitrogen sources.

The expected $\delta^{15}N$ increase with height in the food chain is not absolute, and deviations from this expected value have revealed new information about animal life cycles and feeding habits. Using isotopic techniques, Scrimgeour *et al.*[83] found that beetles, which are pests on local raspberry crops, remain in the soil, between crop rotations, much longer than formerly suspected.

To study the below-ground feeding habits of soil-dwelling invertebrates in Scottish pastures, a multidisciplinary team (soil zoologists, plant biologists and ecophysiologists) was assembled, including scientists from the Macaulay Land Use Research Institute. This was the first, statistically designed spatial and seasonal study of the isotopic signatures of soil invertebrates, and one of the first such studies of soils and plants[30,31]. We found, *inter alia*, that grazing intensity altered below-ground feeding habits.

With continually improving technology at SCRI, and consequently better ability to analyse very small samples, the natural abundance isotope research was extended into plant-pathogen interactions, i.e. animal parasites, nematodes and the effects of nematodes and viruses on plants. During these studies, Dr Brian Boag (SCRI) published[84] the first $\delta^{13}C$ and $\delta^{15}N$ study of the internal parasitic feeding habits within an animal host, which also showed that the rabbit embryo behaves (nutritionally and isotopically) as a parasite on the mother. Using $\delta^{13}C$ and $\delta^{15}N$, Drs Neilson and Brown[85] showed that combined pathogen infection (virus application and nematode feeding) produced a greater physiological effect in *Petunia hybrida* seedlings than that recorded with only a single pathogen infection. Related work[86] established that some nematodes could alter their feeding preferences for plants.

The research at SCRI is closely allied with that of Professor John A. Raven FRS, Boyd Baxter Professor of Biology at the University of Dundee, and with his associates. Professor Raven's research is of a fundamental nature; he has specialised in plant physiology and has used marine algae and higher plants as model organisms. His use of enrichment and natural abundance levels of stable isotopes has revealed much new

and fundamental information about plant physiology and biochemistry. Although he began using natural abundance level stable isotopes to understand plant physiology while still at Cambridge University[87], the first isotope-based research he did in Dundee was enrichment-level studies of $^{18}O_2$ uptake in algae in the light to investigate the Mehler reaction and the (then still controversial) oxygenase activity of RuBISCO *in vivo*[88,89]. This work was followed by an investigation of the pathway(s) of oxalic acid synthesis in higher plants using enrichment studies with $^{18}O_2$ and $^{13}CO_2$ and natural abundance $^{13}C/^{12}C$ ($\delta^{13}C$) measurements[90]. The suggestion that the $\delta^{13}C$ of C_3 plant parasites (which are photosynthetically competent and living on C_4 hosts) can be used to assess the fraction of parasite C obtained from the host[91], has been widely followed up by subsequent studies elsewhere. This higher plant work led to the use of $\delta^{13}C$ data to interpret variations in the water-use efficiency of plants as a function of nitrogen source (ammonium, nitrate, nitrogen gas, ammonia gas)[92,93,94]. Food chain reconstruction from fossil (Cretaceous) data has also been attempted[95].

Using both aquatic plants and algae, Professor Raven's laboratory has used primarily $\delta^{13}C$ to make fundamental discoveries about photosynthesis. The innovative *Chlorella* (alga) work involved the use of carbon dioxide and nitrogen availability in regulating expression of the carbon dioxide concentrating mechanism, again with interpretative aid from stable isotopes[96]. Research on freshwater plants related $\delta^{13}C$ to the inorganic carbon source as determined by other, physiological measurements; these measurements, in combination, led to estimates of the diffusion pathlength for carbon dioxide in the freshwater alga, *Lemanea*, which does not use bicarbonate ions[97,98].

The freshwater algal work led to investigations on the ecophysiology of *Lemanea* with several lines of evidence (including carbon isotope natural abundance), corroborating the diffusion pathlength deduced earlier[99,100], and subsequent work on $\delta^{13}C$ of plants from freshwater habitats[101,102].

The other major theme in Professor Raven's algal work is the ecophysiology of marine micro- and macroalgae. As primary producers, these organisms are fundamental to the entire marine ecology. As is the case for freshwater organisms, the capacity for obtaining carbon from bicarbonate and, apparently, carbon dioxide concentrating mechanisms in general, involves less change of $\delta^{13}C$ relative to source than does diffusive entry of carbon dioxide[98,103,104]. The complication of atmospheric carbon dioxide as well as dissolved inorganic carbon for intertidal algae and plants was also studied[104].

A key event in algal studies was research on a range of marine macroalgae from the east of Scotland, which confirmed the correlation of diffusive entry of carbon dioxide, these organisms having very negative $\delta^{13}C$ values[105,106]. This work also showed that the *in situ* growth rate and $\delta^{13}C$ can, as for the freshwater *Lemanea*, be used to estimate the length of the carbon dioxide diffusion pathway in marine algae such as *Delesseria*. Further work on these algae investigated effects of variations in light supply[107,108], and of variations of inorganic carbon and oxygen supply[109,110], on photosynthetic and growth rates and on $\delta^{13}C$. The taxonomic and geographical spread of the data set subsequently has been extended[111,112] and currently is being analysed prior to publication.

A continuing theme in the work on aquatic macrophytes has been the use of $\delta^{13}C$ for interpreting symbiosis. This work followed from research on the role of the N_2-fixing cyanobacterium, *Anabaena*, in supplying carbon to its symbiont, the (freshwater) fern *Azolla*[98]. Work on the marine lichen, *Lichina*, has helped to define the mechanism of inorganic carbon supply to the cyanobacterial symbiont, *Calothrix*[113]. Raven *et al.*[114] investigated the metabolic relationship between the brown Australasian macroalga, *Hormosira*, and *Notheia*, a brown alga which only grows on *Hormosira* or the closely related *Xiphophora*. This work found, from gas exchange and $\delta^{13}C$ measurements, that the epiphyte could satisfy all of its energy and carbon requirements from its own photosynthesis and was not parasitic. Because *Notheia* contains the sugar alcohol altritol, which only occurs in a few other brown algae, including both *Hormosira* and *Xiphophora* which are relatively distantly related to *Notheia*, it was thought that the altritol in *Notheia* could be derived from *Hormosira* or *Xiphophora*[115,116]. This possibility has now been dismissed using mass spectrometric and NMR analyses of $\delta^{13}C$ and ^{13}C enrichment studies; hence, we now know that altritol synthesis occurs in both *Notheia* and its relatively distant relatives *Hormosira* and *Xiphophora* (and *Bifurcariopsis* and *Himanthalia*)[115].

Kleptoplasty by sacoglossan molluscs living on green (and red) macroalgae is a further example of symbiosis involving marine macroalgae. Here, some of the chloroplasts ingested by the molluscs during feeding

are retained by the sacoglossan in which they continue to photosynthesise for days to weeks (and occasionally months). Raven *et al.*[117] have used $\delta^{13}C$ to estimate the fraction of animal carbon which is derived from kleptoplastic photosynthesis rather than from direct feeding on the host algae in Western Australia.

Finally, work on microalgal cultures led by Dr Andrew Johnston (now at SCRI) has yielded important data on the relationship among the inorganic carbon supply to marine microalgae, their growth rate, and algal $\delta^{13}C$ relative to source inorganic carbon[118,119]. These, and other data, cast doubt on some recent attempts to use $\delta^{13}C$ in marine sediments to 'hindcast' sea-surface, and hence atmospheric, carbon dioxide levels in the past.

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Management of plant parasitic nematodes

D.L. Trudgill

The research of the SCRI Nematology Group has a high international profile. In 1998, it organized the 24th International Nematology Symposium which attracted 400 delegates from all over the world. The SCRI has a world-wide reputation for research on nematode vectors of plant viruses, on potato cyst nematodes (PCN, *Globodera pallida* and *G. rostochiensis*) and for fundamental studies on nematode genetics and secretions. In the past 5 years, we have been involved in seven EU-funded shared cost projects (value to SCRI >£1 million), two grants from the British Potato Council (£250,000), two Link project (£300,000), and various other grants and commercial contracts (>£100,000). Currently, there are eight Ph.D. students in Nematology.

Nematode damage is increasing - nematologists are decreasing Nematodes are hidden in the soil and, consequentially, the damage they cause as crop pests, and their beneficial role in helping cycle soil nutrients, is not widely appreciated. The importance of nematodes to agriculture is continually increasing. As agriculture has become progressively more intensive throughout the world, so the area of nematode infested land, and levels of infestation have increased. Nematicide use has also increased, with all the associated costs, including increased damage to the environment. This has already led to some nematicides being banned, the use of others being restricted, and a search for more acceptable methods of controlling nematodes. In contrast to the increasing importance of nematodes, the number of applied nematologists is decreasing, and there is a swing to more fundamental studies.

Figure 1 *Caenorhabditis elegans* - the first metazoan to have its complete genome sequenced.

Molecular approaches have attracted considerable funding in recent years. However, rapid advances in molecular techniques, particularly in the rate at which DNA can be sequenced, are creating new demands. Sequencing the entire expressed genome of the 'model' nematode *Caenorhabditis elegans* (Fig. 1) could now be done in 3 months, and sequencing of almost the entire human genome was achieved almost 2 years sooner than initially anticipated. But, as almost half of the genes sequenced so far have no known analogues, their functional analysis is now an increasing priority. Progress will be greatest if disciplines collaborate (especially molecular and biological/ecological), and if increased priority is given to biologists and ecologists. As SCRI is a multi-disciplinary Institute, it is well placed to be at the forefront of such research.

Three reasons for nematological research Soil nematodes are of research interest because :

1) nematodes are also a major component of the soil microfauna, and differences in their occurrence, abundance and community structure have the potential to provide unique information regarding the soil environment;

2) they are of practical interest because of the damage caused by plant parasitic species;

3) they are of fundamental interest because
   i) *C. elegans* was the first animal to have its genome sequenced,
   ii) many of the most damaging plant parasitic nematodes have evolved complex interactions with their hosts.

Additionally, the infective stages of many of the nematodes which parasitise mammals, arthropods and molluscs, live in the soil.

Ecology/soil processes

Nematode are the most abundant animals in the world. Soil populations are typically *c.* 100 billion (1 x 10^{11}) per ha. Their communities typically comprise more than 50 species, including plant parasites, the juvenile stages of parasites of animals and insects, fungal and bacterial feeders, omnivores and predators. They are potentially holistic indicators of soil processes as they are active within the soil throughout the whole of the year and are much easier to extract, count and study than bacteria or fungi. The different trophic groups can usually be recognised from differences in the structure of their feeding apparatus. Soil nematodes also comprise different ecological groups. Some have short life cycles and potentially rapid rates of population increase (*r* strategists), others have long generation times and reproduce slowly (*K* strategists). We have shown that the majority of soil nematodes are beneficial as they help mineralise and cycle nitrogen and other plant nutrients, or parasitise fungal, insect and mollusc pests. Only plant and animal parasites are harmful. Two examples from our research of the wider value of studying nematode ecology are given below.

Temperature and development (thermal-time) We showed that there is a linear relationship between temperature and rates of nematode development (Fig. 2). This enabled us to determine the specific thermal-time requirements of nematodes for different developmental processes (Table 1). The cardinal values for development are the base temperature (T_b - below which there is no development) and the heat sum (*S*) (expressed in ^{o}C days above T_b). Differences between nematode species, some derived from the literature, are given in Table 1. It is apparent that values for T_b vary and that some nematodes have a much higher rates of development (smaller value of *S*) than others, *e.g.* the free-living, bacterial-feeding species *C. elegans* can have four generations (43^{o}C days per generation) in the time required for an egg of the plant-parasitic *Longidorus elongatus* to hatch (154^{o}C days).

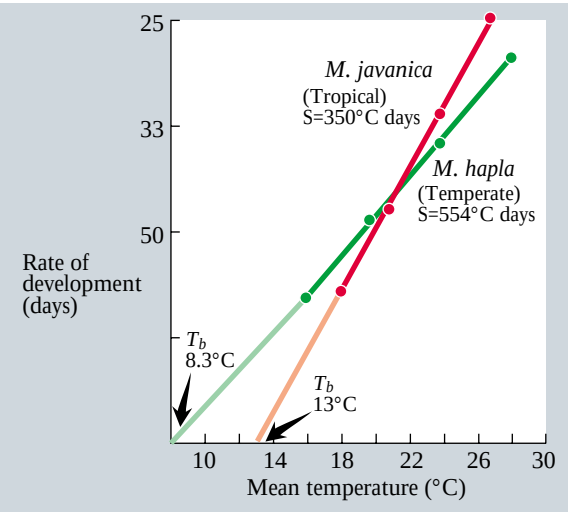

Figure 2 Relation between temperature and rates of development of a tropical *(Meloidogyne javanica)* and temperate *(M. hapla)* nematode.

Species (food source)	*Tb* (°C)	*S* (°C days)
Embryogenesis		
Haemonchus contortus (animal parasite)	6.6	15
Aphelenchus averne (fungal feeder)	8.3	31
Meloidogyne javanica (plant parasite)	13.0	138
Longidorus elongatus (plant parasite)	8.5	154
Complete life cycle		
Caenorhabditis elegans (bacterial feeder)	5.3	43
Goodeyus ulmis (bacterial/fungal feeder)	0.9	114
Meloidogyne hapla (plant parasite)	8.3	554
Meloidogyne javanica (plant parasite)	13.1	343

Table 1 Base temperature *(Tb)* and thermal requirements (*S*; °C days) for embryogenesis and one generation of different nematodes.

Thermal time has been applied to show that root-knot nematodes can have several generations on crops growing in tropical conditions, that potato cyst nematode can be controlled by trap crops lifted at the correct time, and that the northern root-knot nematode (RKN; *Meloidogyne hapla*) does not pose a threat in Scotland. But its most important contribution has been the general hypothesis that differences in *S* within many poikilothermic groups (nematodes, insects, plants) often reflect differences in their ecological strategies and, more widely, that differences in T_b reflect the thermal environment to which each species is adapted. A comparison of the requirements of two species of RKN, temperate *M. hapla* and tropical *M. javanica*, demonstrated that there was an inverse relationship between T_b and *S*, so that as one increases, the other decreases (Table 1). This ensures that each species develops faster than its relatives in the environ-

	Temperature (°C)			
	10	15	20	25
	No of days			
Meloidogyne hapla (temperate)	316	82	47	33
Meloidogyne javanica (tropical)	No development	171	49	29

Table 2 Effect of temperature on minimum duration of one generation for a temperate and tropical species of nematode.

ment to which it is adapted, *e.g.* temperate *M. hapla* develops fastest than tropical *M. javanica* below 21°C, but the converse occurs above 21°C (Table 2).

Nematode communities and populations Agricultural soils are one of our most valuable natural resources, and their long-term, sustainable management is crucial. However, modern agriculture makes increasing demands on soil, including expecting it to cope with a range of 'pollutants'. These include deposition of 'diffuse' pollutants from the atmosphere and a wide variety of pesticides. British soils receive about 30,000 t of pesticides per annum, but we have an incomplete understanding of their long-term, wider effects. Whilst soil nematodes are potentially a holistic indicator of soil conditions, we lack a good understanding of the factors that regulate their community structure and population densities. In 1997 and 1998, large patches of poor growth were observed in some Scottish cereals (Fig. 3). One area of damage was investigated and found to be heavily infested with a migratory ectoparasitic nematode (*Tylenchorhynchus* spp.) whose numbers had increased to 20,000 per kg soil (60 billion per ha), many times greater than is usual. Why this should have happened is unknown, but other plant parasitic nematodes were scarce, suggesting that the community was unbalanced. The

Figure 3 Damage by *Tylenchorhynchus* spp. to spring barley.

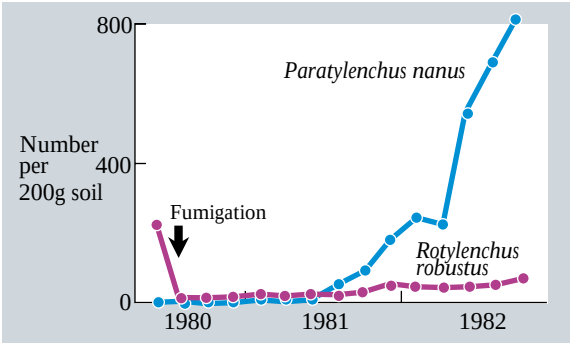

Figure 4 Rapid increase of *Paratylenchus nanus* (r strategist) after fumigation to control *Rotylenchus robustus* (K strategist).

results from some trials with soil fumigants indicate that the numbers of some *r* strategy species of nematodes (e.g. *Paratylenchus nanus*) can increase rapidly after treatment to levels much greater than previously (Fig. 4), suggesting that the *K* strategy species (e.g. *Rotylenchus robustus*) suppress the *r* strategists and maintain a balance.

Nematodes as crop pests

Nematode problems are increasing Modern agricultural practices actively spread nematodes in soil moved by machinery and irrigation water, and by the movement of infected plant material. In the 1970s and 1980s, the two species of potato cyst nematode (PCN) were estimated to infest less than 40% of potato fields in the UK, and the white species (wPCN; *G. pallida*) was much less common than the yellow species (yPCN; *G. rostochiensis*). The widespread and repeated growing of potato cultivars resistant only to yPCN has progressively changed the distribution and importance of the two species. A recent survey of 500 potato fields in England and Wales showed that 64% are now infested compared with only 40% a decade ago, and the white species was now present in 92% of infestations[2].

Similar changes involving other nematodes are occurring throughout the world. Examples include the cropping of virgin land in Burkina Faso, where damage due to RKN, *Meloidogyne* spp. typically appears in the second or third crop (Sawadago, pers. comm.). In Ecuador, a survey coordinated by SCRI of 207 horticultural crops showed that 99% were RKN infested. In the Netherlands, a serious pest of potato in northwest America, the Columbian RKN (*M. chitwoodi*), has recently been identified. The pine wilt nematode (*Bursaphelenchus xylophilus*), which has killed millions of trees in Japan and China, has just been reported

from Portugal. A Chinese Ph.D. student in Nematology, Miss Qing Chen, has just returned from China and reports that the pine forests around her hometown have been devastated since her last visit home (Fig. 5).

Figure 5 Scots pine showing damage caused by *Bursaphelenchus xylophilus* (Figure courtesy of Nemapix).

Once a damaging nematode has been introduced, intensive agriculture, often involving the frequent cropping of good hosts, generally increases the problem. Improved husbandry, which increases plant growth and yields, also provides more food for nematodes, further increasing nematode populations and damage. The difficulties of managing such problems are discussed below.

Nematicides and the problems of controlling nematodes in soil Chemical nematicides are the only means available for most farmers to actively protect their crops from nematode damage. Their use can be viewed from several perspectives. The perspective presented here is that, because nematodes are in soil, they are exceptionally difficult to control with chemicals and, consequently, rates of nematicide application are much higher than for other pesticides. Nematode populations are often huge; populations of PCN have to be >3,000 million eggs per ha before they cause damage, and may exceed 300,000 million per ha. To control such large populations, nematicides have to be mixed thoroughly in the soil. This is a considerable technical challenge because the cultivated soil-layer in each ha weighs *c.* 3000 t and contains up to 500 t of water, 100 t of organic matter, 800 m^3 of air, but <10 kg of nematodes (Fig. 6).

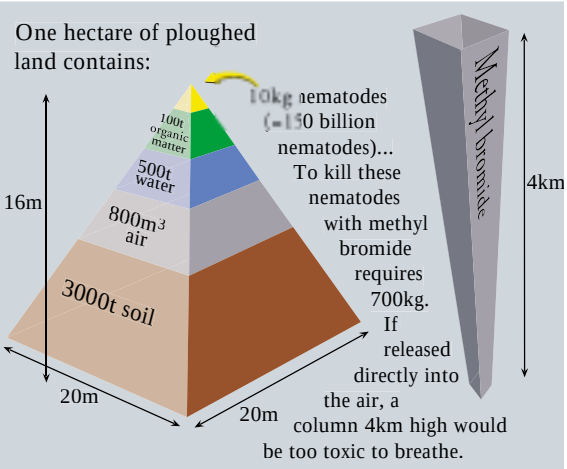

Figure 6 Schematic representation of the relative components of 1 ha of soil, and the volume of air that would exceed 17ppm methyl bromide following a standard rate of methyl bromide.

Nematicides – toxicity There are of two types of nematicide; fumigants that release toxic gases into the soil, and non-fumigants whose active ingredients dissolve and move in the soil water. Nematicides must remain active in the soil for sufficient time to exert the control required. For potato crops infested with wPCN, granular nematicides have to be active for >6 weeks to cover the extended hatching period. To try and over-come problems of incorporation, movement and persistence, nematicides are applied at much greater rates than pesticides applied to control foliar pathogens. Also, some nematicides are exceptionally toxic – only 1 mg of aldicarb (Temik 10G, widely used in the UK on carrots, potatoes and sugar beet) is needed to kill a rat weighing 1 kg (recommended rates of application are up to 3.3 kg a.i. per ha). The fumigant methyl bromide (used to control a range of soil-borne pathogens, particularly RKN, *Meloidogyne* spp.) is both very toxic and applied at a high rate (up to 700 kg per ha). If the recommended rate of methyl bromide was mixed into the atmosphere rather than in

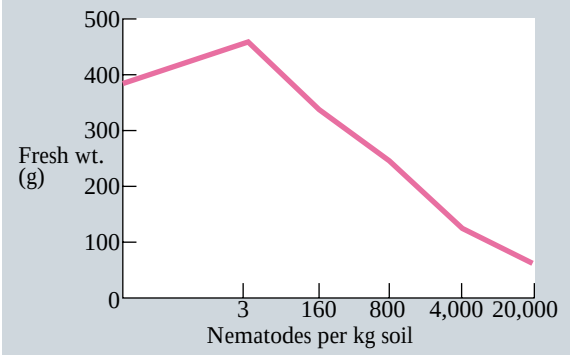

Figure 7 Effect of different numbers of *Meloidogyne incognita* at planting on the total fresh weights of tomato plants after 135 days.

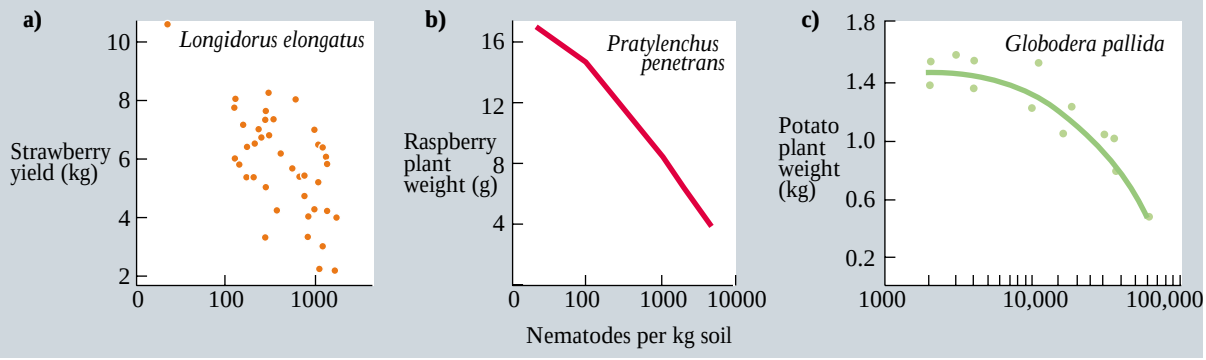

Figure 8 Relation between numbers of nematodes at planting (log scale) and yield for (a) strawberry infested with *Longidorus elongatus*, (b) raspberry plants in pots with *Pratylenchus penetrans* and, (c) potatoes with white potato cyst nematode *(Globodera pallida)*.

the soil, then a column of air about 4 km high above each treated field would exceed the 17 ppm limit above which a respirator should be worn (Fig. 6).

Environmental concerns Toxicity does not necessarily imply an unacceptable threat to consumers or the environment. In practice, where it is approved for use, aldicarb degrades rapidly to non-toxic products and is used safely. But fumigant nematicides, particularly methyl bromide, which is the third most important ozone-destroying chemical, have recognised environmental costs. The UK is a relatively small user of methyl bromide, but elsewhere much larger amounts are used. In Crete, 600 t are used annually, mainly to control RKN damage and wilt diseases, and the USA uses c. 16,000 t. Under the amended Montreal Protocol, the production and use of methyl bromide is being phased-out in most countries, but is still increasing in others[3]. Consequently, what is done to control nematodes in one part of the world can be of direct relevance to the others. Our contribution to developing a biocontrol agent effective against RKN, which could help replace methyl bromide, will be discussed later.

Nematicides also have a direct financial cost. Consequently, farmers are also interested in the development of strategies to effectively manage soil nematodes whilst minimising nematicide use. To be effective, such strategies often require the integration of several control measures and require a deeper knowledge of nematode ecology and biology than is required for chemical control alone. Four examples of our contribution in developing such knowledge are given below.

Modelling nematode damage and management Effective management of nematodes requires an understanding of the relationship between nematode population density at planting and crop growth. In contrast to aerial pathogens whose epidemiology is typically dynamic, nematodes are relatively immobile and reproduce slowly. Consequently, above a certain threshold, nematode damage is usually directly proportional to the nematode population density at planting (Figs. 7 & 8). Below the damage threshold, nematodes may even stimulate plant growth (Fig. 7). We have shown that the amounts of damage caused vary with the crop and nematode involved. *L. elongatus* (the needle nematode) is a migratory ecto-parasite that is particularly damaging to seedling crops such as carrots and perennial crops such as strawberry. In field trials, the threshold for damage to carrots was as few as 20 *L. elongatus* per kg soil, whereas much larger populations were required to damage strawberry (Fig. 8a). *Pratylenchus penetrans* (the root-lesion nematode) is a migratory endo-parasite that tunnels in plant roots. It has a wide host range and, in the mid-1970s, a series of field trials showed that the growth of newly

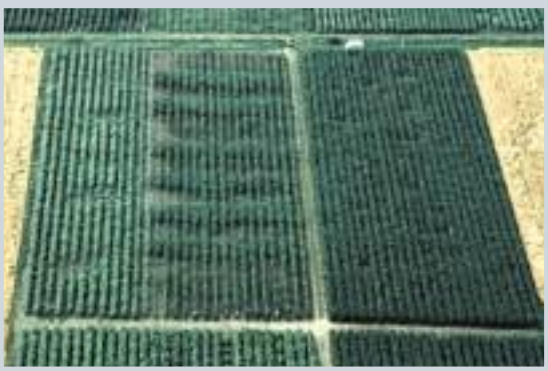

Figure 9 Three-year old raspberry plantation growing at a site with *Pratylenchus penetrans* where alternate strips were treated with nematicides prior to planting.

Figure 10 Tomato root system heavily galled by *Meloidogyne incognita*.

Figure 12 Females of white potato cyst nematode (*Globodera pallida*) on a potato root.

planted raspberry was consistently improved where it was controlled (Fig. 9). The growth of raspberry in a pot test was inversely proportional to the numbers of *P. penetrans* at planting, confirming its pathogenicity (Fig. 8b). *M. incognita* (a root-knot nematode) is a sedentary root endo-parasite that causes attacked roots to swell to form galls (Fig. 10). It has a short life cycle and, unlike most soil nematodes, populations can increase rapidly during one growing season. Consequently, it can be extremely damaging with a threshold as low as 60 eggs per kg soil (Fig. 7). *G. pallida* (wPCN) has a similar biology to *M. incognita*, but it is inherently less damaging because it has only one generation per year. Although the threshold for wPCN damage is c. 2000 eggs per kg soil, field trials showed (Fig. 8c) that population densities often become much larger, resulting in severe damage.

Based on knowledge of the relationship between numbers of nematodes at planting and damage, fields can be sampled prior to planting. Sampling errors tend to be large, particularly when nematodes are heterogeneously distributed, and much work has been done to identify appropriate sampling strategies. Currently, most sampling is barely adequate for making decisions with regard to whether to plant a susceptible crop, or apply a nematicide. Long-term management that seeks to keep nematode population densities below the damage threshold, requires both an understanding of nematode population dynamics, and accurate sampling data to determine whether the management is being effective - and if it is not being effective, where it is failing.

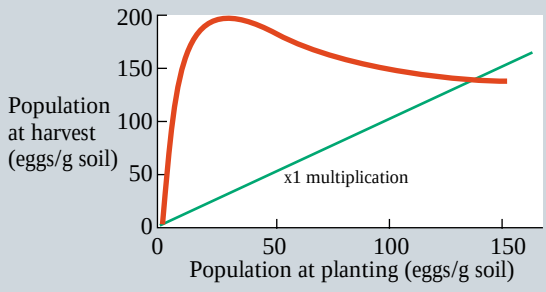

Figure 11 Relation between the numbers of potato cyst nematode at planting and at harvest for cv. Maris Piper with *Globodera pallida*.

For many nematodes, as the nematode population density at planting increases, so the density at harvest reaches a maximum, and then decreases (Fig. 11) because the size of the root system available for nematode feeding and development is progressively reduced. Our research, often in collaboration with others nematologists, has addressed a range of problems including the wPCN, *Globodera pallida*, tropical RKN, *Meloidogyne* spp., fanging damage in carrots, and replanting problems in raspberry. Progress, or otherwise, in managing each of these problems will be briefly discussed.

White potato cyst nematode (wPCN)

Our studies on wPCN (Fig. 12) have provided the database required to model the long-term threat that it poses to potato producers in the UK, and options for its management. The threat it poses is not always obvious because the progressive increase of wPCN is almost imperceptible, especially in seed potato land and ware land on long rotations. Modelling has shown that up to five potato crops are required for populations of wPCN to increase to damaging levels. However, wPCN is increasing everywhere, and is proving much more difficult to manage than the yPCN.

	Main effects of Scion			Stock		
	Cara	P. Dell	P. Javelin	Cara	P. Dell	P. Javelin
Total dry wt(g)	80	67	47	67	69	61
% decrease due to *G. pallida*	28	32	-	21	40	-
Tuber dry wt as % of total	30	51	70	51	54	50

Table 3 The average stock and scion growth of grafts between potato cv Cara and Pentland Dell or Cara and Pentland Javelin and the effect of *Globodera pallida* damage. Results means of 9 harvests.

Tolerance of Damage A basis for modelling the control of wPCN was laid by field trials which enabled us to quantify the effects on crop yield and nematode multiplication of interactions between wPCN population density at planting, soil type and cultivar resistance and tolerance of damage. Soil type was shown to affect rates of juvenile invasion, and, hence, both crop damage and maximum rates of nematode multiplication. Tolerance and resistance were shown to be independent characteristics with some resistant cultivars more tolerant, and some less tolerant of wPCN damage than susceptible cultivars. More detailed studies showed that PCN damage affected potato growth by reduced rates of nutrient uptake. This decreased rates of top growth, the amounts of light interception by the crop canopy, and yield. Vigorous, late maturing cv. Cara, which produces a large canopy, was shown to be more tolerant of damage, but to create long-term problems because it supported much higher populations of wPCN than most other susceptible cultivars. Grafting experiments between early/late maturing, and tolerant/intolerant cultivars showed that both scion and stock genotypes influenced tolerance (Table 3).

Resistance and virulence The efforts of the potato breeders at SCRI, and elsewhere, to breed for resistance to wPCN have been supported by screening, each year, up to 10,000 plants. We have also provided the strategic nematological under-pinning to the breeding programme. Achievements include demonstrating that resistance in *Solanum vernei* and *S. andigena* CPC 2802 is quantitatively inherited. We also showed that the virulence of populations of wPCN differed (Fig. 13) but that different wPCN populations still tended to rank resistant clones in the same order. Consequently, it is acceptable to use just one population of wPCN to routinely screen for resistance. Importantly, clones derived from *S. andigena* were shown to have greater and more consistent resistance than those derived from *S. vernei*, emphasising that breeding should concentrate on the former. However, selection for increased virulence in wPCN was demonstrated following the repeated growing of such clones.

Modelling integrated control of PCN Our prediction that the repeated growing of potato cultivars resistant only to yPCN (*G. rostochiensis*) would progressively select for the wPCN (*G. pallida*) has been dramatically confirmed. Anticipating this change, our research in the 1980s focused on factors important in the management of wPCN. Achievements include confirming in field trials that wPCN damage and population dynamics can be described by simplified density-dependent equations, and demonstrating that these equations can be given predictive value by incorporating the tolerance associated with potato cultivar

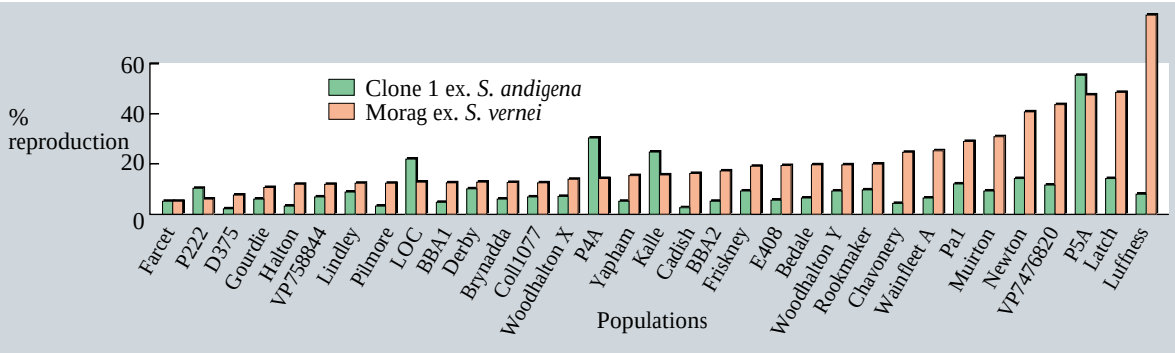

Figure 13 Percentage virulence on two clones of partially resistant potato, compared with susceptible cv. Desirée, of forty populations of *Globodera pallida* from the UK, Europe and South America.

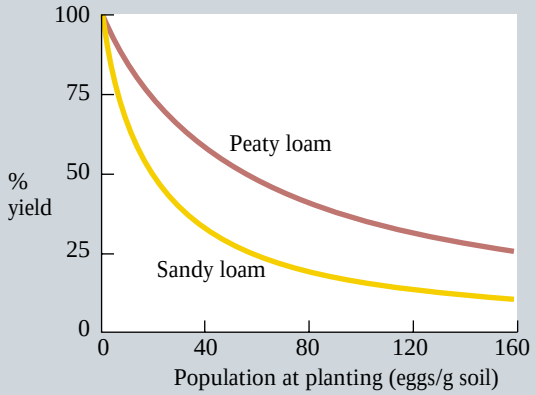

Figure 14 Percentage yield reduction for cv. Maris Piper due to *Globodera pallida* at a peaty loam and a sandy loam site.

and soil type (Fig. 14). The effects on wPCN multiplication of partial resistance and nematicides were shown to be additive – a crucial observation for their use in the integrated control of wPCN (Fig. 15). With funding from the British Potato Council, these results and equations are being used to develop a computer-based programme able to model the impact of different control measures on the long-term management of wPCN. This model shows that nematicides are most effective in managing wPCN when used against small (when the wPCN multiplication rate is at its maximum) rather than large populations (Table 4). It also shows that in high yielding fields, wPCN is unlikely to be controlled without integrating rotation, nematicides and partial resistant cultivars (Fig. 16). Even so, the effectiveness of this integration depends on rates of wPCN population decline between potato crops, nematicide effectiveness, and the virulence (ability to over-come resistance) of the wPCN, all of which are unknown in most commercial fields. To remedy this information deficit, there need to be fundamental changes in the strategy for monitoring wPCN, including the processing of larger samples, and sampling after, as well as before each potato crop.

Before Planting (eggs/g)	After Harvest (eggs/g)	
	No Nematicide	Nematicide
100	147	190
20	190	84
4	84	25
0.8	25	5

Table 4 Effect on *Globodera pallida* population densities (eggs/g soil) at harvest of applying a nematicide (80% kill).

Figure 15 Two strips of nematicides applied across a field infected with potato cyst nematode.

Root-knot nematodes

Three species of RKN, *M. arenaria*, *M. incognita* and *M. javanica*, cause extensive root galling in susceptible crops (Fig. 10) and are amongst the world's most important crop pathogens. They have world-wide distributions in tropical and Mediterranean regions, where they attack and seriously damage almost all the major crops. Coffee, cotton, tobacco, fruit-trees, and almost all vegetable and horticultural crops are damaged. Related species damage cereals, including rice. In the UK, *M. hapla* damages carrots and other horticultural crops, and potatoes are threatened by *M. chitwoodi*, recently identified as causing problems in the Netherlands. Without using methyl bromide, RKN are extremely difficult to manage because of their short generation time (1 month at 25^{o}C), high reproductive rate (2000 eggs per female) and very wide host range (most weeds and crops).

RKN damage in Ecuador During an EU-funded study, coordinated by SCRI, the damage caused by

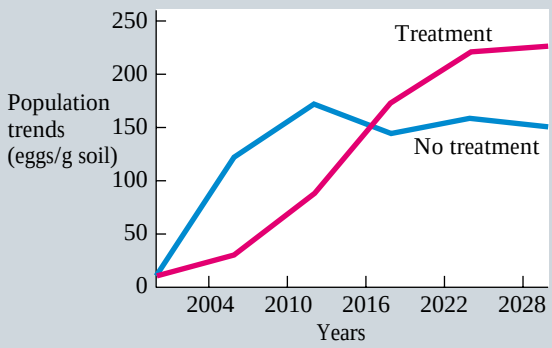

Figure 16 Increases in population density of *Globodera pallida* on cv. Maris Piper on a peaty loam soil and 6 year rotation, with and without a nematicide (80% kill).

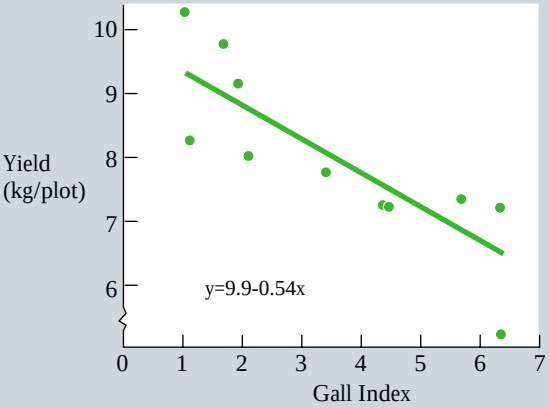

Figure 17 Relation between root gall index due to damage by *Meloidogyne* spp. and yield of tomato in a micro plot trial in Tanzania.

Figure 18 Juvenile *Meloidogyne* spp. carrying spores of *Pasteuria penetrans*. Picture IACR, Rothamsted.

RKN was determined by field trials and surveys in Ecuador, and in several other tropical countries. A total of 207 horticultural crops were sampled in Ecuador (many of them treated with nematicides) and 205 were found to be RKN infected (C. Trivino, pers. comm.). The mean damage (gall) index was 5.5, and the trials in Ecuador and Tanzania showed (Fig. 17) that yield was decreased by between 5% and 6% for every unit increase in the damage index, *i.e.* RKN is decreasing yields in Ecuador by between 25% and 30%.

Biological control of RKN The problems of managing RKN by rotation and with nematicides has encouraged study of alternatives. The project in Ecuador also examined the utility of a bacterium (*Pasteuria penetrans*) as a biological control agent for RKN. This bacterium has several attributes. It has an enormous rate of increase; each infected female RKN releases into the soil c. 2 million spores of *P. penetrans*; the spores persist in the soil for many years; and they are specific, only attaching to the soil migratory, juvenile stage of RKN (Fig. 18). Infected juveniles carry the spores into the plant root when they invade, but the nematodes are not killed until they have developed into large, adult females – each of which is packed with spores.

A survey in Ecuador by Dr Carmen Trivino showed that c. 30% of RKN populations were infected with *P. penetrans*, but that levels of infection were generally low. Laboratory experiments by Dr Mireille Fargette in France showed that the rates of infection probably varied because there are great variations between isolates of *P. penetrans* in their ability to infect RKN, and between populations of RKN in susceptibility to isolates of *P. penetrans*. Attempts to increase suppression of RKN at sites with *P. penetrans* by the repeated and intensive cultivation of crops susceptible to RKN were ineffective. However, the introduction into these trials of small amounts of an exotic isolate of *P. penetrans* resulted in a large increase in the proportion of spore-

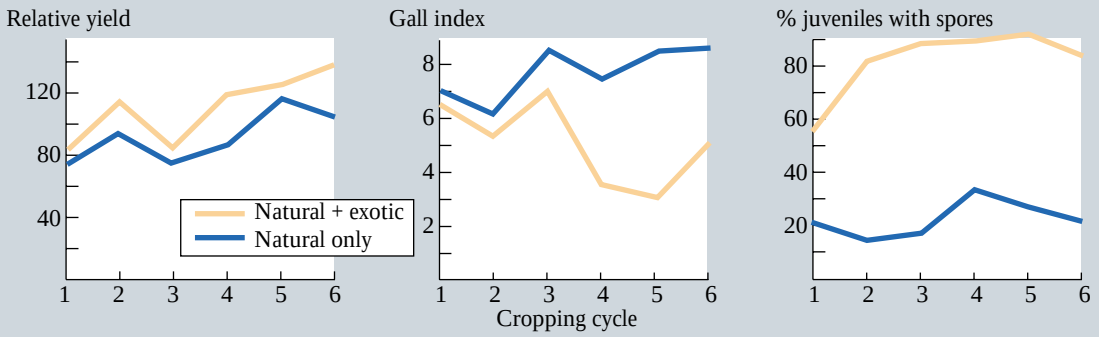

Figure 19 Relative yields of bean and tomato, gall index and percentage of juveniles of *Meloidogyne incognito* carrying spores of *Pasteuria penetrans* over five cropping cycles. Results are for plots with a natural infestation of *P. penetrans*, without and with the addition of an exotic isolate.

Figure 20 'Fanged' and stunted carrots due to nematode damage.

infected juveniles, decreased root galling, and increased yields (Fig. 19). These results strongly suggest that, in natural infections, a balance develops between the host and parasite such that levels of infection remain relatively low. Introducing an exotic isolate of *P. penetrans* that infected most or all of the RKN juveniles, produced an epidemic and greatly increased suppression.

Figure 21 Aerial view of 3-year old raspberry plantation with 'sick' patches.

Fanging damage in carrots and parsnips

Damage to the tap roots of seedling carrots and parsnips leads to 'fanging' (Fig. 20). Fanged carrots have to be removed, decreasing yields and increasing costs. The granular nematicide aldicarb (Temik 10G), applied in the drill at planting, is used to decrease fanging, but the specific nematodes involved were unclear. Studies at 20 sites with Dr M. Groom and Ms E. Horring showed that aldicarb decreased fanging from a mean of 21% to 7%, and that the degree of fanging was correlated ($r^2 = 0.31$, $P = 0.01$) with the numbers of *Longidorus* spp. The presence of RKN (*M. hapla*) and cyst (*Heterodera carrotae*) nematodes accounted for further damage. Despite these results, aldicarb use is not based on an estimate of the potential risk, and many fields are treated unnecessarily.

Replant problems in raspberry

Raspberries tend to be replanted in the same fields, resulting in raspberry 'sick' land (Fig. 21). Although the causal agents were initially unknown, a pre-planting treatment with the granular soil sterilant dazomet (Basamid) was found to minimise replant problems (Fig. 9). Pot tests demonstrated that the root-lesion nematode (*Pratylenchus penetrans*) was a major cause of the problem (Fig. 22), but an unidentified fungus was also involved at some sites. In pot experiments with soil from problem fields, deep-freezing soil markedly improved subsequent raspberry growth. Treating the soil with the fungicide benomyl or the nematicide aldicarb slightly improved growth but, when applied together, they improved growth as much as deep-freezing (Table 5). These results confirmed that raspberries can suffer replanting problems, and suggest that rotation may be of value. However, once *P. penetrans* has been introduced, it is likely to persist as it has a wide host range. A survey of planting stocks showed that a substantial proportion (20%) carried *P. penetrans* and, based on these results, spawn nurseries were sampled to ensure freedom from *P. penetrans*.

Fundamental studies

Continued progress in managing nematodes has to be under-pinned by relevant fundamental and basic studies. Such studies usually also have wider scientific relevance. Two examples are given, both involving PCN.

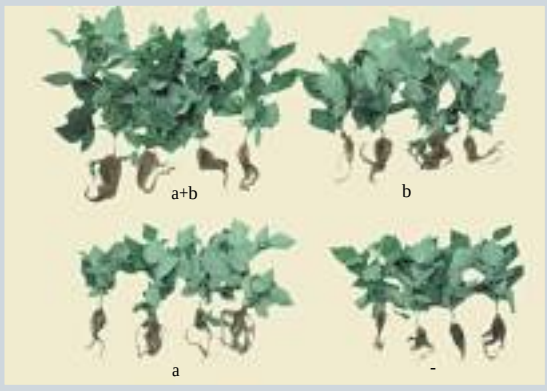

Figure 22 Raspberries grown in soil from a 'sick' plantation treated with a nematicide (aldicarb; a), a fungicide (benomyl; b), or both together.

Experiment/Site	Untreated	Nematicide	Fungicide	Nematicide + Fungicide	Frozen
	Mean plant weight (g)				
1.	18	49*	40*	-	48*
2.	16	24	23	49*	46*
3.	7	6	15*	24*	-
4.	9	13	23*	40*	-
5.	15	13	24*	36*	-
6.	48	39	52	61*	-
7.	20	45*	24	58*	-
8. (sterilised soil)	20	21	23	21	-

*Significantly different from untreated P>0.01.

Table 5 Soils from fields with raspberry re-plant problems treated with a nematicide, fungicide or frozen (-22°C). Results are from different experiments and are mean raspberry plant weights.

Heterogeneity in PCN and RKN

Potato cyst (PCN), and root-knot (RKN) nematodes have been widely spread around the world by agriculture. PCN came from the Andean region of South America, but the origins of the most widespread and damaging RKN species (*M. incognita, M. javanica*) are unknown. Each species of PCN and of RKN has been sub-divided into 'pathotypes' and 'races' respectively that differ in their host range. It was suggested that some of the variation in virulence of the wPCN (*G. pallida*) on resistant cultivars of potato (Fig. 13) was due to the introduction of different populations and gene-pools from S. America. A range of molecular techniques was used to explore this hypothesis. At the same time, the hypothesis that variation will be greater with sexually reproducing wPCN than in parthenogenetic RKN was also tested.

RAPDs Short regions of DNA from a range of UK, European and S. American populations of wPCN were amplified at random using the polymerase chain reaction (PCR). Because juvenile wPCN are so small (0.5 mm), many 1000s of nematodes were required to provide the DNA for this analysis (this contrasts with studies on larger organisms, *e.g.* plants, where all of the required DNA, which is guaranteed to be homogeneous, can be obtained e.g. from a single leaf). Whilst there were differences in RAPD profiles between some 'pathotypes', the European populations of *G. pallida* formed a heterogeneous group distinct from two S. American populations and from populations of *G. rostochiensis* (Fig. 23a). Similar studies with populations of six species of RKN from widely distributed origins produced fewer and more reproducible bands, and there was less variation within species, especially for *M. incognita* (Fig. 23b). We concluded that, with wPCN, the variation observed reflected substantial heterogeneity within each population, whereas with RKN, the less complex and consis-

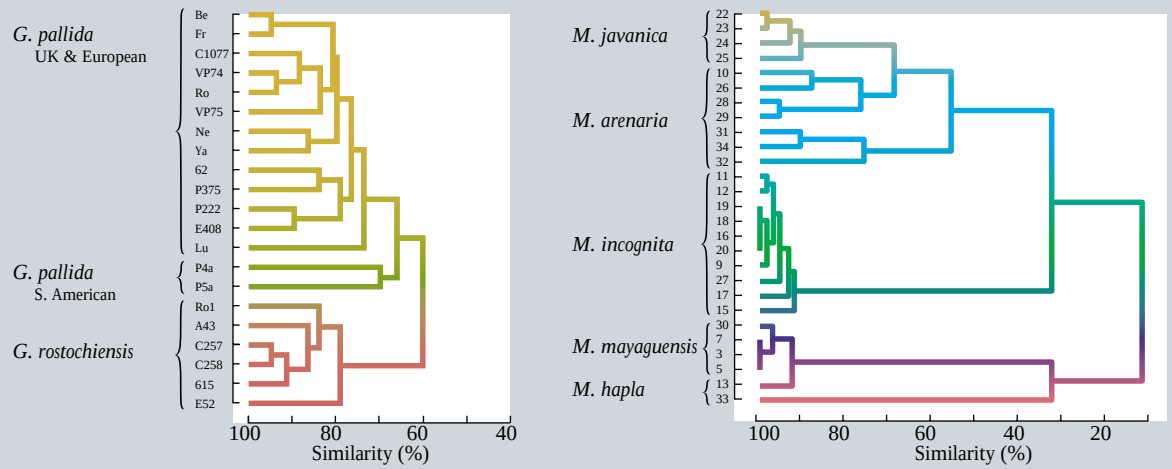

Figure 23 Dendogram summarising RAPD results for populations of (a) both species of PCN and (b) six species of RKN.

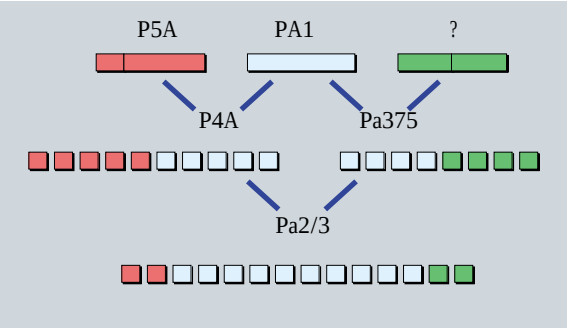

Figure 24 Differences in ITS ribosomal DNA in populations of *Globodera pallida*. Populations of the common UK pathotype Pa 2/3 contain three types of ribosomal DNA that may have resulted from population hybridization.

tent banding patterns reflected the homogeneity of each population that is a consequence of parthenogenetic reproduction.

Ribosomal DNA (rDNA) For nematodes, the nuclear genome contains multiple copies of tandemly repeated rDNA, which can be amplified by PCR. The internal transcribed region of the rDNA was amplified from various populations of wPCN and the products digested with a range of enzymes (endonucleases) that cut the DNA only where specific sequences occur. This process produces fragments of rDNA which may vary in length. Populations of the wPCN from South America and from Northern Ireland, which represented different pathotypes (P4A, P5A, and Pa1), were distinct, whereas, populations from England were very similar (Fig. 24). However, the English populations appeared to contain the greatest number of restriction sites, apparently resulting from different types of ITS regions occurring within individual nematodes. This suggests that they may be derived from a mixture of distinct gene pools that had interbred. In contrast, for the RKN, *M. incognita*, *M. javanica* and *M. arenaria*, this region has very little sequence variation even when these species are compared. This suggests a recent divergence.

Mitochondrial DNA (mtDNA) The suggestion that there might have been several introductions of *G. pallida* into the UK, was examined by studying the mitochondrial DNA (mtDNA) of a range of populations from the UK and South America. In animals, mtDNA occurs in the mitochondria as a circular molecule coding for 12-13 protein genes and 24 transfer RNAs, which requires a minimum of 13,000 bases. MtDNA is ideally suited for studying colonisation by animals because it is inherited maternally, it evolves more quickly than the nuclear DNA, and each cell contains many identical mtDNA copies. Consequently, distinct introductions of PCN from different parts of South America were likely to have differences in their mtDNA that could be correlated with virulence differences. The results showed that with the exception of the exceptionally virulent 'Luffness' population and the avirulent Pa1 from N. Ireland (Fig. 13), UK populations of *G. pallida* were not distinct, suggesting that they derive from a single introduction.

However, the two populations from South America differed greatly (Fig. 25). The mtDNA from the P4A population was even more diverse than the UK populations, and could have been the progenitor of the UK populations. The P5A population was so different that it suggests that it may be a new species.

Unique organisation of PCN mtDNA. During these studies, the mtDNA of *G. pallida* was found to be organised uniquely, posing fundamental questions regarding the inheritance and functioning of mtDNA. Instead of a single circular molecule of >13,000 bases,

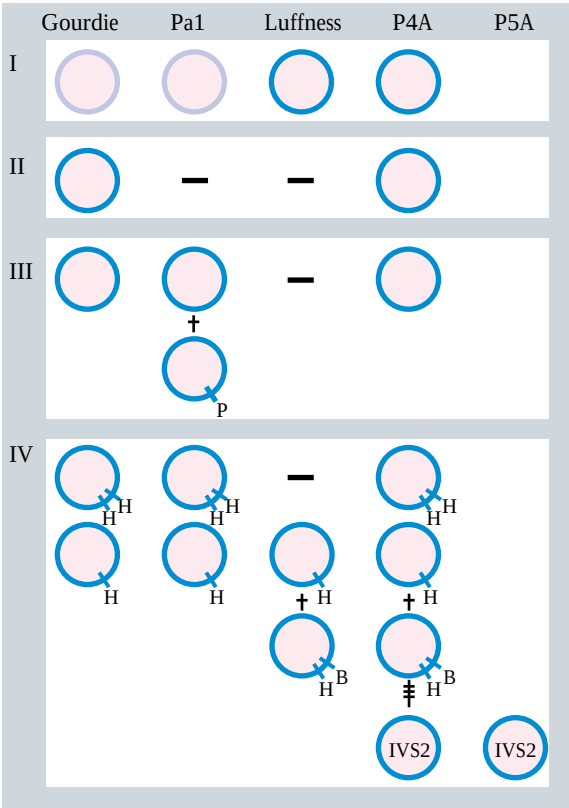

Figure 25 Occurrences of four mitochondrial mini circles in a typical UK *Globodera pallida* population (Gourdie), in two distinct populations from the UK (Pa1, Luffness), and two populations from S. America (P4A and P5A).

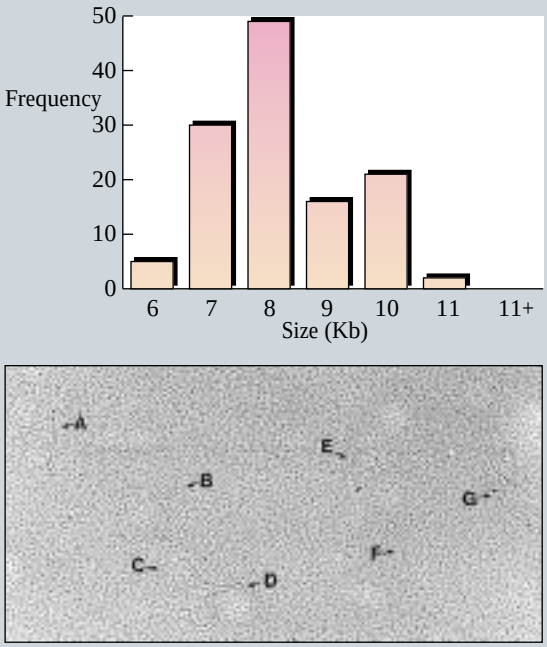

Figure 26 Electron microscope photograph of mitochondrial mini circles of *Globodera pallida* (A-G), and histogram of their estimated size distribution.

the mtDNA of all the populations studied was comprised of several smaller molecules of 6,000 to 10,000 bases (Fig. 25). Initially, we were concerned that these were artefacts or relatively rare mutants, but their occurrence and lengths were confirmed under the electron microscope (Fig. 26) and by sequence analysis.

Nematode-plant interactions

Some nematodes induce specialised feeding sites in their hosts. These provide the nematodes with greatly increased amounts of food, enabling them to have high rates of reproduction and, consequently, become serious crop pests. They induce these changes by injecting, through their hollow stylet, secretions from oesophageal gland cells into the root cells around their heads (Fig. 27). These cells become enlarged, mulinucleate and much more active. Understanding the initiation and regulation of these profound changes in plant gene expression is of fundamental interest to both animal and plant scientists, and should also provide novel options for control. Plant resistance to nematodes, and mechanisms of nematode virulence (ability to circumvent plant resistance) are other topics of wide fundamental interest with potential practical relevance.

Secretions Studies on nematode secretions are an important area of collaborative research involving colleagues in the UK and Europe. Secretions of nematodes are important for a variety of reasons, including the changes they induce in the plant root cells (see above) which provide endoparasites such as *Globodera* and *Meloidogyne* with an abundant supply of food. Studies using plant promoters coupled to the GUS reporter gene have shown that, in inducing these changes, the nematode manipulates plant gene expression (Fig. 28). The secretions thought to be responsible originate from one or more of the three gland cells associated with their feeding apparatus. These gland cells also produce secretions involved in root penetration, and in the formation of the 'feeding tube' – a complex structure attached to the stylet-tip - that is secreted into the plant cell prior to each bout of ingestion. Nematodes also produce secretions from the two chemosensory amphids on their head, from the 'excretory pore', and from glands associated with the reproductive system. Proteins are also found coating

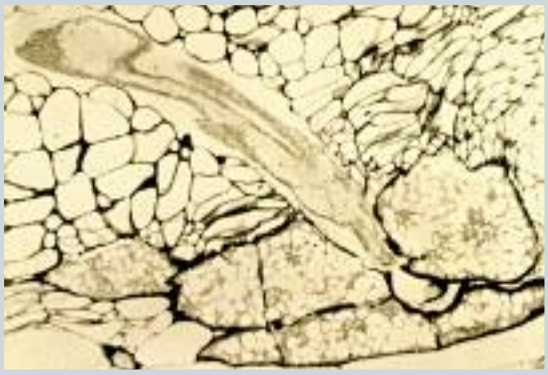

Figure 27 Longitudinal section along a root containing a giant cell system associated with a developing juvenile *Meloidogyne*.

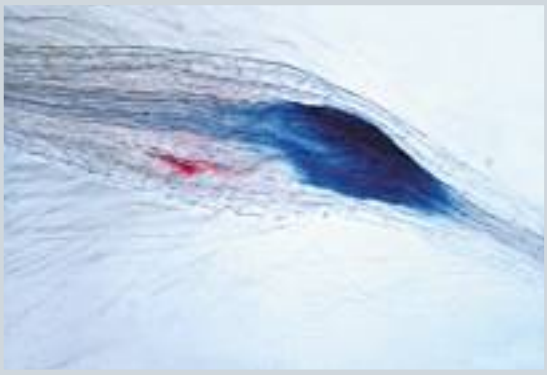

Figure 28 Expression of GUS reporter gene (blue staining) in a feeding site induced by developing *Meloidogyne* juvenile (red).

Figure 29 Surface of juvenile *Globodera pallida* stained red by the specific attachment of an antibody raised against a secreted fatty acid-binding protein.

the nematode surface (Fig. 29) that may be secreted directly through the cuticle. We developed various techniques to collect and study these secretions, but the analysis of gene expression using ESTs (expressed sequence tags) has been especially productive. This has led to the identification of a pectate lyase, the first gene of this type to be cloned from any animal. We have also identified genes encoding a thioredoxin peroxidase, glutathione peroxidases and a secreted fatty acid-binding protein. Studies of the sites of expression and properties of these genes suggest that one of the peroxidases and the fatty acid binding protein may be involved in protecting the nematode from host defences. Of particular interest is the indication that nematodes have been recruiting genes from other organisms. The cell wall degrading enzymes (cellulases and pectate lyases) strongly resemble orthologous genes from *Erwinia*. While there is evidence that horizontal gene transfer has occurred in these cases, the mechanisms by which this has happened remain unclear. Researchers at SCRI are now focusing on the development of systems that will allow the function of potentially interesting nematode genes to be tested *in vivo*.

Figure 30 *Longidorus* nematode feeding on a galled root-tip. Note the sand grain (bottom right).

Virus vectors

The nematologists at SCRI have been at the forefront of research on virus transmission by nematodes since the inception of this relatively young research area. This has partly been achieved by close collaboration with virological colleagues and this unique combination of research skills has resulted in numerous requests both nationally and internationally for assistance and collaboration with nematode and virus problems. The research is highly focused and extends from fundamental core-funded studies to externally funded, pre-commercial PCR-based molecular diagnostics (PCR-MDs).

Some of the achievements include developing methods for conducting transmission tests with *Longidorus*, *Xiphinema* and trichodorid spp., whereby each step in the transmission process (rates of feeding, virus ingestion and retention during the acquisition phase, and rates of feeding and virus infection during the transmission phase) can be quantified (Fig. 30). Using this approach, it became clear that many reports of nematode transmission were of doubtful validity. We established criteria for demonstrating virus transmission and, based on these, almost two thirds of the reports of viruses being transmitted by specific nematodes were rejected. Subsequently, new associations and crop problems have been identified by SCRI staff, including *Paralongidorus maximus* as a vector of an atypical strain of *Raspberry ringspot nepovirus* in vineyards in Germany; *Longidorus arthensis*, a previously undescribed

Figure 31 A cherry tree showing typical bare-branch symptoms caused by *Cherry rosette virus* transmitted by *Longidorus arthensis.*

Figure 32 Yellowing diseased in barley cv. Express caused by *Arabis mosaic virus* transmitted by *Xiphinema diversicaudatum*.

species, as the natural vector of a new nepovirus in cherry orchards in Switzerland (Fig. 31); *X. diversicaudatum* as the vector of a strain of *Arabis mosaic nepoviruses* causing a yellowing diseases in barley in Switzerland(Fig. 32); and *X. intermedium* and *X. tarjanense* as vectors of *Tobacco* and *Tomato ringspot nepoviruses*. We have shown also that *Tobacco rattle tobravirus* transmitted by trichodorid species is widespread not only in northern Europe, but also in northern Greece and Portugal, and that the nematode also causes direct damage (Fig. 33).

By analysing rates of virus transmission, we demonstrated large differences between nematode species in the rates at which they would transmit their associated viruses and, in Europe, a high degree of specificity between viruses and their vectors. This specificity of association sometimes extended even to specific nematode populations and minor serological variants of the viruses. Collaborative studies with colleagues in North America, who are seeking to control virus

Figure 33 Stubby root system caused by the direct feeding of trichodorid nematodes.

Figure 34 'Spraing' disease in potato caused by *Tobacco rattle virus* transmitted by trichodorid nematodes.

problems in vines and fruit crops, have demonstrated the converse. The virus-vector species of *Xiphinema* in North America each transmit two or three distinct viruses, and each virus is transmitted by more than one species of nematode. However, within a species, some populations may not transmit virus, or may transmit only one, rather than two or three, viruses.

A recent success has been the development of techniques for studying the specificity of the transmission of the distinct strains of *Tobraviruses* by species of trichodorid nematodes. We are the only centre in the world able to conduct such studies and have demonstrated that each strain of *Tobraviruses* has only one or two species of trichodorid nematode as its vector (Table 6). Consequently, in potato fields with a 'TRV-spraing' problem (Fig. 34 & Fig. 35) in the tubers and where typically two or three trichodorid species will be present, only one species may be transmitting the virus.

These specialised techniques (Fig. 36) also have been

Figure 35 Potato crisps made from: left, healthy potato; right, potato with 'spraing' disease.

Nematode	Tobravirus	Strain
P. allius	TRV	USA
P. anemones	PEBV	English
	TRV	PaY4
P. minor	PRV	Brazil
	TRV	USA
P. nanus	TRV	PRN
P. pachydermus	PEBV	Dutch
	TRV	PRN (PpK20)
		PaY4
P. teres	PEBV	Dutch
	TRV	Oregon
P. tunisiensis	TRV	Italian
T. cylindricus	PEBV	English
	TRV	RQ
		TcB28
T. primitivus	PEBV	TpA56
	TRV	TpO1
T. similis	TRV	Ts-Belgian
		Ts -Dutch
		Ts-Greek
T. viruliferus	PEBV	English
	TRV	RQ

Table 6 Associations between *Paratrichodorus* and *Trichodorus* species and serologically distinguishable strains of *Pea early-browning* (PEBV), *Pepper ringspot* (PRV), and *Tobacco rattle* (TRV) tobraviruses.

used successfully in fundamental investigations, in collaboration with the virologists, to study the genetic determinants of nematode transmission of tobraviruses. This has revealed that the virus coat protein and one or two non-structural proteins are involved. Using these same techniques, it was demonstrated that coat protein-mediated transgenic resistance is ineffective when TRV is naturally transmitted by trichodorid nematodes.

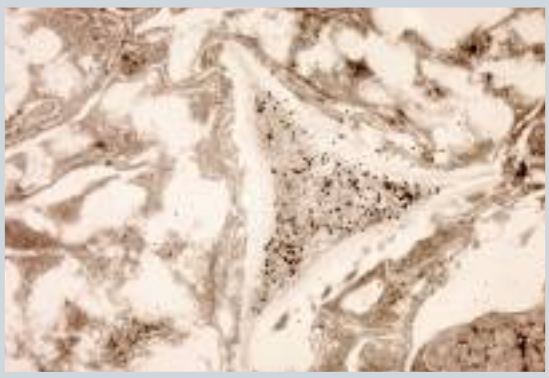

Figure 36 Immunogold (black circular colloidal gold particles) labelling specifically attached tubular *Tobacco rattle virus* particles at the site of virus retention in the feeding apparatus of a trichodorid nematode.

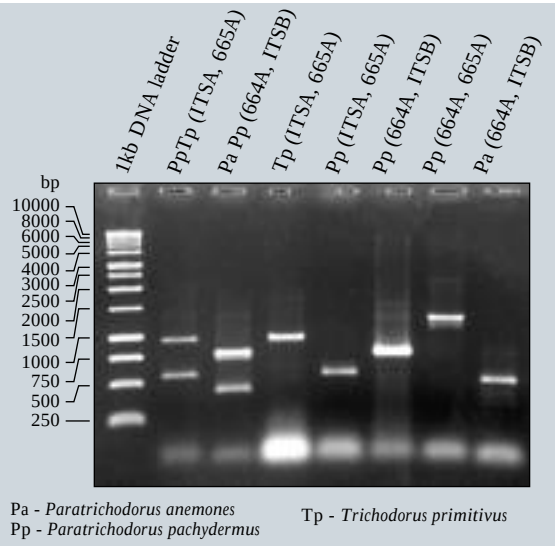

Figure 37 A PCR based molecular diagnostic using several primer sets distinguishing three trichodorid species that occur commonly in Britain and northern Europe.

Most recently, PCR-MDs have been developed for pre-plant and post-harvest detection and identification of trichodorid species (Fig. 37) and serologically distinguishable strains of TRV. These techniques can be used to detect and identify individual trichodorid species in nematode populations extracted from soil samples, and TRV has been reliably detected from

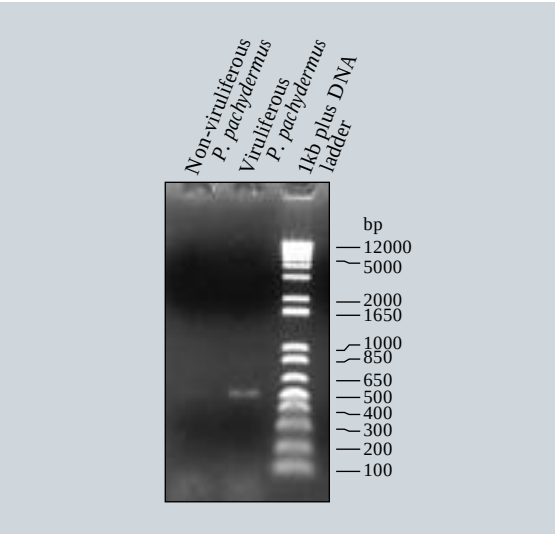

Figure 38 A reverse transcriptase PCR based molecular diagnostic using a universal primer set for detecting *Tobacco rattle virus* revealing the presence of the virus in an individual trichodorid nematode.

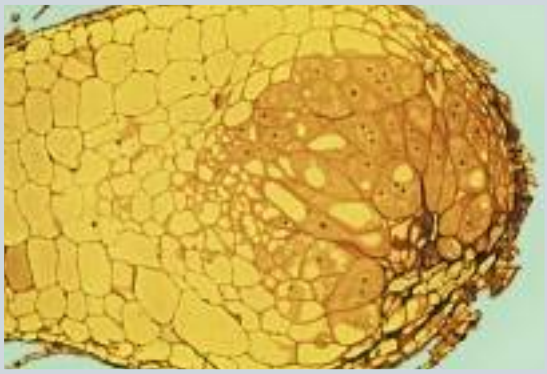

Figure 39 Root tip gall induced by *Xiphinema index* containing multinucleate cells. Photo courtesy of U. Wyss.

individual nematodes (Fig. 38). Current research is focused on obtaining trichodorid species-specific primers for use in PCR-MDs.

The Future

I have deliberately tried to give a relatively non-technical presentation of nematology, and to demonstrate the difficulties of managing nematodes compared with above-ground pathogens and pests. Nematicides are likely to be required to integrate with other methods to protect many crops for the foreseeable future, and more needs to be done to ensure that they are used effectively and with minimum impact on the wider environment. Nematode-plant interactions are an exciting area of research where molecular studies analysing patterns of gene expression are likely to be especially productive and informative. Some *Xiphinema* spp. induce galls containing modified cells similar to those induced by RKN (Fig. 39), and these large nematodes provide a unique opportunity for studying the expression of their genes during different phases of their feeding. Plant resistance clearly has a central role in improving the management of soil nematodes, but often only as one component in an integrated system. Many are pinning their hopes on transgenic resistance and, for several reasons, we have concluded that seeking to transfer 'natural' resistance genes is the best way forward. We are collaborating with German colleagues to isolate a resistance gene (*Hero*) from tomato with a view to studying its expression in potato. Current EU-funded research is exploring changes in gene expression in plants in both susceptible and resistant interactions. However, there are many uncertainties regarding the long-term durability of all types of resistance, and isolating nematode virulence alleles and understanding their modes of action is a priority. For heterogeneous nematode species, such as wPCN, selection for increased virulence seems probable. But, resistance to more homogeneous species (e.g. the single pathotype of the yPCN introduced into the UK), and particularly for the mitotically parthenogenetic RKN, resistance may be surprisingly durable. However, even when one damaging species is controlled, experience indicates that there is often another, only slightly less dominant species of nematode waiting to take its place. Consequently, nematologists need to understand better the processes involved in the regulation of nematode communities and populations, and the processes involved in spread and colonisation. As c. 40% of soil nematodes reproduce asexually, whereas almost all aquatic and animal parasitic nematodes reproduce sexually, it is clear that the soil environment poses special problems for its inhabitants, including how to find a mate, and how to colonise a distant field. The soil environment also poses particular problems for nematologists, including how to sample effectively, and how to study processes such as gene-flow.

Hopefully, the foregoing provides an insight into the difficulties of studying and controlling soil pathogens, but also examples of quality science and progress. Whilst 'citation indices' are a major criterion for judging scientific quality and impact, specialist nematologists will tend to be at a disadvantage because much of their research is destined to be published in nematological journals with a limited circulation. Even so, papers from the group have been published recently in *Nature*, *Genetics*, *Genome*, *Functional Ecology* and *New Phytologist*, and members of the group have relatively high citation indices. This, and our extensive portfolio of external funded projects, has been achieved by integrating the different aspects of our research, including fundamental and applied, molecular (e.g. sequence) and biological (function), and by ensuring we are attractive partners in collaborative projects because of our expertise, particularly as nematologists.

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Oxygen, free radicals, antioxidants and food - some views from the supply side

B.A. Goodman & J.R. Hillman

Introduction to free radicals and antioxidants

Free radicals are chemical species (excluding transition metal and rare earth ions) with unpaired electrons. They are often, but not always, highly reactive, and in biological systems usually have only a transient existence. The reasons for free-radical reactivity lie in the electronic structure of atoms and molecules; the structure of dioxygen, O_2, and the products of its reactions are of particular importance.

All aerobic systems utilise metabolic processes, which are based on free-radical reactions, but some of the products of such reactions can be dangerous, if not properly controlled. Many chemical species are involved in the control of oxidative chemical processes in all biological systems, and these are often referred to as *antioxidants*. The words *antioxidants* and *free-radical scavengers* are often used interchangeably, but antioxidants are not necessarily free-radical scavengers, and free-radical scavengers are not necessarily antioxidants. For example, an extremely important antioxidation reaction is the control of hydrogen peroxide, H_2O_2, which is a precursor of the highly reactive hydroxyl radical, $\cdot OH$, but H_2O_2 is not itself a free radical.

Although biological processes are often complex, they are usually highly specific, and the roles played by different molecules possessing, for example, antioxidative properties, can be extremely varied. The general expressions 'antioxidant' and 'free-radical scavenger' are not, therefore, particularly helpful in discussions of health and nutrition. At the present time, it would be better if both were avoided as far as possible, and instead, to concentrate on descriptions of specific chemical reactions, if our understanding of this extremely important area of science is to develop.

Background to the electronic structure of oxygen

The energy states for an electron in an atom are characterised by four quantum numbers (referred to in order of decreasing magnitude as *n*, *l*, *m*, and *s*), which correspond to the electronic shell, orbital shape, orbital direction and electron spin. The principal quantum number, *n*, has integer values, 1,2, 3, *etc.*; *l* can have values n-1, n-2, …0; *m* can have values 0, ±1, ±2, … ±l; and *s* has values of ±1/2 (called m_s). It is also conventional to use letters instead of numbers to describe *l*, and directional coordinates to describe *m*. Thus,

l =	0	1	2	3	4
	s	*p*	*d*	*f*	*g*

so that in terms of *n* and *l*, electronic orbitals are referred to as *1s*, *2s*, *2p*, *3s*, *3p*, *3d*, *etc*. In *s*-orbitals, *m* = 0, and the radical distribution of electron density is spherical. There are three distinct *p*-orbitals (*m* = 0, ±1), with identical radial, but different directional characteristics. The *p*-orbitals are generally depicted as p_x, p_y, and p_z, which represent complex combinations of the quantum descriptions. Similarly, there are five *d*-orbitals (*m* = 0, ±1, ±2), which are depicted as d_{xy}, d_{xz}, d_{yz}, $d_{x^2-y^2}$, d_{z^2} (Fig. 1). Each of these orbitals may contain two electrons, differing in their values of m_s, usually just referred to as 'spin'.

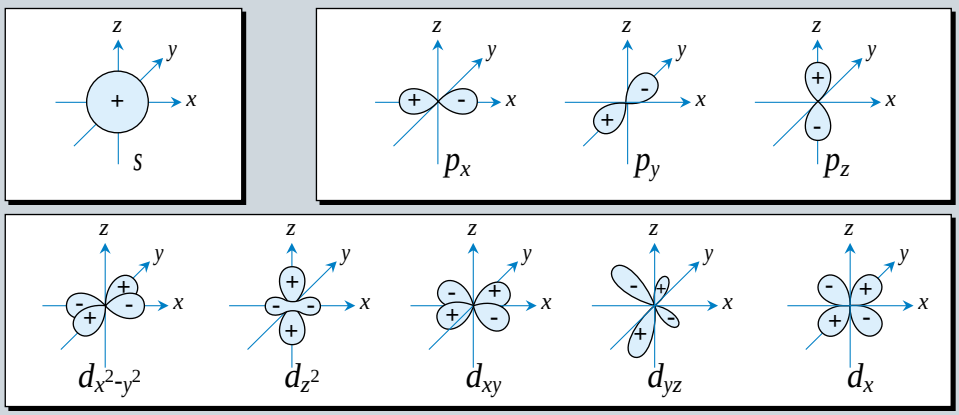


Figure 1 Diagrammatic representation of the radial distribution of electrons in s, p, and d atomic orbitals.

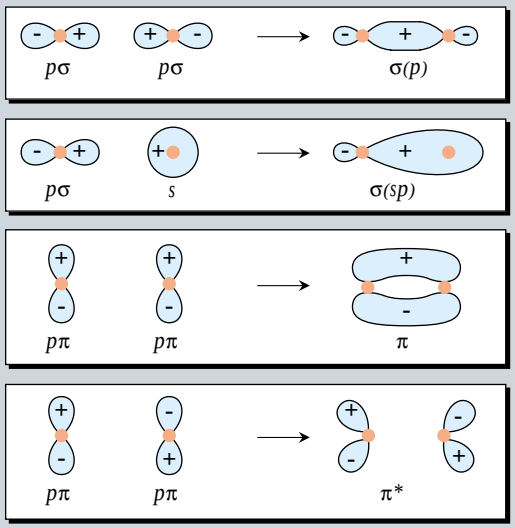


Figure 2 The formation of some simple 2-centred molecular orbitals by combinations of atomic orbitals.

There are a number of rules, which govern the distribution of electrons within atoms. The most important state that (i) no two electrons can have an identical set of quantum numbers, and (ii) the electronic orbitals in multielectron atoms (or ions) are filled in order of decreasing stability. Thus, for example, a nitrogen atom with seven electrons has the electronic configuration $1s^2\ 2s^2\ 2p^3$. The *1s*- and *2s*-orbitals are completely filled, whereas only three of the six *2p* states are occupied. The occupancy of these orbitals is determined by Hund's rules, which state that electrons

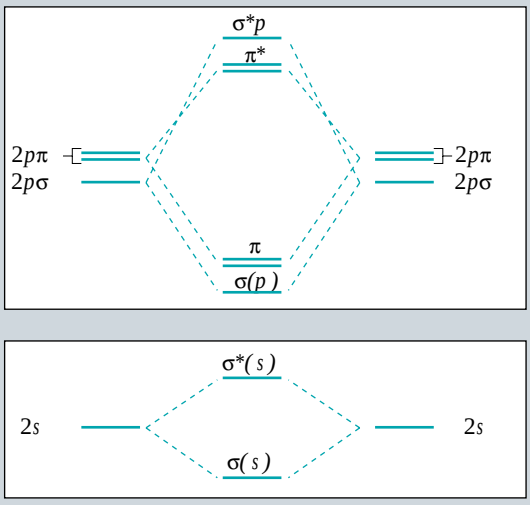


Figure 3 Schematic energy level diagram for molecular orbitals formed from two identical atoms of the 1st row of the Periodic Table (ignoring the core 1s electrons).

with the same *n* and *l* values occupy orbitals with different values of *m* and, as far as possible, have the same values of m_s (*i.e.* their spins are not paired). Similarly, the oxygen atom has the electronic configuration $1s^2\ 2s^2\ 2p^4$ with one *p*-orbital containing a pair of electrons and the others each with a single electron with the same spin. Isolated atoms are not generally stable (except for the inert gases helium, neon, argon, *etc*., which have filled electron shells) and they react to form molecules with more stable electronic structures. Molecular orbitals are formed by combining atomic orbitals on two or more atoms to produce a new set of orbitals (Fig. 2), and a typical molecular orbital energy level diagram for two identical atoms of the 1st row of the Periodic Table is shown in Figure 3.

The population of molecular orbitals is governed by the same rules as those for atomic orbitals. Thus, in the N_2 molecule the orbitals are completely filled up to and including the π-orbitals; all electrons are, therefore, 'paired'. The two additional electrons in O_2 are located in the π*-orbitals. Their combined spin, *S* (Σm_s), can be either 1 or 0 depending on whether the individual spin vectors have the same or opposite sign. It is common to refer to the spin states by their multiplicities, *2S+1*, so the two states for O_2 are $^3O_2$ and $^1O_2$ (triplet or singlet oxygen). Hund's rules then make $^3O_2$ the more stable state. Thus, molecular O_2 is a free radical with two unpaired electrons, a property which is the key to understanding the free-radical chemistry of oxidation processes.

The involvement of free radicals in oxidation processes

The complete reduction of molecular oxygen (*i.e.* oxidation of substrates) involves the transfer of four electrons *per* oxygen molecule to generate the oxide, O^{2-} ion, which has the stable $1s^2 2s^2 2p^6$ electronic configuration:

$$O_2 + 4e^- \rightarrow 2O^{2-}$$

However, intermediate levels of reduction can (and often do) occur. The one-electron reduction of O_2 produces the superoxide free radical anion, $O_2^{\cdot -}$, which contains three electrons (2 paired, 1 unpaired) in the π*-orbitals in Figure 3. Further reduction produces the peroxide ion, O_2^{2-}, in which the π*-orbitals are fully occupied *(i.e.* there are no unpaired electrons).

Peroxides are broken down in the presence of oxidisable transition metal ions, and the hydroxyl free radical (·OH) is generated (the Fenton reaction):

$$\text{e.g. } Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH$$

The ·OH radical is highly reactive and is able to abstract a hydrogen atom (·H) from a wide range of

organic compounds, generating a carbon-centred free radical in the process:

$$R\text{-}H + \cdot OH \rightarrow R\cdot + H_2O$$

These reactions are of particular importance in biology and in the stability of foods, because of their roles in the decomposition of unsaturated lipid molecules. There is, however, a wide range of other reactions in foods that involve free-radical processes, some probably detrimental, some beneficial, and others benign as far as quality perceptions are concerned. Some of these are discussed later in this article, but generalisation is not possible with the current status of our knowledge.

Food quality – what are the most important criteria?

The relative importance of the various food quality criteria differs in different parts of the world. In the UK, in common with other developed countries, however, *safety* is perceived as of overwhelming importance. In addition, microbial contamination causes greater concern than chemical components, although at the current time, the prion agents for transmissible spongiform encephalopathies (TSEs) represent a major problem. However, the public has little ability to use safety as a criterion in food selection, and has to place a huge element of trust in the food producers and suppliers. The recently established Food Standards Agency has a crucial role in ensuring that this trust is justified.

Sensory properties, summarised as appearance, texture, aroma and taste, along with cost and responses to marketing activities, are the main selection criteria that are used for food purchases. With fresh fruit and vegetables, sensory properties, along with relative cost, are the over-riding factors determining choice. In contrast, marketing activities are probably the main factor determining the choice of prepared products, although sensory properties, especially taste, are factors, which determine the extent of any further purchases of a product.

Where then do *nutritional properties* feature as criteria? Except where they are used in marketing tactics, they would appear to be seldom a factor in consumer choice at the present time. A major reason for this is a lack of relevant data upon which the public can make informed choices. Although processed and many prepackaged food products contain typical analytical values for a number of their components, it is not clear, to most people at least, how these should be used in formulating a healthy diet. In the case of fresh products, the impracticability of performing sample analyses at the point of sale invariably means that the public has no information on their chemical compositions. Even at a research level, for most plant foods there is a dearth of analytical data on how the composition of different varieties of particular plant products *vary with environmental conditions during growth and different post-harvest storage regimes.*

For certain types of food product, selection may be based on medicinal properties, and the production of functional foods is anticipated to be a major growth area in the future. There is also growing evidence that specific food products that are already widely available can provide protection against certain ailments (*e.g.* coffee can provide protection against allergic reactions). Other products, purchased because of physiological effects that are produced when they are consumed, may have side effects that can be either beneficial or detrimental. For example, small quantities of alcohol consumed on a regular basis may have beneficial effects on the cardiovascular system, although excessive consumption is demonstrably detrimental to health.

Oxidation processes in foods – impact on quality

Oxidative processes generally lead to a depletion in the levels of antioxidant molecules in stored foods. This is particularly important for fresh fruit and vegetables, which represent the major sources of vitamins in many diets. In Scotland, the decline in ascorbic acid (Vitamin C) contents of potatoes during storage is of particular significance, since for many people this represents the major source of this essential vitamin. Post-harvest storage conditions may, therefore, have a key role in micronutritional quality.

Levels of micronutrient molecules, such as the antioxidant vitamins, in fresh plant materials can be affected greatly by the plant being subjected to biotic or abiotic stress processes. An example is shown in Figure 4 of the decline in ascorbic acid content of leaf tissue from plants as a result of prolonged high humidity conditions. Similar effects have been seen for other stress processes, including the apparently healthy regions of plant tissue subjected to biotic damage. *Oxidative reactions caused by stress processes can, therefore, have a marked effect on the composition of food products.*

In foods in general, it is now generally accepted that consumption of saturated fats is linked to heart disease, whereas unsaturated lipid molecules are thought to have beneficial effects on the consumer.

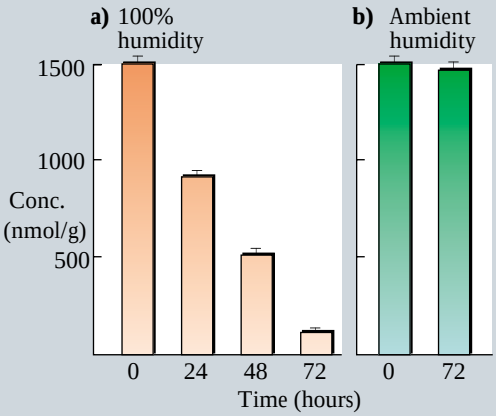


Figure 4 Variation with time of the ascorbic acid contents of *Phaseolus vulgaris* leaves from glasshouse grown plants (a) subjected to 100% humidity in incubation boxes, and (b) control samples at normal humidity levels.

Unsaturated lipids, however, have much lower stability with respect to oxidative processes than their saturated counterparts. The carbon-centred free radicals ($L\cdot$), derived from reaction of $\cdot OH$ with lipid molecules, react with oxygen to form lipid peroxy radicals:

$$L\cdot + O_2 \rightarrow LOO\cdot$$

Lipid peroxy radicals initiate a chain reaction with other lipid molecules:

$$LOO\cdot + LH \rightarrow LOOH + L\cdot$$

Decomposition of lipid hydroperoxides occurs through heating or catalysis by transition metal ions:

$$LOOH + M^{n+} \rightarrow LO\cdot + M^{(n+1)+} + OH^-$$
$$LOOH + M^{(n+1)+} \rightarrow LOO\cdot + M^{n+} + H^+$$

The lipid peroxidation process can be terminated by the reaction of two radicals with one another:

$$L\cdot + L\cdot \rightarrow L\text{-}L$$
$$LOO. + L\cdot \rightarrow LOOL$$
$$LOO\cdot + LOO\cdot \rightarrow LOOL + {}^1O_2$$

${}^1O_2$ as described above is the unstable form of O_2 and highly reactive. It readily reacts with other lipid molecules, for example, to produce lipid peroxides, which continue the reaction described above.

In addition to the reaction sequence described above, autoxidation of lipids results in the formation of off-flavours and other undesirable chemical compounds. The lipid peroxy radicals can also undergo intramolecular rearrangement to produce endoperoxides. These are then transformed into endoperoxy hydroperoxides after further rearrangement and reaction with oxygen. Finally, the endoperoxy hydroperoxides decompose in the presence of metal ions to produce malondialdehyde and other low-molecular-mass fragments. Aldehydes and ketones generally have distinct sensory properties, and the unattractive sensory properties of some of these lipid peroxidation products make them sensitive markers of oxidation processes. Addition of antioxidant molecules to food products can delay these lipid decomposition processes, thus increasing the shelf-lives of the products. Improvements in product quality at the point of consumption may not necessarily follow, however, especially if the extended lives of the products are spent in storage.

Oxidation reactions in foods are not limited to the lipid components; reactions involving proteins, carbohydrates and nucleic acids are also extremely important. One of these is the Maillard or non-enzymatic browning reaction, which involves the condensation between reducing sugars and the free amino groups of proteins. The Maillard reaction is ubiquitous in nature, but is particularly important during the processing and storage of foods. It is responsible for changes in flavour, colour and nutritive value during thermal processing, such as during roasting of meat, baking of bread, or brewing of coffee. The initial stage of the Maillard reaction involves the formation of a Schiff's base (aldamine) between amino and carbonyl groups (Fig. 5), which may rearrange to an Amadori compound (1-amino-1-deoxy-2-ketose). Amadori compounds may then react further by several pathways, including enolization, dehydration, aldol condensation and oxidative degradation. Many of the

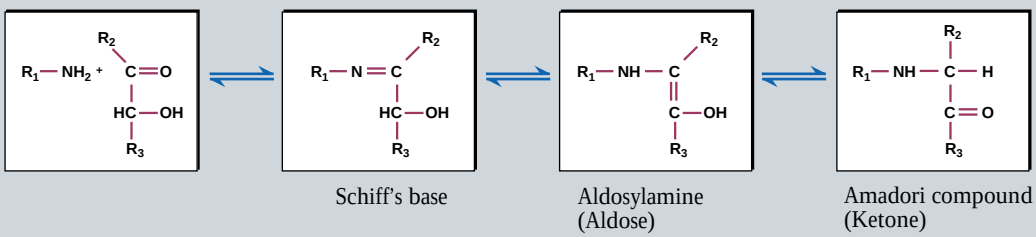


Figure 5 Chemical reactions involved in the initial stages of the Maillard reaction.

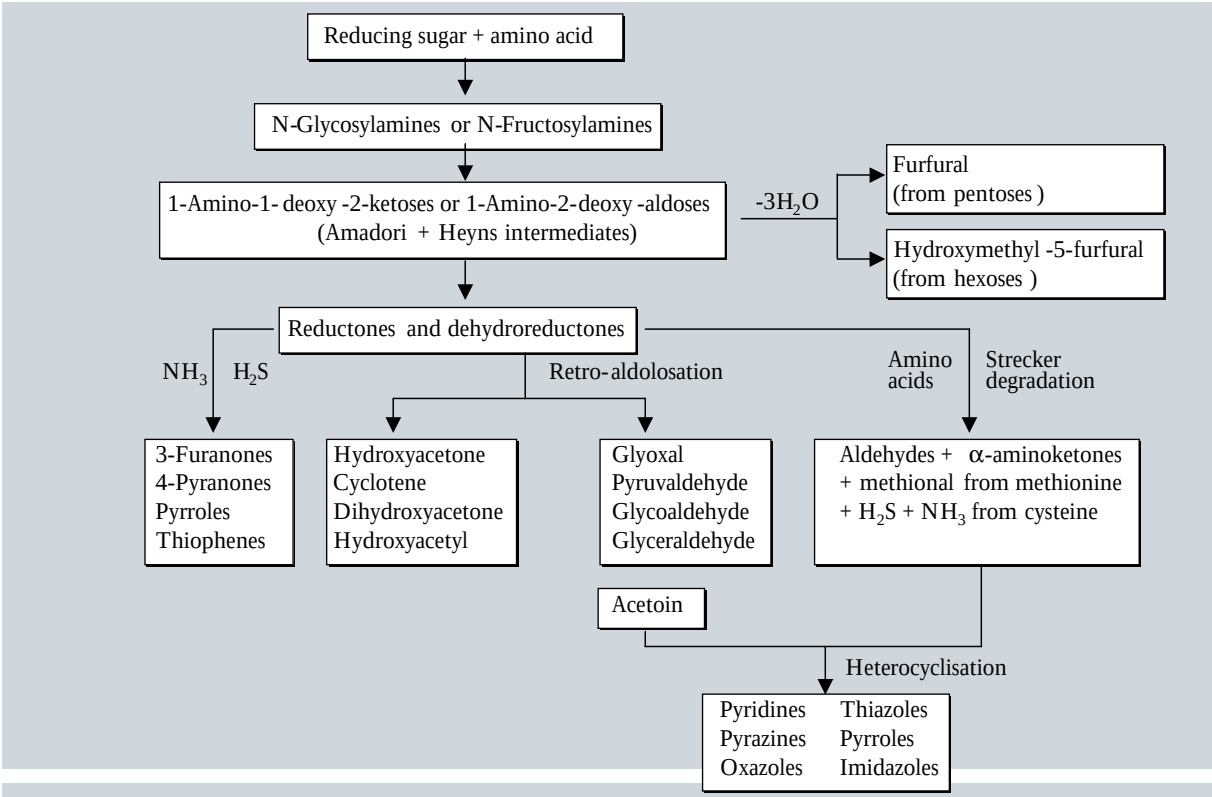


Figure 6 Reactions schemes for the formation of aroma compounds in foods as a result of Maillard reactions.

low-molecular-mass products of Maillard reactions have important sensory properties and biological activities, and various types of reaction, which lead to the formation of aroma compounds in food products, are summarised in Figure 6. The final stages of the Maillard reaction are complex and involve the condensation of many low-molecular-mass compounds into high-molecular-mass polymers, known as melanoidins, which have considerable variability in their compositions and structures.

Oxidation processes in food are, therefore, by no means always detrimental to quality. Many Maillard reaction products behave as strong antioxidants, and, as can be seen with the examples in the previous paragraph, some are also responsible for desirable sensory properties. In this respect, it is often the temperature of the reaction, which is important, and the 'best' products may be obtained by heating at the 'right' temperature for the 'right' period of time. Usually, we do not understand the chemistry, and the statements in the previous sentence are derived from generations of empirical measurements. *Many food preparation activities are still perceived as arts rather than science, and there is a need to develop our understanding of these important processes.*

Oxygen and biological systems – living with free-radical processes

For most organisms, free radicals are an inevitable consequence of living in an aerobic environment, and many fundamental metabolic processes proceed *via* free-radical mechanisms. In photosynthesis and respiration, for example, a series of free radicals are involved in the basic biochemical reaction schemes. Despite such fundamental processes, the crucial role played by free radicals in biological processes is often ignored, and free radicals are frequently seen in terms of their chemical reactivity and ability to bring about molecular change. In the context of health and nutrition, such change is often equated with being detrimental, despite its necessity in evolution and adaption to continually changing environments. One of the keys to survival of any organism, however, is the prevention (or minimisation) of free-radical damage to itself, whilst utilising free radicals for its own benefit (for providing energy and inflicting damage upon competitor organisms).

In order to avoid the damaging consequences of uncontrolled free-radical reactions, all aerobic organisms have evolved a vast array of protective mechanisms, which operate under normal conditions. Many

Generic name	Specific compounds
Vitamin A	Retinol Retinal Retinoic acid
Vitamin B_1	Thiamine
Vitamin B_2	Riboflavin
Vitamin B_6	Pyridoxine Pyridoxal Pyridoxamine
Vitamin B_{12}	Cobalamin derivatives
Vitamin C	L-ascorbic acid
Vitamin D	Cholecalciferol
Vitamin E	α-,β-,γ-,δ-tocopherol α-,β-,γ-,δ-tocotrienol
Vitamin K	Phylloquinone and derivatives
Biotin	Biotin
Folic acid	Tetrahydrofolic acid and derivatives
Niacin	Nicotinic acid

Table 1 Major vitamin molecules.

of these are enzymatic, and free-radical neutralisation is accompanied by a change in oxidation state of one or more transition metal ions in the enzyme. In addition, there are a number of organic molecules, which can play important roles in controlling free-radical processes in biological systems. The best known of these molecules are the vitamins (Table 1), but there are other molecules, many uncharacterised, which are believed to possess beneficial antioxidant properties. *There is now considerable interest in identifying and testing the efficacy of such molecules in protection against a number of causes of premature death, including cardiovascular disease and cancers.*

Free radicals, antioxidants and health: are deteriorative processes inevitable?

Disease states may result either from attack by competitor organisms (pathogens), or from the deterioration or breakdown in the functioning of essential components of the body. Free-radical-induced damage has been widely implicated in disease processes, but this should not be seen as surprising, since any interference with normal metabolic processes would be expected to have implications for free-radical reactions. Physical damage or pathogen attack both invoke a free-radical response in both plants and mammals. Also, the presence of elevated free radical levels in tumours may be primarily a reflection of a higher metabolic turnover in malignant tissue rather than being associated with the source of the disease. There is, therefore, an important problem of distinguishing between 'cause' and 'effect', and this represents a major difficulty in understanding free-radical aspects of the chemistry of disease processes.

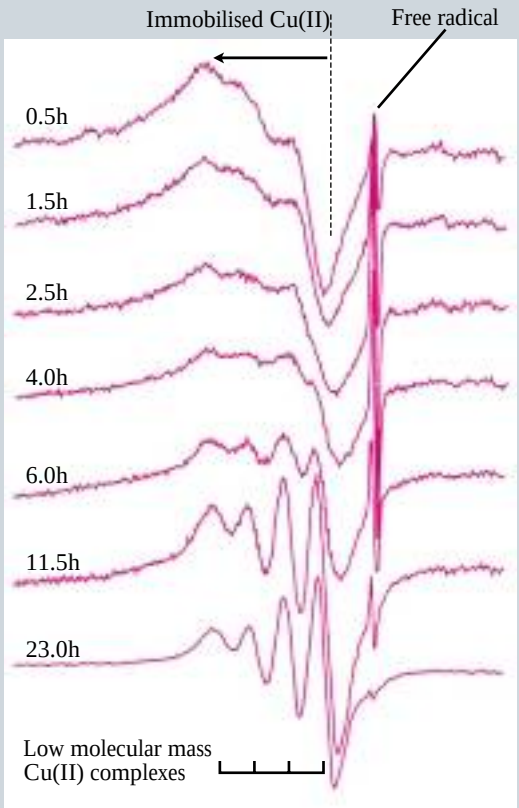


Figure 7 Electron paramagnetic resonance (EPR) spectra of wheat roots at various periods following a short exposure (30 minutes) to toxic levels of Cu(II) (10^{-5}M $CuSO_4$). Note: The free radical signal increased in intensity initially as the plant attempted to respond to the challenge, then decreased to virtually zero as it failed and died.

In contrast, the free-radical theory of ageing holds the premise that deteriorative ageing processes are the result of cummulative free-radical damage. Hence, *anything, which can slow down this free-radical damage, should have a beneficial effect on the ageing process.* A good example here is the maintenance of fluidity and structural integrity of cell membranes. Lipid-soluble antioxidants, such as tocopherols, play an important role in protecting the membrane lipids, and this fact is now widely recognised in the formulations of anti-ageing creams. Although the effectiveness of such preparations may still be a matter of debate, the protection of cells against antioxidative damage is a major reason why the various antioxidant vitamins are essential dietary components.

The oxidative free radicals involved in ageing processes are short-lived, and it appears that they are

chemically identical to those involved in normal metabolic processes. *Contrary to popular opinion, however, there is not a build-up of free-radical activity* (or even levels of stable free radicals) *as a result of ageing*. In fact, just the opposite occurs, and senescing tissues show decreased levels of free radicals as a result of slower metabolic turnover. Death actually results in a virtual cessation of free-radical production (see Fig. 7).

In addition to the long-recognised vitamins, many other molecules found in foodstuffs have antioxidant properties. In recent years, there has been a great deal of effort to determine the extent to which they may be bioavailable, and to identify whether or not there is a positive health role for them. Although the vast majority of chemical species present in all foodstuffs is unknown at the molecular level, a current strategy is to explore in detail the properties of those molecules that can be isolated relatively easily. A positive response in test systems produces an identification of (potentially) 'desirable molecules', which can be used to produce 'healthier' food products. Since most of such products are of plant origin, many plant breeders aim to produce new varieties in order to satisfy projected consumer demand for foods enriched in these 'healthy molecules'. Caution is required in this approach, with respect to antioxidants especially, because it is often not possible to monitor the knock-on effects of changes in selected biochemical pathways. As a consequence of their need to survive in an environment of solar radiation and a vast range of other abiotic stresses, plants produce naturally a plethora of antioxidant molecules in their various organs. Enhancement of the levels of production of one type of molecule, therefore, carries the possibility of depression in the production of another, which performs a similar function in the plant, but which might be a more effective dietary component for humans and livestock. *Development of appropriate analytical techniques for large-scale metabolic profiling, should in the future allow a much higher fraction of the metabolic products to be characterised, and consequently to result in valuable improvements in our understanding of the functions of a much wider range of food components.*

The antioxidant argument, as commonly presented, is also a gross oversimplification of the complex range of reactions in which such molecules are involved. Health properties of foods cannot realistically be equated to their overall antioxidative capacities, since if that were the case, healthier food could be produced simply by addition of cheap synthetic chemical antioxidants, such as butylated hydroxytoluene (BHT). Unfortunately, the answer to improving health is not that simple. *Life is complex, and anyone looking for simple answers, is likely to get the wrong ones.*

The crucial role of plants in health and nutrition

The importance of plants in human nutrition was established at the beginnings of civilisation and, for most of human history, plants have represented the principal means for the control of disease as well as the provision of nutrients for healthy living. Several specific molecules have been shown to possess beneficial properties for maintenance of good health or treatment of disease, but, in most cases, the nature of the association between food and health is of a long-standing empirical nature. Nevertheless, around 30% of current world-wide sales of therapeutic agents are derived from plants or micro-organisms, and some 60% of the drugs currently used in cancer treatment are of natural origin. In Asia, reports of medicinal properties of plants go back 3,000 years, and formulations based on plants still account for a considerable fraction of traditional Asian and African medicines. There is also a growing awareness in Western countries of medicinal properties associated with specific plants or plant-derived products. In addition to the frequent reports of beneficial health properties associated with red wine, tea, and coffee, there have been many other reports concerning medicinal properties of individual plant products. Recent examples include claims that broccoli and apples help to prevent cancer, cranberries provide an effective treatment of urinary infections, tomatoes reduce blood clotting, black pepper prevents hair greying, and kiwi fruit are good laxatives, to name just a few.

There is a current tendency to attempt to explain beneficial health effects of foods in terms of protection provided by particular chemical components (*e.g.* resveratrol in red wine), even though the chemical composition of much of the product remains unidentified. This is indeed an important concept, because current legislation requires detailed information on the identities and quantities of any additives to foods, but very little is known about the precise chemical nature of most of the natural components. For example, consider the case of coffee. This is probably the most extensively characterised of any foodstuff and has been the subject of many hundreds (if not thousands) of man-years of investigation. There are estimates of between 4,000 and 15,000 for the number of chemical components in the beverage; around 1,000 of these have been identified, less than 20 have been subjected to basic toxicological evaluation, and only one (caffeine) has had its physiological effects thor-

oughly investigated. There is, therefore, a high level of ignorance about the natural chemical make-up of even the most common foods, and their *acceptability for consumption is based primarily on historical evidence of safety*. Consequently, *advancing our understanding of food composition would appear to be a priority area for investigation with modern technology, if the nutritional quality is to be developed as a food selection criterion.*

In humans, some antioxidant molecules are synthesised within the body, but many are derived from food. Plants, because of their abilities to synthesise large quantities of antioxidant molecules, have a special place in human nutrition. However, many of the chemical species in our bodies are not components of the foods we eat. Some are generated through reactions between different food components and others during digestive processes. Research, in which single food components are tested for their contributions to health, should consider the point that their chemical reactions could be quite different when presented to the body in a whole foodstuff. Even the consideration of individual foodstuffs is still an inappropriate oversimplification to understanding human nutrition. *It is unrealistic simply to present foods as 'healthy', or 'unhealthy', although there are clearly healthy and unhealthy diets (lifestyles).*

Of particular significance are the simplistic dietary claims which prey on public concerns about their own mortality, especially where emotive issues such as cancer and heart disease are concerned. The beneficial role of antioxidants in the diet as a result of their free-radical scavenging activities is an example of an oversimplified message, and much greater effort should be directed to understanding the relevant chemistry of foods and their transformations during digestion.

To what extent do we need improved food quality, or just better information?

There is now considerable interest in the production of plants with enhanced nutritional quality as a mechanism for improving the overall health of the population. This is currently a shared objective of the agricultural and food-processing industries, and major research efforts are being devoted to the breeding of plants (using both conventional and molecular techniques) to produce nutritionally enhanced products. There have been some notable successes as, for example, in the production of Vitamin A-enriched rice, which is starting to make an impact in China and SE Asia, but in the developed countries, there are currently more problems relating to over-consumption of food than to malnutrition. In this respect, Scotland is no exception with some 60% of the population estimated to be overweight or obese.

There is now more choice of foods than at any time in history, and in every supermarket there is a wider variety of food products than could have been imagined by our grandparents. How then do we transfer these opportunities to the dinner table? The answer has to be through knowledge, experience and education. At present, the public does not know the identities of most of the natural chemical components of foods, and clearly has little or no knowledge of their behaviour in the digestive system. Even when attention can be focused on one or more molecules with known nutritional properties, there is usually limited understanding of the extent to which they affect other (frequently uncharacterised) species. *We now have biological techniques, which allow us to make rapid changes in the composition of our foods, but substantial investments are required in analytical techniques to monitor fully the consequences of these changes.* Although it is relatively easy to screen out potentially toxic products, the subtleties in the differences in composition of most novel and conventional foods, means that thorough evaluation of the effects on humans is still only possible through the observation of generations of consumers. *There is, therefore, a desperate need for the development of good models for the prediction of the consequences of dietary changes, especially with respect to antioxidant molecules, if we are to reap the benefits from recent advances in biological techniques.* Fortunately, with the continuing advances in computer power, the technology to achieve such sophisticated models is already on the horizon.

Can health effects of food be considered in isolation, or should diet be based on lifestyle?

It is now a priority in many developed countries to advance understanding of the role of diet in the maintenance of health. This should, however, be seen as a long-term programme, and it must be recognised that there can be no quick fix. Inappropriate diets in childhood have been linked to diseases of middle age and, because of maternal inherited traits, it may take generations to develop a scientific understanding of the principles our forebears derived by trial and error. This is all made more difficult by living in an age of constant change – new foodstuffs, new methods of processing, pre-prepared meals, *etc*. *The development of new chemical- and bio-assay systems should be a great asset in advancing our knowledge of novel foods, but it is of crucial importance that these analytical approaches are*

Country	Life expectancy at birth (years)		Probability of dying between 15 and 59 years	
	Males	Females	Males	Females
UK	74.7	79.7	11.1%	6.7%
Ireland	73.3	78.3	11.6%	6.7%
France	74.9	83.6	14.6%	5.9%
Germany	73.7	80.1	13.6%	6.7%
Italy	75.4	82.1	10.9%	5.1%
Spain	75.3	82.1	12.9%	5.4%
Sweden	77.1	81.9	8.9%	6.0%
USA	73.8	79.7	14.8%	8.5%
Japan	77.6	84.3	9.5%	4.8%
Australia	76.8	82.2	9.4%	5.3%
China	68.1	71.3	17.0%	12.5%
India	59.6	61.2	27.5%	21.7%
South Africa	47.3	49.7	60.1%	53.3%

Table 2 Selected life expectancy data from the WHO World Health Report 2000.

developed in parallel with the food technology, if a fuller understanding of the relationships between diet and health is to be achieved.

In recent years, much attention has been given to the 'French paradox', and dietary differences between Mediterranean and North European countries have been invoked to explain longer life expectancies in France compared to the UK. There have also been claims that consumption of red wine is the explanation for this effect. It is interesting then to examine closer recently published data by the World Health Organisation (Table 2). Firstly, there is a relatively small difference between life expectancies for males in France and the UK, and both are similar to those of other major countries in Europe. Furthermore, the probability of premature adult death (i.e. between 15 and 59 years) for French males is one of the highest in Europe. In contrast, the life expectancy of the French female is the longest in Europe. *If it really exists, the 'French paradox' is, therefore, a gender-related issue*. It is completely inappropriate to just consider diet to explain life expectancy data, and to ignore environmental contributions to health, such as the climate, housing conditions, and working practices; nor should genetics be discounted. Hopefully, the next few years will bring huge advances in our knowledge of some of the subtleties in the relationships between genetics and health.

It is also unreasonable to assume that every individual has the same dietary requirements. The diet of an office worker will not be adequate for a professional athlete, and an athlete's diet will probably not be beneficial to the office worker. For many years we have had information on the energy requirements for different occupations, but, although various governments produce recommended daily allowances for a number of vitamins, detailed information on a wider range of micronutrient requirements is not currently available. *Increases in understanding how diet could be adjusted in the context of lifestyle in order to achieve optimum health effects should be regarded as a priority in nutritional research.*

What is the case for nutritionally enriched foods and dietary supplementation?

There is now a considerable demand in the developed countries for food supplements, despite the fact that our supermarkets now have year-round stocks of fresh produce from all over the world. The need for dietary supplementation, therefore, has never been less than it is at the present time, but does it still convey a real benefit? Unfortunately, except for a few special cases where individuals are subjected to severe stress conditions, the answers are largely unknown. Nutritionally enriched foods are beneficial to people in areas of the world where only a limited range of foodstuffs is available, but there is debate as to what fraction of the population of the UK could benefit from such products.

It can be argued that the increased use of synthetic antioxidant food preservatives in the latter half of the 20^{th} century has been responsible for general improvements in the health of people in developed countries. Also, the longer active life-spans in the USA compared to the UK is sometimes attributed to nutritional supplementation, although there is no significant difference in actual life-spans. There are, however, many other lifestyle differences between these countries, and at the present time it is not possible to confirm or refute such claims. Indeed, public attitudes to physical activity might be a major factor, not to mention the availability of facilities, and in some parts of the country, a more amenable climate. Nevertheless, the perceived importance to health of various antioxidant molecules has led to the development of an industry devoted to the production of dietary supplements, the objective being provision of the means to avoid nutritional deficiencies.

The cost of routine dietary supplementation is not insignificant, and this raises the question as to when it is valuable, especially since nutritional requirements

are dependent on a number of lifestyle factors. Also, there is a question of the extent to which conventional foods can be used to alleviate the need for dietary supplementation. The problem is identifying what sort of improvement is desirable and how this can be achieved. Apart from the vitamin molecules, the present state of our knowledge on many dietary supplements is limited, and detailed chemical compositions of popular supplements, such as for example ginseng or *Ginkgo biloba*, are largely unknown. Without such information, it is not possible to begin a programme of identifying active ingredients (if activity is associated with individual molecules) or of developing an understanding of the factors which influence the qualities of different samples of these products.

Meanwhile, the European Commission is concerned about claims relating to the effectiveness of food supplements. It is relatively straightforward to determine toxicological effects by following the methods used for screening pharmaceuticals, but it is much more difficult to establish the efficacy of various formulations. The approach used to establish the essential requirement of vitamins, by testing their abilities to remove adverse symptoms from subjects suffering from severe deficiencies, is largely irrelevant to the populations in developed countries. As stated above, there is an urgent need for the development of good micro-nutritional models in order to remove some of the evangelical approaches from research in diet and health.

Natural versus added antioxidants in the diet

Popular current opinion is that the key to beneficial nutritional properties of plants is the antioxidants, which are produced in large quantities in order to aid survival in an aerobic environment. The logic behind this thinking is that humans need protection against the detrimental effects of oxidative stress reactions, and that plants have evolved mechanisms for synthesising the appropriate chemicals to enable them to survive in an environment of acute oxidative stress. There is then the thought that what works for plants, should also work for humans, although it neglects the production of toxic plant components. Generalisation is, therefore, potentially dangerous, and the safety of all plants has to be considered on an individual basis. Fortunately, for most food plants this has been done on an empirical basis over thousands of years.

There is good epidemiological evidence that a diet rich in fruit and vegetables provides health benefits over one that is devoid of fresh plant products. The role of fish, especially oily or uncooked fish, should also be considered in a healthy diet, since Japan, with its high levels of fish consumption, has the highest life expectancy of any developed country. Specific health claims have been made for several individual products (see above), whereas others are thought to make an overall contribution to health. Such statements should, however, be treated with caution, since an over-reliance on a limited range of food products can be seriously detrimental (*e.g.* deaths from excessive carrot consumption). It has been recognised recently that excessive consumption of vitamin molecules can be detrimental to health, and it is likely that excessive consumption of any type of food product is undesirable.

Because of its importance, the validity of the link between antioxidant molecules in the diet and human health should be carefully scrutinised. There is a strong current view that this link is an established fact, but is such confidence really justified? The basis for the concept lies in the essential role of vitamins, but the current status of other 'antioxidant' molecules in foods still remains in the 'non-proven' category.

The acceptability of chemical food additives has changed appreciably since the 1980s, when there was concern about the presence of synthetic preservatives in foodstuffs and their supposed adverse implications for health. These molecules were antioxidants and were (and still are) used to inhibit oxidative degradation processes. Positive benefits were not generally recognised then, and even now there seems to be a conception that the natural products are in some way superior. Sometimes, of course, that may be the case, but it can not be taken as a generality, since many of the most toxic compounds known to man are of a natural origin. Also, humans do not live in biological isolation, but in an environment in which there is a constant competition with other biological organisms. We may be the dominant mammalian species on this planet, but we have by no means developed control of all micro-organisms. Our battle against them involves the use of toxins, which are more lethal to them than to ourselves, *i.e.* in the right circumstance toxic materials are beneficial, whereas nutritious materials can be detrimental, because they could help the organisms with which we are in conflict. *A full understanding of the roles of various foodstuffs is, therefore, an extremely complex issue, and at the present time there is a need to keep an open mind about the health implications of individual food components.*

Techniques to measure free radicals, antioxidants and free radical scavengers

Free radicals can be detected directly by electron paramagnetic resonance (EPR) spectroscopy (Fig. 8), a technique that is designed to study specifically molecules with unpaired electrons. The same technique, therefore, is able to characterise the paramagnetic transition metal centres in some antioxidant enzymes. As mentioned in earlier paragraphs, many free radicals have extremely short half-lives and, except where high steady-state levels are generated, cannot easily be studied directly in biological samples. There are, however, various practical procedures that allow EPR to be used for the study of unstable radicals. One of the most useful of these is 'spin trapping', where a molecule, known as a spin trap, is added to a reaction medium and reacts specifically with unstable radicals to produce adducts, which are themselves radicals, but with greater stability. In many cases, the spectral parameters of these radical adducts allow identification of the original radical. Other techniques for the study of unstable radicals include stabilisation by rapid quenching to low temperature, or generation within the spectrometer by irradiation or mixing the reactants which produce the radicals in the spectrometer using a flow system.

Figure 8. Electron paramagnetic resonance spectrometer.

There are various approaches available for the study and characterisation of antioxidant molecules. Accredited chemical analytical methods are available for the routine determination of the known vitamin molecules, and assay kits have been produced for measurement of activity of various enzyme preparations. Measurements of uncharacterised antioxidant molecules are more difficult and a range of assays has been produced with the objective of assessing the overall antioxidant capacity of a sample. In reality, such assays measure the ability to carry out a specific reaction and are far removed from any assessment of the contribution to health of a particular food product. Nevertheless, they have some value in screening exercises. In recent years, there have been rapid developments in chromatographic techniques for molecular separation. These have been paralleled by advances in techniques for molecular characterisation, such as mass spectrometry (MS) and nuclear magnetic resonance (NMR). Coupling these techniques should in the near future lead to rapid progress in identifying a new generation of molecules that are able to perform specific antioxidant reactions in foodstuffs, such as the inhibition of lipid autoxidation.

Different approaches are required to determine free radical scavengers, depending on whether they are known molecules, or uncharacterised mixtures. In the first case, a standard analytical chemistry approach can be used, but dealing with the latter is more difficult. As mentioned at the beginning of this article, the expression 'free radical scavenger' is not particularly helpful in the context of food and health. Some radicals, such as ·OH, are highly reactive and unspecific in their reaction. With these radicals, there is no such thing as a specific scavenger, although the nature of the reaction products can be definitive for a particular radical (spin trapping is a specialised example). Other radicals are much less reactive, and may be scavenged specifically. In such a situation, however, *different free radical scavengers are required for reaction with different types of radical*. The enzyme superoxide dismutase (SOD) is an example of a specific scavenger for the $O_2^{\cdot -}$ radical.

The future – what are the nutritional priorities for the 21st Century?

The framework for the human genome has now been established and, within the next few years, a good knowledge of the natural variations in individuals will likely be obtained. Identification of the functions of the various genes will then follow, thus opening up the possibility for routine treatment of the adverse consequences of many genetic weaknesses – perhaps identified through screening at birth. The pharmaceutical industry offers a route to the treatment of the

genetic basis of diseases, but economic factors are likely to limit its availability. In a practical sense, there is a need to learn how to adjust lifestyles to counter genetic susceptibilities. Diet and nutrition should, therefore, represent the foundation upon which it will be possible to devise 'recipes' for each individual that will provide the optimum dietary conditions for a healthy life. This might well include functional foods as a practical link between basic diets and conventional medicine.

In order to achieve these aims, huge advances need to be made in the characterisation of our foodstuffs and identification of a much wider range of food components than is presently available. This will need to be supplemented by data on the physiological effects, not just as individual molecules, but of food ingredients in combination with one another, in the digestive system. Simultaneously, information needs to be generated on the natural variation in the levels of physiologically active molecules, and the extent to which these can be controlled in the food production pathways. This type of *analytical chemical knowledge will provide the scientific basis from which it should be possible to generate progressive improvements in the nutritional qualities of our foodstuffs, and hopefully to produce a general extension in the period of high-quality life for individuals.*

Conclusions

Understanding the health implications of diet and other lifestyle factors offers the most practical approach for a long-term increase in the overall health of populations. At the present time, there is good information on the functions of a relatively small number of essential molecules in the diet, but a paucity of knowledge of the chemical composition and physiological effects of many of the components of our foods. Generation of this knowledge is only a first step, however, since for it to be effective, the public must both understand and accept the information. This latter may yet prove to be the most formidable obstacle, given the current willingness of many people to risk health damage by consuming substances known to have long-term adverse effects.

There is also the question of the extent to which it is reasonable to expect individuals to monitor their daily intake of a wide range of nutrients. Particular attention may be beneficial when bodies are physiologically stressed (*e.g.* after major illness, professional athletes, *etc*.) and recovery rates may be enhanced by the consumption of certain types of molecule. In such situations there may be positive advantages in using dietary supplements. Alternatively, the stress generated by worrying too much about details of a diet may negate potentially beneficial effects for some individuals. As a society, a long-term aim should be to use education to encourage individuals to adopt healthy lifestyles naturally, without thought.

There is an argument that changes in food compositions are currently happening too quickly for non-clinical effects to be detected, but this is a trend that is likely to continue for the foreseeable future. Potential problems must be seen and addressed in the context of a rapidly changing world. There is no such thing as a risk-free change, or even existence, and at the present time, we have to place a large degree of trust in risk/benefit analyses. It is imperative, then, that such exercises are conducted diligently, and independent of short-term profit motive. However, there will inevitably be mistakes (just as there have always been), and all reasonable steps must be taken to ensure that they are minimised, including the encouragement of open and informed debate on on-going science and its implications. Nevertheless, *no change is not a no-risk scenario.* The dynamic nature of the world means that increasingly more innovative solutions are required to address a rapidly evolving set of problems. In order to achieve this, it is vital that all relevant branches of science are developed simultaneously.

The final take-home message is that the science underlying the links between food, diet and health is exceedingly complex and demands greater investment, if our understanding is to be increased to a level that will produce tangible benefits in terms of human health. Such an investment at the present time will represent a valuable legacy for future generations.

Genetics

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

Genetics touches every aspect of biology – the boundaries between genetics, molecular biology and biochemistry have become diffuse and their application and exploitation in viral, microbial, animal, plant and environmental science provides the scientific knowledge base for current and future research at SCRI. The new Genetics Division brings together expertise in plant breeding, genetics of crop plants, molecular genetics, genomics and gene expression. Interactions, both within the Division, and with chemists, biochemists, pathologists, cell biologists and environmental scientists in other Divisions, is the key to producing high quality research in the future, which is competitive nationally and internationally.

The success of breeding programmes has been based on an understanding of the biology and genetics of particular plant species, their interactions with pathogens, the ability to phenotype traits, plant handling and field trialling skills, and quantitative genetic and statistical analyses. Capabilities and expertise in gene cloning, gene expression and plant transformation, and the development of germplasm and molecular markers, built up over the last 10 years at SCRI, provide the basis for establishing structural and functional genomics research. This will greatly impact our understanding of the developmental and evolutionary biology of plants underpinning traits of commercial importance. As the sequences of most plant genes are identified through genome sequencing of *Arabidopsis* and rice, and high throughput genomics approaches to gene discovery and function, new opportunities arise in both fundamental and applied genetic research. The new genetics will ultimately allow us to dissect the molecular and biochemical events which give rise to complex phenotypes of economic importance, and identify genes involved in these processes. In the future, more emphasis will be needed on genetic and biochemical approaches to address gene function of isolated genes for such exploitation. In the meantime, the development and extensive application of marker systems will provide ever-increasing resolution of genome organisation, more precise mapping of molecular markers, and the potential to assess variation in wide germplasm. SCRI maintains large germplasm collections of potato, blackcurrant, raspberry, and, to a lesser extent, barley. One of the future goals of the Genetics Division is to characterise fully the germplasm genetically and phenotypically to provide information for breeding objectives in plant improvement programmes in the short, medium and long term.

At present, molecular markers are being utilised to develop and assess marker-assisted selection of parents and progeny in barley. The application of markers to established, accelerated, breeding methods in potato will give a greater understanding of the mechanisms behind the success of these methods, providing the potential for further improvement. Taken together

with identification of genes involved in major traits, the assessment of genetic and functional variation of alleles in diverse germplasm and gene-specific markers, the potential for targeted and rapid plant improvement based on extensive parent and progeny marker-assisted selection will continue to increase. The efficiency of the breeding process will aid in overcoming some of the limitations of traditional plant breeding, such as breeding barriers and unfavourable gene linkages, and provide incredible opportunities for targeted plant improvement not feasible by traditional approaches.

The vast quantities of data being assembled locally and internationally require us to build a Bioinformatics infrastructure and capacity. Data on markers, genotypes, genetic maps, DNA sequences and expression profiles are being generated locally and need to be assembled in a highly usable and accessible format. Local databases must be compatible with other national and international databases. Scientists at SCRI must have access to information on all aspects of relevant biology to inform research programmes and their direction. There is no doubt that genomics and bioinformatics will change the way in which scientific problems in the future are addressed.

To address gene function, a battery of techniques are being or have been established. A key technology is plant transformation where over-expression or antisense knock-out are used to assess function. The ability to transform with isolated genes also provides a second route to plant improvement, overcoming breeding or crossing barriers and allowing introgression of specific genes. This depends on our ability to transform the mandate crops and to isolate genes and understand mechanisms of gene expression and its regulation.

The expansion in structural genomics must also be paralleled by increased molecular, genetic and biochemical analysis of key biological processes and by increased capabilities in gene function technologies such as virus-induced gene silencing and RNAi (Biochemistry/Cell Biology and Pathology Divisions). Plant genomics will be paralleled by research on pathogens and pests, and plant-pathogen interactions in the Pathology Division. The technologies to analyse relationships between cultivars and related species, and to identify novel sources of genetic diversity, can also be applied in studies assessing the effects of environment on genetic diversity within plant systems (Plant, Soils and Environment Division). The increased effort will require the establishment and management of interactions between innovative scientists. The establishment of generic genomics tools and technologies using mandate crops, in particular barley and potato, provides SCRI with flexibility in the future to apply the technology to other plant or crop species in response to changing patterns in agriculture.

Against a background of reduced profitability in agriculture, plants will remain a source of new products for processing and food industries. The diversification of plants to produce novel compounds for downstream industries will depend on innovative ideas from teams of chemists, biochemists, molecular biologists, geneticists and breeders with knowledge and expertise in metabolic and biochemical pathways, gene systems, gene expression and introgression of genes and traits into advanced and adapted germplasm. The challenge for the future is to maintain the knowledge of specific plant/crop systems, the ability to assess and measure phenotypic variation accurately, and breeding expertise, and marry these to the exponentially increasing knowledge and capabilities of the new genetic and genomic approaches.

Genomics Unit A major objective of plant genomics is to discover all of the genes in an organism and determine their location on genetic and physical maps. By correlating the location of genes with the location of loci affecting traits, it is possible, after a number of parallel studies, to establish the role of a gene in a given process (the candidate gene approach for gene identification). Our studies focus on both potatoes and barley. Using potato as an example, over the last year we have made four significant advances towards these objectives. First, using a combination of AFLP and SSR markers and a diploid segregating population, we have constructed an ultra-high density genetic map (UHD map) of the potato genome comprising over 6,000 mapped markers (in collaboration with colleagues in The Netherlands, France and Spain). The target is 10,000 markers, which represents approximately 10 markers per centiMorgan (cM is the unit of genetic distance). Second, we have constructed bacterial artificial chromosome libraries of one of the parental clones (BAC libraries contain large size DNA inserts and are a key component of physical mapping strategies) and have developed a pooling strategy which allows us to identify groups of BAC clones which support the amplification of the 6,000 AFLPs already placed on the genetic map. Thus, in principle, we can quickly build thousands of short physical contigs (a contig is a group of physically over-

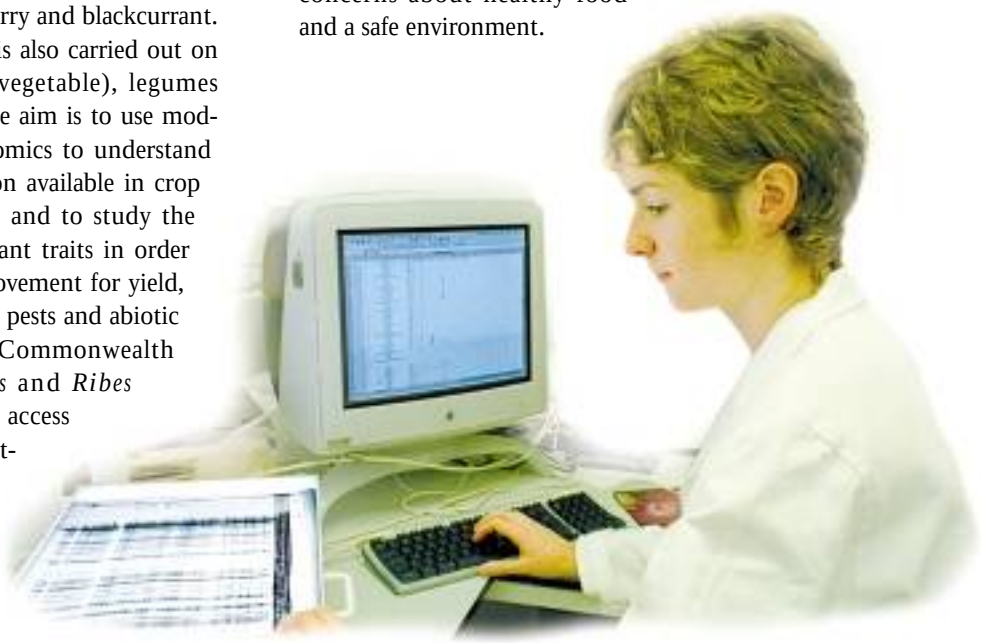
lapping large DNA clones) and link them directly to the genetic map. Currently, we are using this strategy to build a physical map of a region of the potato genome covering approximately 2-5 cM of the genetic map, which is known from previous studies at SCRI and elsewhere to contain a number of genes influencing a wide range of phenotypic characters such as dormancy, yield and disease resistance. Once the physical contig is built, we will sample sequence the entire region to identify all of the genes and, if funding allows, proceed with complete genomic DNA sequencing of this region. Third, we have completed a pilot expressed sequence tag (EST) project which has generated the partial DNA sequence of approximately 2,500 potato genes. By exploiting the physically mapped BAC clones, we will be able rapidly to position the majority of the ESTs directly on to the physical and genetic maps. From the alignment of these maps, we will ultimately be able to relate the position of genes (and identified but uncharacterised contigs containing genes) with the position of genetically mapped phenotypic traits. Genes which are functionally consistent with having a role in the trait, are expressed at the relevant time and, in the relevant tissues, can be considered strong candidates for being components of the trait. Candidate genes will then have to undergo a range of other studies involving plant transformation. Finally, the volume and complexity of this type of data demands that sophisticated methods for acquisition, archiving, analysis and interpretation of biological information be developed and implemented. Over the last year, significant progress has been made towards the automated processing of molecular markers, DNA sequence and BAC fingerprint data. Approaches for integrating such diverse data types and archiving these in an accessible and informative manner are currently being explored.

Gene Expression Unit Regulation of gene expression at the post-transcriptional level is complex and subtle. A major achievement of the RNA Processing Group over the last 2 years is the characterisation of a plant mini-exon system. In contrast to vertebrate mini-exon systems, inclusion of the potato invertase mini-exon is regulated by strong plant intron splicing signals instead of specific regulatory elements. This splicing system is very sensitive to sequence changes in the splicing signals, and provides the best system to date for systematic characterisation of plant splicing signals, which has previously been very difficult. In addition, proteins which bind to the invertase transcripts *in vitro* are being examined. In parallel, in collaboration with scientists in Switzerland and Austria, who have isolated genes for a number of protein splicing factors, proteins which alter splicing behaviour *in vivo* can be characterised. Thus, the potato invertase mini-exon system is proving extremely useful in addressing outstanding questions on plant pre-mRNA splicing. A number of other mini-exons (as small as 3 nt) have been described in plants. Whether such transcripts depend on similar mechanisms or involve novel regulatory elements and factors is important to determine, as such systems may open the door on splicing and post-transcriptional gene regulation.

The continuing focus on gene expression during the malting process of the barley grain has led to developments in several areas. A foundation unigene set derived from 2000 expressed sequence tags (ESTs) forms the basis for initial microarray studies on gene

expression during the malting process. Genetic diversity is being determined for a subset of these unigenes by their amplification and sequencing across a variety of barley germplasm, from mapping parents to landraces and wild barley. During this exercise, the prevalence of single nucleotide polymorphisms will be established and opportunities explored for their practical utilisation. Key ESTs have generated new research programmes, both at SCRI and elsewhere, on individual genes of interest both from pure and applied science viewpoints. New mapping and transgenic technologies are also being developed for the exploitation of this gene sequence information. This research aims to develop the interface between traditional hypothesis-driven experimental molecular biology and high-throughput non-biased data collection strategies central to genomics efforts as described above. It is anticipated that this will be fertile ground for practical crop improvement programmes.

Applied Genetics Unit Research in the Applied Genetics Unit is focused on SCRI's mandate crops, comprising barley, potato, raspberry and blackcurrant. Contract research and breeding is also carried out on brassicas (oilseed, forage and vegetable), legumes (*Faba* bean) and strawberry. The aim is to use modern molecular genetics and genomics to understand and exploit the genetical variation available in crop species and their wild relatives, and to study the genetics of economically important traits in order to underpin scientific crop improvement for yield, quality and resistance to diseases, pests and abiotic stresses. SCRI maintains the Commonwealth Potato Collection and *Rubus* and *Ribes* germplasm collections, and has access to wild barley germplasm. Malting quality in barley, processing quality in potatoes, and berry quality in soft fruit are all currently important targets. As most of these economically important traits display continuous variation, the emphasis is on developing the theory and practice of quantitative trait locus (QTL) analysis and of molecular marker-assisted selection (MAS). The Unit has developed and mapped doubled haploid populations of barley and F1 and backcross populations of potatoes at the diploid and tetraploid level, and is developing F1 populations of blackcurrant and raspberry. A diagnostic marker has already been developed for BYMV resistance in barley and is being used widely in commercial breeding programmes in Europe. The research leads to improved parental material for use in commercially-funded breeding programmes, and also to faster, more efficient and novel components of breeding programmes which can be tailored to meet the requirements of commercial customers. It is through these commercial contracts that we are able to produce finished cultivars that meet the ever increasing demands of farmers, growers, processors and supermarkets, as well as addressing public concerns about healthy food and a safe environment.



Development of Recombinant Chromosome Substitution Lines - a barley resource

W.T.B. Thomas, A.C. Newton, A. Wilson, A. Booth, M. Macaulay & R. Keith

The goal of SCRI's barley research programme is to identify the genes controlling commercially relevant characters such as yield and quality. This is currently a stepwise process in which regions of the genome controlling the characters are localised through relating expression of a target character (phenotype) to the genetic constitution of individuals (genotype). The process is called QTL mapping, but the results are fairly imprecise, with it being impossible to decisively refine a QTL to an interval of less than 30 cM, about a third of a barley chromosome arm. This means that association of QTL with candidate genes will also be imprecise and will therefore hinder the exploitation of genomics programmes such as the barley transcriptome resource being developed at SCRI. Another problem associated with QTL analysis is that the first generation mapping procedure, simple interval mapping (SIM), which is implemented by software such as MAPMAKER/QTL, can give misleading results. The development of techniques to account for variation in other parts of the genome, composite interval mapping (CIM), which is implemented by software such as MapQTL, MQTL, QTL Cartographer and PLABQTL, has led to some improvement in QTL location. This is illustrated by mapping plot yield and heading date data from a trial of random lines from the cross Derkado x B83-12/21/5, for which a genetic map had been constructed, carried out at SCRI in 1999 (Fig. 1). For heading date, what appeared to be one QTL has been resolved into three separate QTLs, two alleles from Derkado delaying heading date and one shortening it. The two QTLs delaying heading date are over 30 cM apart from the results of composite interval mapping and the QTL shortening heading date is also nearly 30 cM from the next nearest heading date QTL. Despite these three QTLs being spread out over a distance greater than half a barley chromosome arm, there was no indication of more than one QTL from simple interval mapping. When yield is considered, the situation is even worse for there was no indication of any significant yield QTLs on the chromosome from simple interval mapping, yet composite interval mapping revealed two QTLs some 20 cM apart. As these QTLs were linked in repulsion in the parents, i.e. one parent possessed the increasing allele at one locus and the other parent possessed the increasing allele at the other locus, simple interval mapping was not able to detect any effect in this region. Composite interval mapping has clearly helped to improve the identification of QTLs affecting key traits in barley.

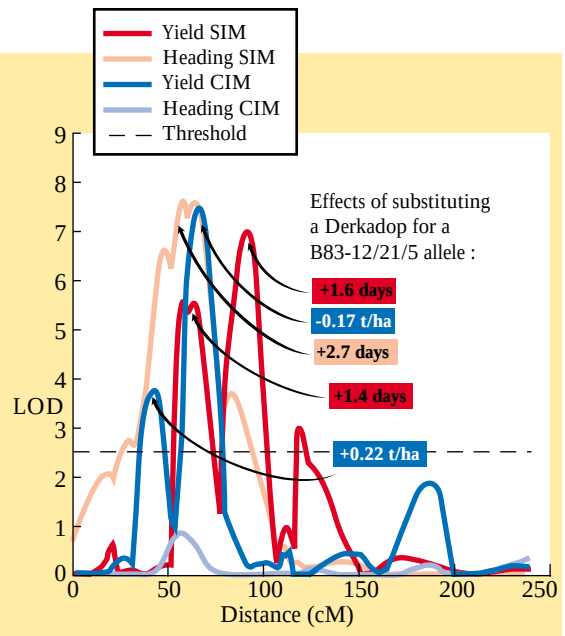


Figure 1 Derkado x B83-12/21/5 Chromosome 7H Scans 1999.

The problem with QTL identification by composite interval mapping is, however, that one is unsure whether the effects detected are real or due to over parameterisation. It is also unclear whether, as in the case of the two variates shown in Figure 1, there are two closely linked QTLs, one affecting heading date and the other yield, or just one QTL with a pleiotropic effect. Another problem is that the effects associated with particular QTLs can also be overestimated. One way to solve this problem is to design alternative populations in which to estimate QTL effects. Paterson *et al.* (1990)[1] proposed the development of a series of isolines in which small segments of a donor genome are introgressed into a common recipient to give a library of lines which covers the whole donor genome. Such isolines are called Recombinant Chromosome Substitution Lines (RCSLs), which are depicted graphically in Figure 2. RCSLs

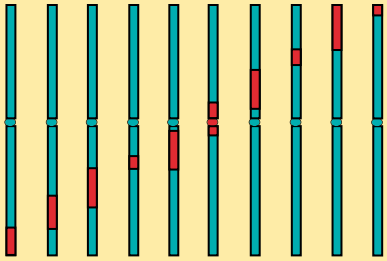


Figure 2 Set of ten Barley Recombinant Chromosome Substitution Lines with over lapping segments of donor genome (red) introgressed into a common recipient background (green).

enable fine mapping of the genome and will solve many of the problems identified above. In *Brassica oleracea*, phenotypic and genotypic analysis of RCSLs has not only confirmed the location of QTLs revealed by mapping studies but has also revealed extra loci[2]. At SCRI, we are developing a series of barley RCSLs to address some key questions in deployment of molecular markers for gene mapping:

1. Can we reveal more QTLs controlling economically important traits?

2. Will the use of RCSLs improve the estimation of effects of individual loci?

3. Are closely linked QTLs affecting different characters really two different loci or due to errors in the estimation of one pleiotropic locus?

4. If there are two loci closely linked in repulsion, can the linkage be broken to produce a marked improvement in phenotype?

We are also posing more fundamental questions through appropriate choice of donor parents and recipient. We have chosen the recently recommended spring barley Chime as the recipient parent as it represents the best available balance between malting for brewing and distilling, yield, and agronomic suitability. One donor parent is Regina, representing a diverse cultivated barley from the winter gene-pool, which has been shown to be quite distinct from the spring. The RCSLs from this donor will therefore be used to investigate the differences between the two gene-pools. Two other donor parents are a Syrian land-race (supplied by S. Ceccarrelli, ICARDA) and an *Hordeum spontaneum* accession from Israel (supplied by E. Nevo, Hebrew University). Previous research at SCRI has revealed the narrowing of the genetic base that has occurred in developing current North-West European spring barley germplasm and these last two donors have been chosen to examine some further questions:

1. Is there useful variation associated with the loci involved in domestication that can be exploited through marker-assisted introgression?

2. Do novel alleles in the donor genotypes at loci associated with economically important QTLs represent novel QTL alleles, some of which may be beneficial?

From Figure 3, it can be seen that not only are there considerable difference between the donor and recipient genotypes, there are also whole regions where the Syrian land-race and the *Hordeum spontaneum* accession possess novel marker alleles. We will therefore be able to use this material to answer these important questions that are central to the targeted use of exotic material in germplasm development. Our initial goal is to develop 'coarse focus' sets of lines with large portions of genome introgressed from each donor, using our library of SSR markers to monitor the process. We then propose to construct 'fine focus' sets of lines from the appropriate 'coarse focus' lines to study closely a target area of the barley genome. We will make these sets of 'coarse' and 'fine focus' lines available to the wider barley research community for further study and development.

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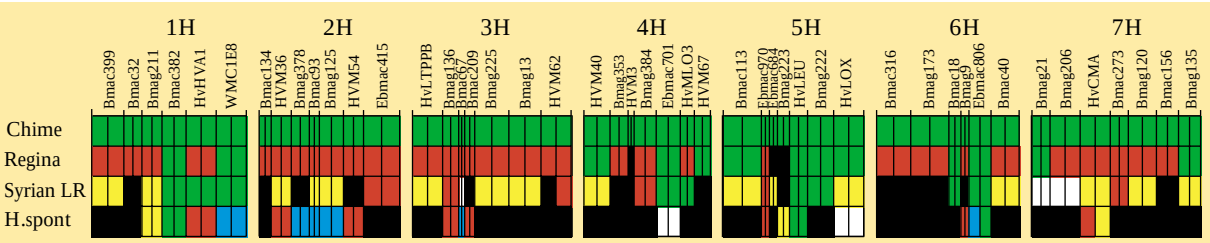

Figure 3 Graphical genotypes of recipient and donor genotypes. Black indicates novel allele. White indicates missing value.

Nutritional value and flavour of the cultivated potato

G.R. Mackay, M.J. De,Maine, D. Muir[1], G. Ramsay & M.F.B. Dale

The European form of cultivated potatoes, group Tuberosum of species *Solanum tuberosum*, as classified by Dodds[1], took approximately 200 years to evolve from its presumed ancestral form *Solanum tuberosum* group Andigena (Dodds), following the latter's introduction into Europe at the time of the Spanish Conquest of South America. In the next 200 years, it became a staple food and provided the classic example of the dangers of over-reliance on a single food commodity during the mid-19th century when late blight devastated the crop and caused the Irish famine. Nevertheless, the potato has spread from Europe to the rest of the world, and is now the fourth most important food crop after wheat, rice and maize. The potato is capable of producing a higher yield per unit area of highly nutritious food in a shorter growing season than all of the major cereals. The tubers provide an excellent balance of carbohydrates, vitamins and minerals and potato protein is superior in quality to that of cereals, despite being deficient in the sulphur bearing amino acids cysteine and methionine (Table 1). According to the US Department of Agriculture, "a diet of whole milk and potatoes would supply almost all the food elements necessary for maintenance of the human body." It is, therefore, hardly surprising that, in the last 30 years, the proportion of the world's potato crop grown in less developed countries has increased from 11 to 30 per cent and is still rising.

Demand in the more affluent societies of North America and Europe remains more or less constant, though the proportion of the crop processed into French fries and crisps is rising constantly. With an average consumption of approximately 100 kg *per capita* per annum, the potato remains an extremely important component of human diet in the UK.

Historically, practically all research into improvement of potatoes has been directed towards removing biotic and abiotic constraints on productivity; breeding for resistance to pests and disease for example[2]. In terms of selection for quality, such efforts have largely been directed to the reduction in antimetabolites, such as glycoalkaloids, eliminating after-cooking-blackening and improving fry colour[3], the assumption being that, nutritionally, there is very little variation if any between different cultivars. Similarly, in most conventional breeding programmes, it is impossible to taste all the clones in the early generation when most genetic variation is eliminated. Thus, flavour can only be assessed on the final few clones, very close to submission to statutory trials as potential new cultivars,

Constituents	Content
Calories	**100 K**
Protein	**3 g**
Carbohydrate	**23 g**
Fat	**Trace**
Recommended daily allowance	
Dietary Fibre	**12%**
Vitamin C	**45%**
Vitamin B	**15%**
Folic Acid	**8%**
Iodine	**15%**
Potassium	**20%**

Table 1 Average nutritional contents of a single medium size (*c.* 150 g) potato tuber.

[1]Hannah Research Institute

when the breeder is unlikely to discard a clone in which he/she has invested so much time and resource unless it has an obvious taint that no-one who eats it will fail to notice.

The burgeoning interest, in the UK at least, in 'flavoursome' old varieties may not be based on sound scientific evidence but it is a commercial reality and the success of special purpose salad or punnet types such as Anya[4] suggests more effort on quality and flavour may be necessary if modern high yielding, disease-resistant varieties are to satisfy end user needs and succeed in the marketplace. Moreover, special purpose varieties which fulfil the needs of a higher added value niche in the market may represent a more attractive option to growers than standard ware potatoes at current price levels.

From Table 1, the importance of potato as a source of vitamin C is self-evident. During some of our recent research, we have produced evidence of genetic variation for both quality and flavour that could be amenable to selection and improvement if desired. In a recent SERAD-funded FF project, designed to look at nutritional traits in genetically modified (GM) potatoes, we found statistically significant variation between the non-GM controls for most nutritional traits. For example, in the case of vitamin C, one unnamed clone had more than twice the level of vitamin than the cultivar Desiree and, though there were marked differences due to site and season, the cultivars Pentland Crown and Pentland Squire also had consistently higher levels than Desiree. Another study of over 40 different tetraploid *S. tuberosum* clones and cultivars indicated a five-fold range in their vitamin C contents, from 0.2 mg/g freeze-dried matter (FDM) to 1.3 mg/g FDM (see Fig. 1). These results were from material following 6 weeks storage at 4°C. It is known that vitamin C content in potatoes, and also in many perishable foodstuffs, declines during storage. The decline may be up to 50% and so such results as in Fig. 1 will be a product of both the inherent levels of vitamin C and also the effect of the genotype on the stability of vitamin C during storage. Studies are continuing to examine genotypic differences, environmental responses and also the analyses of both parents/lines and derived progeny for molecular markers associated with this important nutritional trait.

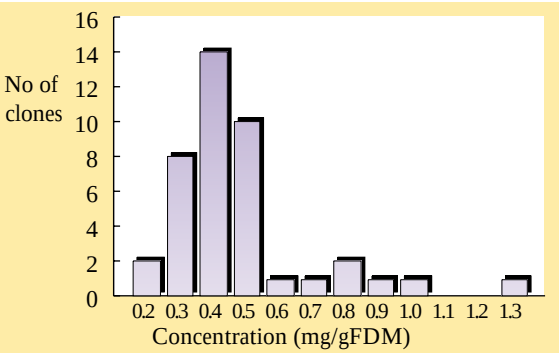

Figure 1 Distribution of vitamin C content in 40 different tetraploid *S. tuberosum* clones and cultivars.

Figure 2 Diversity of Group Andigena tubers.

Starch is the major component of potato dry matter. It is generally accepted that the amylose component of potato starch ranges from 18-23% but, in a very limited sample of SCRI breeding material, we have identified clones with amylose levels down to 16% and as high as 30%. Amylose/amylopectin ratios will affect the properties of the starch which could include organoleptic characteristics too but these have not been exploited.

In South America, at least seven forms of cultivated potato are still grown and some attract a premium because of their eating qualities.

Broadening the genetic base of the European potato by the use of landrace and wild material has permitted great improvements in pest and disease resistance over many decades. Until now, few attempts have been made to include flavour in the list of traits under routine selection by breeders, and hence widening of the genepool has had a negligible effect on this trait. The potential to expand the range of flavours, textures and cooking qualities in material adapted to UK conditions is now evident. As European tastes become more adventurous, exploiting a wider range of variation for these traits is increasing in priority. Collections containing landrace material of cultivated

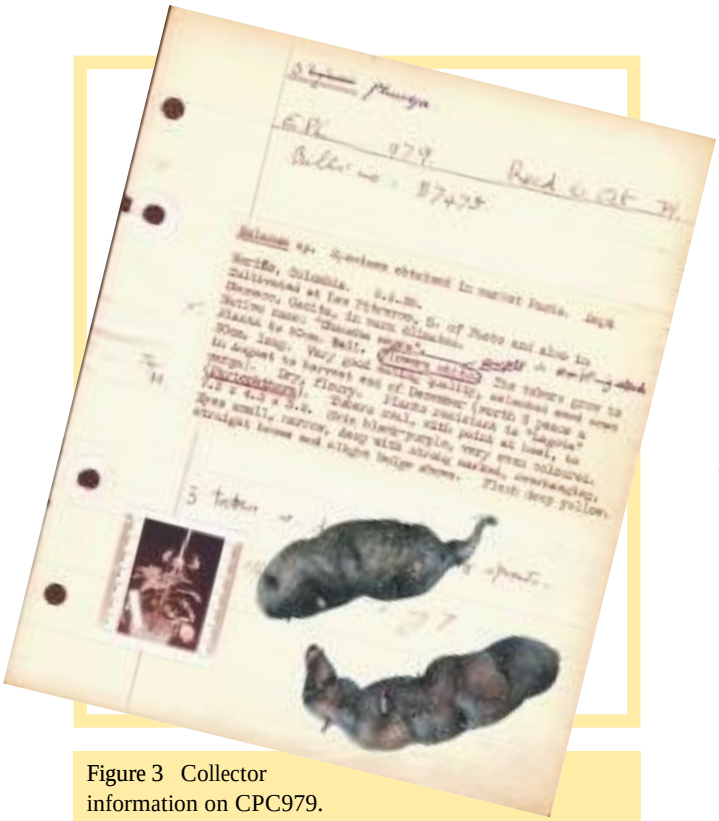

Figure 3 Collector information on CPC979.

accessions, such as the Commonwealth Potato Collection (CPC), can provide a tremendous wealth of variation for flavour and cooking quality traits as well as wide variation in tuber form and colour (Fig. 2). Such variation is generally greater in landraces from the central Andes, such as those from Group Stenotomum and Group Andigena, than those from Group Tuberosum.

One pointer to particularly useful accessions can be found in some of the original notes made by collectors as they gathered material for the collection. Group Phureja line CPC979 for example, known as 'Chaucha Negra' to the Colombians who gave the material to the collectors, is noted for its dry, floury tubers and its very good eating quality (Fig. 3). A challenge for the future is the development of efficient means to transfer any of these interesting traits into genetic backgrounds adapted for European conditions.

Varieties of Group Phureja are widely grown in Colombia, Ecuador, Bolivia and Peru, where they are prized for their delicate yellow flesh, flavour and speed of cooking. They are generally grown for domestic use rather than as a cash crop, as they have lower yields, small tubers and sprout early in store, although 'papas amarillas' attract a premium in Lima supermarkets. Work, which began at the John Innes Institute in the late 1950s and was transferred first to the Scottish Plant Breeding Station and then to SCRI, has produced a population of Phureja which is adapted to long-day (UK) growing conditions. This was achieved by a process of cyclical mass selection and produced genotypes with higher yields, improved tuber size and longer dormancy than unadapted material[5]. The excellent cooking qualities of Phureja, however, have been retained. Some companies in the potato industry have now recognised the superior flavour inherent in this unique material, and three clones have been submitted to National List trials with a view to marketing them as novel cultivars[6].

Moreover, in collaboration with the Hannah Research Institute, it has been possible to quantify these flavour differences. A team of ten trained tasters was used to develop a suitable vocabulary of descriptors for each characteristic for use on both Phureja and Tuberosum potatoes. Useful flavour descriptors were found to be: creamy, sour, salty, bitter, metallic, and earthy; mouth-feel descriptors were: floury, sticky, dry, smooth, and grainy; and appearance descriptors were: yellow, white, cream, grainy and shiny. Intensity of each character was also recorded. Principal Component Analysis of the scores given to all sensory attributes, using the covariance matrix, found that the first two Principal Components accounted for 40% and 30% of the variance and a sensory space map of the scores for the individual samples on the first two dimensions was produced (Fig. 4). This distinguishes between the two parental groups, Tuberosum (CON cluster) and Phureja (PHU cluster). The data also showed that it was possible to distinguish between individual Phureja clones for some characters. Phureja material had a higher intensity of flavour and colour than Tuberosum with testers preferring Phureja. Clones from a hybrid progeny of a cross

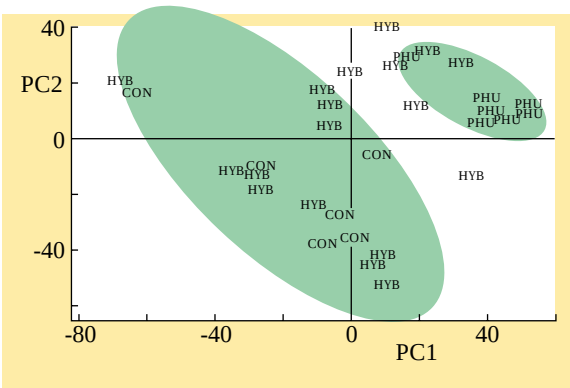

Figure 4 Sensory space map - all quality attributes.

between a dihaploid of Tuberosum (2x) and a Phureja clone were found to have characters which spanned the range between the two parental groups, indicated by the HYB plots in Figure 4.

That it was possible to repeatedly distinguish between the two parental groups of potatoes on flavour and other sensory characters, and that Tuberosum-Phureja hybrids have intermediate scores, indicates a genetic basis for the differences. Although tasting of large segregating populations is impractical, it should be possible to find molecular markers associated with these characters. Conventional breeding methods can be used to produce diploid (by the use of dihaploid Tuberosum) and tetraploid (by the use of unreduced gametes from Phureja) hybrids and, using marker aided selection, identify superior clones with Phureja flavours. Thus, there is the potential for accelerated transfer of flavour and other genes from Phureja into Tuberosum using molecular markers. Other positive quality characteristics which have been found in Phureja are generally lower levels of glycoalkaloids than in Tuberosum, less after-cooking-blackening, and enzymic (tyrosinase) browning. Enhancing Tuberosum with Phureja genes for cooking quality would introduce these desirable characteristics into varieties that are higher yielding, have larger tubers, improved tuber shape, long dormancy and, hopefully, resistance to diseases and pests, the strategic objective of most research to date[7].

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Gene discovery in potato

W. De Jong[1], J. Davidson, G. Bryan, P. Birch, R. Waugh & D. Marshall

All characteristics of potato, including tuber shape, size, number, colour, skin finish, texture, flavour, cooking characteristics, utilisation of nutrients, susceptibility to low-temperature sweetening and resistance to pathogens, are controlled to a greater or lesser extent by its genes. Like other higher eukaryotes, potato probably encodes 50,000 to 100,000 genes, the vast majority of which have never been characterised. Obviously, knowing what the genes look like (in sequence terms), where each is encoded in the genome, and what each gene does, would greatly enhance our ability to improve potato, both through conventional crossing and selection schemes, and via targeted genetic modification.

Large international teams have recently determined the complete nucleotide sequence (and thus gene content) of two model organisms, yeast (15×10^6 base pairs) and the soil nematode *C. elegans* (70×10^6 bp). The first complete genome sequence of a higher plant, *Arabidopsis thaliana* (120×10^6 bp), has just been completed, and the sequence of the human genome (3×10^9 bp) is already available as a 'first draft'. This incredible volume of sequence information, and parallel developments in miniaturization and information management technology, are radically altering the questions that researchers can ask. For example, it is now possible, using microarray technology, to simultaneously measure the expression level of every yeast gene on a single microscope slide. To obtain a quick overview of how yeast regulates its metabolism, investigators have grown yeast under a wide variety of conditions, measured the expression level of every gene under each condition, and, by determining which genes are coordinately regulated, provided a remarkably detailed understanding of yeast metabolic control. Using the complete sequence as a guide, international teams are also in the process of systematically knocking out each gene, and then evaluating each mutant yeast line for altered behavior.

The current cost of sequencing, although much lower than just a few years ago, is still too high to justify sequencing the entire potato genome (1×10^9 bp), but it has nevertheless reached a point where large scale efforts targeted at specific portions of the genome can be considered realistically. In most higher eukaryotes, only a small portion of the genome actually codes for genes (for example, assuming that the average gene is 1000-bp in length, only 5-10% of the potato genome would consist of genes). What the rest of the genome does is not well understood – some of it performs necessary structural functions (e.g. sequences comprising the centromeres, telomeres etc.), while other sequences probably help to regulate the expression of the genes themselves, and some sequences appear to be 'junk' DNA (e.g. transposons). The regions corresponding to genes are unique in that these sequences are transcribed into messenger RNA, exported to the cytoplasm, and then translated into proteins, the functional actors of the cell. By isolating messenger RNA, reverse transcribing it into complementary DNA (cDNA), and then sequencing cDNA clones, it is possible to focus sequencing efforts on the genes themselves. The use of this approach on a very large scale was pioneered in 1993 by The Institute for Genomics Research (TIGR), based in the USA, who called the resulting sequences 'expressed sequence tags', or ESTs. Prior to their report, the scientific community generally assumed that sequencing was

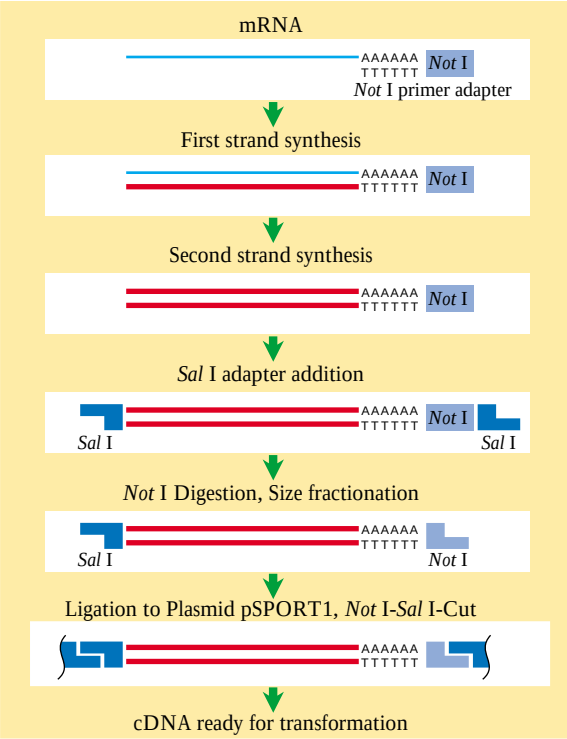

Figure 1 Summary of the cDNA cloning procedure.

[1] Cornell University, Ithaca, New York

Figure 2 Biomek 2000

best conducted by completely sequencing one gene at a time. TIGR demonstrated, however, that with automation and an emphasis on high throughput, more sequence information could be obtained per unit time/unit cost by sequencing fragments of randomly selected genes, even when the inevitable redundancy is taken into account. Since their seminal report, the EST approach to gene discovery has been applied to many organisms, and is now generally recognized as a logical first step in genome characterisation.

To date, despite its importance as the world's fourth most important food crop, very little effort has been directed at sequencing the potato genome. To help fill this gap, we recently initiated an EST program in potato. So far, we have constructed three cDNA libraries, one from shoots, and two from tubers, and have sequenced approximately 1000 clones from each library. An outline of the cloning procedure used is shown in Fig. 1. All of the sequencing has been from the 5´ end of randomly selected cDNA clones, to maximize the chance that at least some protein coding sequence would be detected in each clone. Sequencing this relatively large number of clones has required the development of some automation, which has so far been focused on template preparation, but will eventually be applied to sequencing reaction set-up and downstream data processing. In particular, with a Biomek 2000 robot (Fig. 2), we now routinely and inexpensively isolate template DNA from 384 bacterial cultures at a time, using an alkaline lysis isolation procedure first described by Bruce Roe of Oklahoma State University (http://www.genome.ou.edu/protocol_book/protocol_index.html). All of the sequences have been determined by examining reaction products on an Applied Biosystems 377 sequencer. With our current protocols, one technician can easily generate over 1000 sequences per month. The volume of sequence data has necessitated the development of a wide range of bioinformatics support at SCRI, to automate removal of vector sequences and homology searches to determine if any genes of known function are related to each EST, and to organize all of the information and database search results in an easily accessible, user friendly database – SPUDBase.

Since its inception, the potato EST program has served primarily as a tool of 'gene discovery', a source of sequences that can be browsed for genes of value to other projects at SCRI. For example, several ESTs have been identified that are clearly related to known disease resistance genes. Since we are currently trying to characterise genetically several sources of resistance to the white potato cyst nematode, each of these ESTs has been mapped, to determine whether any co-localize with resistance loci. In another project looking for potato genes activated during the defense response against the late blight pathogen, *Phytophthora infestans*, colleagues isolated a cDNA fragment corresponding to an induced gene. This cDNA was not full-length, nevertheless comparing it against our sequence database revealed that a full-length cDNA clone had already been characterised in the EST program.

Over the past few years, colleagues at SCRI have developed over 150 simple sequence repeat (SSR) markers from potato. Because of their highly-polymorphic, multi-allelic nature, SSR markers are very well suited for genetic studies in highly heterozygous, autotetraploid potato (see Ann. Rep. 1996/7, 96-98). About 30 of the 3000 EST sequences obtained to date have been found to contain SSRs. Most of the gene-derived SSRs are trinucleotide repeats, which is not surprising since only trinucleotide motifs can contract or expand without altering the reading frame of the gene. An example of an EST containing a microsatellite is shown in Fig. 3. In collaboration with colleagues at the International Potato Center in Peru, we are currently investigating how frequently these coding sequence SSRs reveal polymorphism, and for

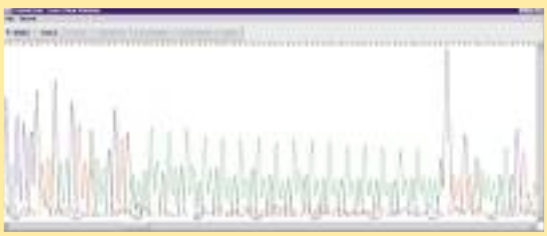

Figure 3 Sequence electropherogram of EST containing Simple Sequence Repeat with17 repeats.

Figure 4 Alignment of SAMDC sequences.

those which do, determining genetic map locations. At least in theory, SSR markers based on coding regions should be useful in a wider range of germplasm than other SSRs, because primer annealing sites will evolve more slowly in coding than non-coding sequences.

One of the more interesting results of the exploratory sequencing so far is that several ESTs closely related to well known potato genes are even more closely related to genes from other species, suggesting a hitherto unrecognized ancient polyploidization or gene multiplication event in potato. For example, the potato gene encoding S-adenosyl methionine decarboxylase (SAMDC) has been intensively studied at SCRI[1]. Two overlapping ESTs (950 and 980) bear obvious sequence similarity to potato SAMDC, but appear to be more closely related to SAMDC from Japanese morning-glory *(Ipomoea)* and other plant species. Figure 4 shows an alignment of the two SAMDC-like potato ESTs against SAMDC sequences from the databases. This suggests the possibility of the existence of a second potato SAMDC gene - whose function still needs to be determined at the biochemical level. This was not suspected before it was sequenced, although in hindsight, it may provide an explanation for the residual SAMDC activity observed in transgenic potato plants expressing the original SAMDC in an antisense orientation, even though SAMDC transcripts could not be detected. Other interesting potato ESTs may represent a second hexokinase gene and a possible second sucrose phosphate synthase gene.

Sequencing several thousand potato genes is only a beginning, but still a very useful foundation. In the future, we hope to build on this work in at least three ways. The first is to use non-redundant ESTs to develop a very dense gene map of potato. In a typical mapping experiment, genes controlling a trait are localized to a chromosomal region that may contain anywhere from tens to thousands of genes. Knowing what genes are present in that region will greatly accelerate functional correlations between traits and genes. Secondly, high density microarrays of potato ESTs will be constructed to allow the expression of thousands of genes to be monitored simultaneously in a wide variety of experimental conditions, e.g. during tuber transition from dormancy to sprouting, or during response to pathogen attack. Thirdly, as increased sequence information becomes available and bioinformatics expertise develops, it will become possible to conduct large scale comparisons of all potato ESTs with the completely sequenced *Arabidopsis* genome. A significant issue in working with any crop plant is how to use functional information obtained in the intensively studied model plant *Arabidopsis*. Over the next five to ten years, systematic mutant screening will allow functions to be assigned to literally thousands of genes in *Arabidopsis*. When a gene in *Arabidopsis* is known to have only one clear relative in potato, inferences can be made with some confidence, but it becomes more complicated when the gene is part of a gene family in either or both species. It will also be possible to compare the extent of conservation of genome structure between potato and *Arabidopsis*. We have already seen occurrences of groups of several potato ESTs showing 'hits' to the same large segment ('contig') of *Arabidopsis* genome sequence. If we can demonstrate that such sets of ESTs, or a subgroup thereof, are closely linked in potato, suggesting some degree of genome collinearity, this will further increase the applicability of sequence information from the model to crop plant systems. Thus, large scale potato EST sequencing will help to provide a much needed context for judging gene relationships between the two species, and thus ensure that better use can be made of the very rapid progress in *Arabidopsis* and other model systems.

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SSR frequency and occurrence in plant genomes

L. Cardle, M. Macaulay, D.F. Marshall, D. Milbourne, L. Ramsay & R. Waugh

Until recently, bioinformatics could give only a vague picture of plant genomes due to the short fragmentary nature of the plant DNA sequences available from public databases. As large scale sequencing projects are revealing more and more long, contiguous DNA sequences, the overall genetic structure of plants will become increasingly clear, providing better genetic models for the development of strategies for experimental studies.

Here we demonstrate a bioinformatic analysis which takes advantage of the significant number of long, contiguous sequences recently deposited in the international sequence databases. We have re-evaluated the distribution of Simple Sequence Repeats (SSRs), a sequence feature of eukaryotic genomes which are an important source of polymorphisms for genetic studies, and shown that SSRs are far more common in plant genomes than previously estimated.

The ubiquity of SSRs and their usefulness as genetic markers has been well established over the last decade. In mammalian systems in particular, SSRs have been the marker of choice for several years, and well developed SSR-based linkage maps are available for a number of species. The usefulness of SSRs has also been demonstrated for a variety of plant species and this has prompted the initiation of SSR discovery programmes for the majority of agronomically important crops. However, to date, a number of limitations have existed with SSR discovery in plants, including a lack of DNA sequence in databases, a perceived low abundance of SSRs (compared to mammals) and differences in the most common types of repeat found.

Source	*Arabidopsis thaliana*	
Subgroup	Genomic (P1 & BAC)	ESTs
Number of sequences	306	36199
Number with ≥ 1 SSR	305	1040
Repeat type:		
Mononucleotide	1471 (33)	103 (10)
Dinucleotide	1333 (30)	254 (24)
Trinucleotide	1350 (30)	706 (66)
Tetranucleotide	236 (5)	7 (<1)
Pentanucleotide	83 (2)	0
Total SSR content	4473	1070
Total length (kb)	27011.3	14808.0
Average distance (kb)	6.04	13.83

Numbers in brackets show percentage of total SSR content

Table 1 SSR survey of *Arabidopsis* genomic and EST sequence data.

Previous analyses of plant DNA sequence database entries for all possible SSR motifs have revealed frequencies ranging from one every 29kb to 50kb, depending on species. Oligonucleotide hybridisation studies have suggested figures in the range of one SSR every 65kb to 80kb. These results contrast sharply with those for humans, with an estimate of one SSR every 6kb on average.

Despite this relative difference in abundance, the perceived advantages of SSRs as markers are such that plant geneticists have resorted to screening large numbers of clones, or developing selective SSR enrichment techniques, in order to generate sufficient numbers of SSRs. Given the interest of the plant genetics community in SSRs as genetic markers, we have been particularly concerned to establish methods of rapidly identifying robust and informative SSRs linked to genes of agronomic significance.

Frequency and distribution of SSRs in *Arabidopsis thaliana* Three hundred and six non-redundant, genomic DNA sequences longer than 10 kb, and over 36000 EST sequences were retrieved from the EMBL nucleotide database (on 24/06/98) and searched for the presence of SSR motifs. All but one of the long genomic DNA sequences contained at least one SSR, and each clone possessed 10 SSRs on average (Fig. 1a, see Fig. 2 for an example of SSR distribution in a clone). In contrast, only 3% of ESTs contained an SSR, which is similar to the proportion previously found in rice ESTs[1]. Overall, the average distance between SSRs in *Arabidopsis* genomic DNA was approximately 6 kb compared to a figure of 14 kb for ESTs (Table 1).

By examining the detailed features tables available for 51 of the 306 *Arabidopsis* genomic sequences, a con-

siderable difference in the distribution of SSR motifs was found between introns, exons and intergenic regions (Fig. 1b). Almost two-thirds of SSRs were found in intergenic regions (and the majority of these were either mono- or di-nucleotides), 14% were found in exons, and 23% in introns.

Of the exonic SSRs, 91% were tri-nucleotides, reflecting repetitive amino-acid sequence motifs, although there was no simple pattern of motifs in relation to different protein classes. The remaining 9% was made up of 10 di-nucleotides, one mono- and one penta-nucleotide repeat. A more diverse range of motif lengths was found in introns, with similar proportions of repeat types being found in intergenic regions. The proportions found within the data examined indicated that over 40% of all trinucleotide repeats are exonic in *Arabidopsis.*

SSR distribution in other plants In similar analyses of the 52 genomic DNA sequences (>10 kb) from species other than *Arabidopsis*, 38 were found to have at least one SSR motif. The overall average distance between SSRs for these species was 6.8 kb, almost identical to that found in *Arabidopsis* alone.

A number of contiguous sequences of over 30 kb were available for inclusion in this study (from barley, tomato, rice and potato). Using all available data from these species, the estimated SSR frequency was one every 7.4 kb in barley, 7.1 kb in tomato, 7.4 kb in rice, and 6.4 kb in potato genomic DNA. Despite the relatively small number of sequences available, the similarity in SSR frequency with *Arabidopsis* suggests that one every 6 - 7 kb may be a good general estimate for SSR frequency in the type of plant DNA sequence studied here (*i.e.* large insert DNA clone sequences containing a gene of interest).

The study reported here has made use of the recent submission of a large volume of contiguous DNA sequence emerging from the *Arabidopsis* genome sequencing project, to allow an estimate to be made on sequence that is not skewed towards coding regions. This, together with the detailed annotation on a large proportion of the data, has shown that not only are SSRs at a higher frequency than previously estimated, but also that the frequency of the SSRs varies within the genome, with exonic and intronic sequences making up roughly 55% of the genomic sequence but containing only 37% of the SSRs. This is particularly evident in exons which make up 31% of the genomic sequence but contain only 14% of the

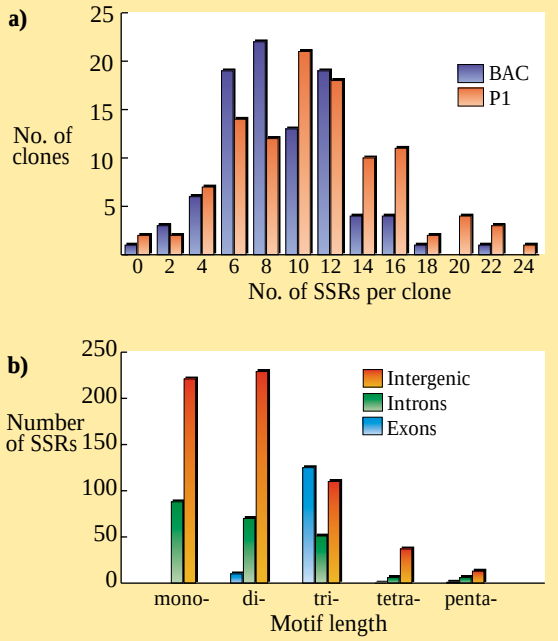

Figure 1 Frequency of SSRs in *Arabidopsis.* a) Actual number of SSRs in a collection of P1 and BAC clones, b) Number of SSRs of different repeat lengths in intron, exon and intergenic sequence.

SSRs, 91% of which are trinucleotide. This finding corresponds well with the lower frequency of SSRs and the preponderance of trinucleotide repeats found in EST sequences compared to genomic sequences[1].

An experimental approach We developed and tested the hypothesis that sequencing random subclones (from e.g. a BAC clone) provides an effective strategy for identifying single or clustered SSRs in targeted genomic DNA. In demonstrating the approach in a barley BAC clone, only one SSR met our 'repeat length' definition and two were slightly short. Nevertheless, polymorphism at one SSR enabled the BAC clone to be mapped to a chromosomal position, which was confirmed by the use of another short SSR known to be upstream of the gene sequence. The discovery of one SSR that meets the criteria above in 36 runs of 400 bp, represents a frequency of one SSR every 14.4 kb, somewhat lower than the estimated values above. However, the polymorphism found in these short SSRs and others, implies that the minimum repeat length used for the search of public databases was possibly too conservative.

In species where BAC or P1 libraries are already available, they represent a ready source of SSRs which are intrinsically 'high value' for several reasons. BAC

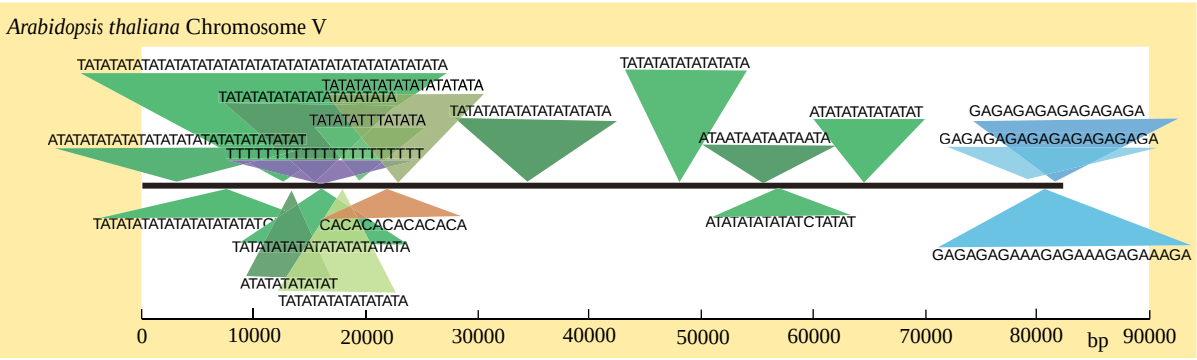

Figure 2 Distribution of SSR sequences in P1 clone MCL19 (84,510 bp).

clones linked to genes of interest can be selected by hybridisation directly or to any closely linked low copy DNA-based marker, and locus-specific SSRs can be developed quickly and efficiently, by the sample sequencing approach described. Even when the actual sequence of the target gene is known, the use of this approach may produce SSR markers more easily deployable than markers based on the actual gene sequence.

In addition, the presence of multiple linked SSRs on a single BAC or P1 clone facilitates the detection of multiple SSR 'haplotypes'. Haplotypes are considerably more descriptive than single markers and are particularly suited for applications such as marker-assisted selection. They are also suitable for many other areas of biology such as biodiversity assessment and population genetics with multiple multi-allelic SSRs giving comparable discrimination to that of Single Nucleotide Polymorphisms (SNPs) in analyses.

Conclusion The findings presented here demonstrate that SSR frequency in plants is considerably higher than previous estimates, with a frequency of one SSR every 6 – 7 kb which is equivalent to that described in mammals. However, differences in the frequency have been found between different regions of *Arabidopsis* relating presumably to differing evolutionary constraints. Although the frequency of SSRs in gene rich regions of other plant species appears to be similar to that of *Arabidopsis*, it is anticipated that genomic regions containing high copy number DNA will have a different profile. In addition, differences between SSR frequency and motifs in available EST data suggest that there are particular genome specific constraints in coding regions. Outside these structural or evolutionary considerations, the reported findings have immediate practical value with the development of a strategy for the targeted isolation of single or multiple, physically clustered SSRs near any mapped gene of interest. This will impact on genetic studies of the increasing number of plant species for which large insert libraries are available.

A complete description of the above study can be found in L. Cardle et al. (2000). Computational and experimental characterization of physically clustered simple sequence repeats in plants. *Genetics* 156(2), 847-854.

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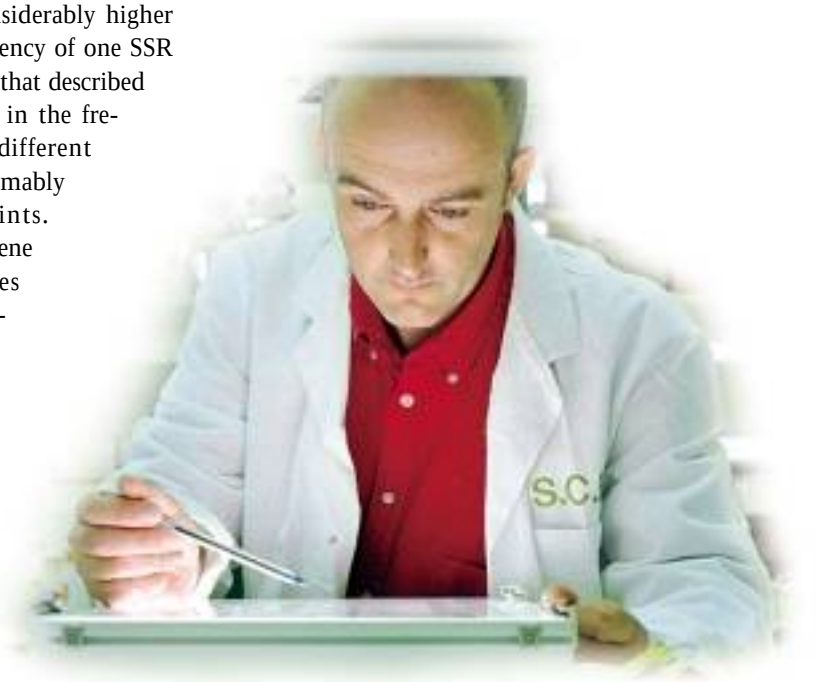

How small is an exon – does size matter?

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

The removal of non-coding introns from pre-mRNA and the splicing together of translatable exon sequence is an essential requirement of gene expression and one area of post-transcriptional processing where gene expression can be regulated. The process of pre-mRNA splicing is mediated by a RNA and protein complex, called the spliceosome, that assembles on the pre-mRNA before removal of the intron takes place. Commitment to splicing requires the recognition of splice sites by factors prior to assembly of the major spliceosomal components. The accurate selection of these splice sites, in a pre-mRNA background that may contain many potential splice sites, is essential to obtain the correct splicing of exons. Two mechanisms of splice site choice, intron and exon definition, have been identified in eukaryotes that allow the accurate selection of splice sites during splicing. In exon definition, splice sites on either side of the exon are recognised by interactions between factors at the 5' splice site and the 3' end of the upstream intron. Because of the smaller size of plant introns, where only ~10% of introns are greater than 600 nt, exon definition was not expected to occur in plant splice site selection. However, we have, firstly, identified a number of splice site mutants that lead to exon skipping which is highly indicative of exon defining interactions operating in plants. Secondly, we have shown that, when two introns are found on the same transcript, one intron can enhance the splicing efficiency of the other, supporting the idea of interactions between the introns across the exon (Ann. Rep. 1996/97, 102-103). It is clear, therefore, that exon bridging interactions have an important role to play in plant splicing.

For exon defining interactions to occur, there is a minimum exon length of ~50 nt required before hindrance between the factors at the splice sites limits any interaction (Fig. 1). Despite this limitation, there are a number of examples of exons that fall below this minimal exon length, called mini-exons. In vertebrates, additional signals, in particular intron splicing enhancers, are required to promote the inclusion of a mini-exon. We have previously described the constitutive inclusion of a conserved 9 nt mini-exon in potato invertase mRNA transcripts. The elements required for the inclusion were found in the upstream intron (Ann. Rep. 1997/98, 71-73). Inclusion of this mini-exon has been studied further and detailed mutational analysis highlights the importance of a branchpoint consensus and an associated U_{11} sequence in the 3' end of the upstream intron. An

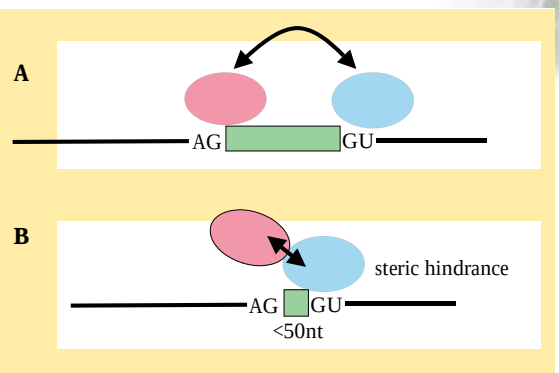

Figure 1 Steric hindrance in mini-exons
A) When an exon (box) is greater than 50nt, factors at the 3' and 5' splice sites (AG and GU respectively) are able to interact through exon definition. B) When exons are less than 50nt, factors are unable to interact and exon definition is inhibited through steric hindrance.

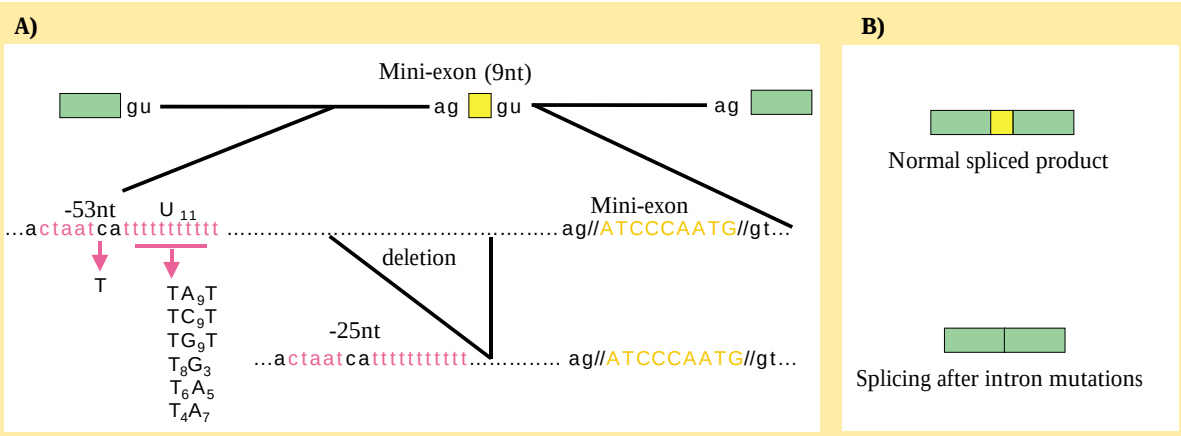

Figure 2 Detailed mutational analysis of invertase mini-exon splicing.
A) The structure of the mini-exon sequence is shown along with mutations to the branchpoint positioned –53nt from the 3' splice site and to the U_{11} element. A deletion between U_{11} and the 3' splice site brought the –53nt branchpoint and its associated U_{11} sequence to a position –25nt from the 3' splice site (boxed sequence). B) Splicing analysis of all these mutants in tobacco protoplasts led to skipping or exclusion of the 9nt mini-exon, while the normal transcript included the mini-exon.

A→U mutation to the branchpoint –53 nt upstream upstream from the 3' splice site skipped the mini-exon (Fig. 2). This shows that the upstream branchpoint nucleotide is essential for inclusion of the mini-exon. A U_{11} sequence 3 nt downstream of the putative branchpoint was mutated to a string of A, C or G nucleotides, and the number of Us were reduced to 8, 6 and 4 (Fig. 2). Virtually all mutations lead to non-inclusion of the mini-exon. This shows the essential nature of the U_{11} element for splicing of the mini-exon and a requirement for this element to be U-rich.

A striking feature of the branchpoint and U_{11} is its distance from the 3' splice site. This arrangement of branchpoint/U-rich region located at an extended distance (>25nt) from the 3' splice site is conserved among plant invertase genes. Reduction of this distance, such that the putative branchpoint was now 25 nt upstream from the 3' splice site, led to skipping of the mini-exon, indicating a need for the branchpoint to be further upstream than normal (Fig. 2). Thus, inclusion of the mini-exon appears to involve a bridging interaction between factors at the branchpoint/U-rich region and the 5' splice site flanking the mini-exon.

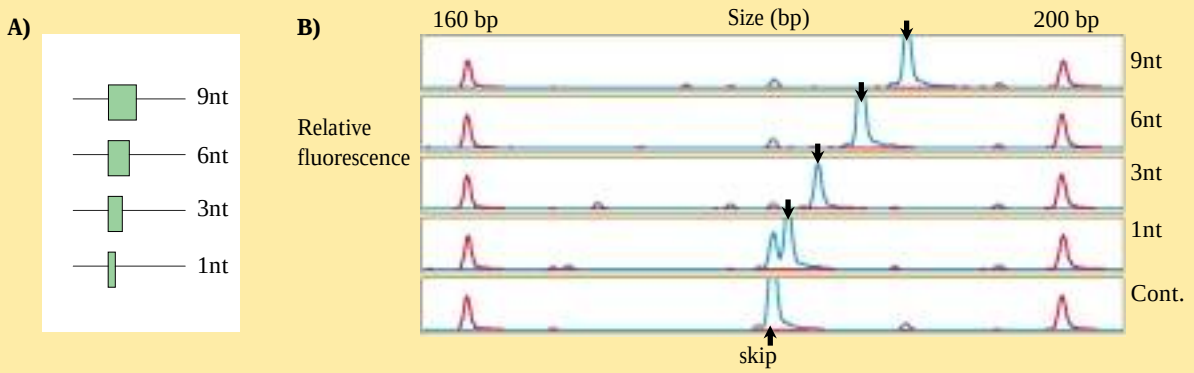

Figure 3 Splicing analysis of shortened mini-exon sequence.
A. The mini-exon sequence shown in Figure 2 was reduced in length from 9nt to 6, 3 and 1nt. The boxes represent the mini-exon.
B. RT-PCR analysis of the shortened mini-exons was performed using a fluorescent primer and the products separated on a gel. Bands found on the gel are represented here as a spectrogram of relative fluorescence against the size in base pairs (bp) of the RT-PCR product. Size marker peaks are above the 160bp and 200bp markers. The position of an RT-PCR product that represents a skipped product of 180bp is indicated. Inclusion of the differently sized mini-exons in the RT-PCR products are shown by arrows above the relevant peaks (189nt, 186nt, 183nt and 181nt for the 9, 6, 3 and 1nt exon respectively).

That the 9 nt exon is included in all invertase mRNAs suggests that the signals which we have defined represent very strong splicing signals. Therefore, it is possible that the sequence of the mini-exon is not important for its inclusion and that the signals could splice other exon sequences. To address this, the mini-exon was reduced in length from 9 nt to 6, 3 and even 1 nt in length. The 6 and 3 nt mini-exons were accurately and efficiently included while two-thirds of the final mRNA transcripts contained the 1nt mini-exon (Fig. 3). Efficient inclusion of a 1 nt exon sequence is quite exceptional and highlights the strength of the mini-exon signals. It also shows that the mini-exon sequence is irrelevant for splicing and that, in this system at least, the small size of the exon is not important. The challenge is to determine whether these signals can act as splicing enhancers to manipulate splicing and regulate expression. At present, we are looking for proteins that interact with the pre-mRNA elements involved in invertase mini-exon inclusion and are investigating other naturally-occurring plant mini-exon splicing systems.

Cereal gene mining and manipulating

G.C. Machray, C.J. Zhang, T.M.A. Wilson, D. Davidson, B.E. Harrower, P.E. Hedley & W. Powell

Research on cereals in the Gene Expression Unit has focused primarily on barley but also features work on wheat for very different but equally compelling reasons. Barley is of paramount importance to agriculture and the local economy in Scotland and the North of England, where most of the crop is utilised for the production of malt which, when processed to produce whisky, remains the major UK export earner from the food and drink sector. Wheat rivals rice as the leading crop in world-wide production, and is becoming increasingly important in the less-developed world as a staple to meet the food demands of an ever-increasing population. These local and global drivers have led to the two research programmes described in this article. They have different objectives but share common technologies and each has already benefited from the other - the potential for exploitation of this research in the future across the cereals, including rice, is tremendous.

Virus-resistance in Chinese wheat Wheat is a staple food for approaching 40% of the world's population and, in China, one half of its 1.2 billion people rely on the wheat crop for their major food needs. China is thus one of the world's major wheat producers, along with the nations of the former USSR and the USA. The crop is grown on 30 million hectares of arable land in China, and production has trebled over the last 30 years. These impressive gains have been necessary to match population growth, but are continually threatened by disease caused by pests and pathogens, with resulting annual crop losses estimated at about 50%. Reduction in these losses would enhance both food security and the sustainability of this production.

Prominent in the losses incurred in the Chinese winter wheat crop through viral disease is a 10-30% loss in yield of winter wheat resulting from infection by viruses transmitted by soilborne fungi. *Soilborne wheat mosaic virus* (SBWMV) and *Wheat spindle streak mosaic virus* (WSSMV), which cause the bulk of this loss, are both transmitted by the fungus *Polymyxa graminis* in soil. Preventing the infection of roots by the application of fungicide is difficult, expensive, environmentally undesirable and impracticable because the fungus can survive as resting spores in soil for many years. Once a field is infected with viruliferous fungus, there is no way to eradicate the virus. Therefore, the only simple and economical method to control these viruses is by growing virus-resistant wheat cultivars in the infected fields.

Plant breeding for virus resistance involves the identification of resistance genes and their introgression into elite genotypes. There follows an ongoing struggle for supremacy between the breeder and the pathogen, which seeks to evade control. The breeder's resistance

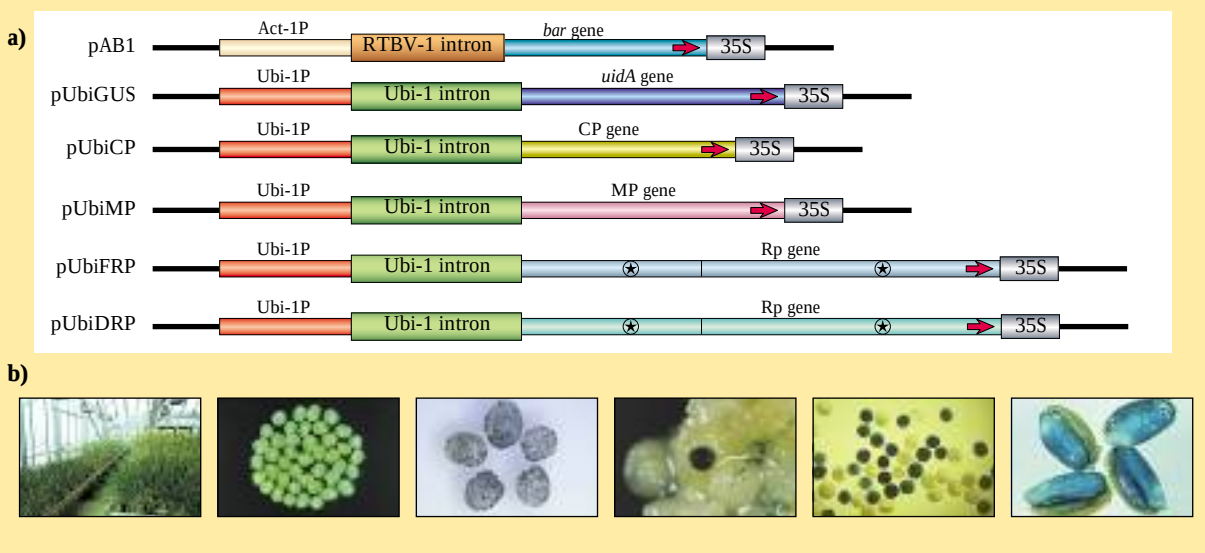

Figure 1 a) Gene constructs for Chinese wheat transformation. b) Stages in the transformation of wheat.

armoury has been enhanced recently by transgenic methods and, particularly, by the finding that, in dicotyledonous plants (dicots), the expression of a single viral gene as a transgene within the plant can inhibit the spread of virus throughout the plant. Genes for viral coat protein, movement protein and replicase have been used in this strategy, which has been developed at SCRI against potato viruses and has proven effective (see SCRI Annual Report, 1993). We have extended this approach to monocotyledonous cereals by transforming these genes from SBWMV into both model cultivars of wheat and cultivars used in breeding programmes in China.

Genes are introduced into wheat by a biolistic approach, using immature embryos as the target tissue. Example constructs are shown in Figure 1. It features genes encoding SBWMV coat proteins, driven by a constitutive promoter from an ubiquitin gene, including the first intron of the same ubiquitin gene, which stabilises expression in monocots. The construct DNA is coated onto gold particles and introduced into cells of the immature wheat embryo by bombardment. Optimal conditions for this introduction are established by determining the best conditions for transient expression, from a similar construct, of a reporter molecule (GUS) (Fig. 1). Gold particles used for bombardment can be coated with the DNA of both constructs leading, after regeneration and selection, to the generation of stable transgenic wheat plants containing both genes, and which express the GUS reporter as well as the coat protein (expression from pollen and endosperm for example, and transgenic plants, are also shown in Fig. 1). Using this approach, we have been successful, as confirmed by Southern blotting, in transforming the model cv. Bobwhite and three Chinese cvs., Yangmai 93-111, Yangmai 94-141 and Yangmai 2980, used in Chinese breeding programmes. Genes for coat protein, movement protein and replicase, in sense, antisense and defective gene constructs, have all been introduced into these varieties. This EU-funded research includes partners from Europe (IACR, Rothamsted and the Max Planck Institute, Koln) and leading Chinese agricultural research centres who will conduct resistance testing of the transformed wheat to determine if the success achieved with this approach in dicots. has been emulated.

Gene expression in malting barley The focus of our work on barley is related to quality rather than disease resistance. Most of the barley grown in Scotland is processed into malt which supports a £2.4 billion per annum whisky export industry. The production of the optimum barley grain for malting is the result of a great variety of complex biological processes. These range from the deposition of starch in the developing endosperm, and all the plant physiology and biochemistry which support this, to a rapid and even germination of the grain achieving the desired hydrolysis of complex carbohydrate to substrates utilisable by yeast. SCRI's breeders and geneticists have made great inroads into putting these processes into a genetic framework and, aided by molecular marker technology, in applying this knowledge to practical breeding goals. It is clear from their work that properties such as malting quality are governed by multiple genes whose contributions will require dissection and description before any understanding of these complex phenotypes can be reached. Fortunately, a new set of tools which can be applied to these problems is now on stream - these include large-scale gene discovery programmes and high volume parallel gene expression analysis. We have begun the application of both technologies to barley, together with the development of barley transformation which will be essential to the determination of function. When combined with existing strengths in genetic and phenotypic analyses of barley, new strategies for the analysis of complex traits can now be assessed.

The gene discovery element of our malting barley research began with the sequencing of DNA from some 2,000 genes expressed in the grain 3 days after the commencement of the malting process. This was achieved by the construction of a cDNA library from the mRNA population present at this stage and single pass sequencing from individual clones derived from this library to derive expressed sequence tags (ESTs). The process was remarkably informative with little redundancy in the sequence information obtained. The primary analysis of this information (after quality checks) was comparison to existing public databases of all known genes. The results of this analysis can be

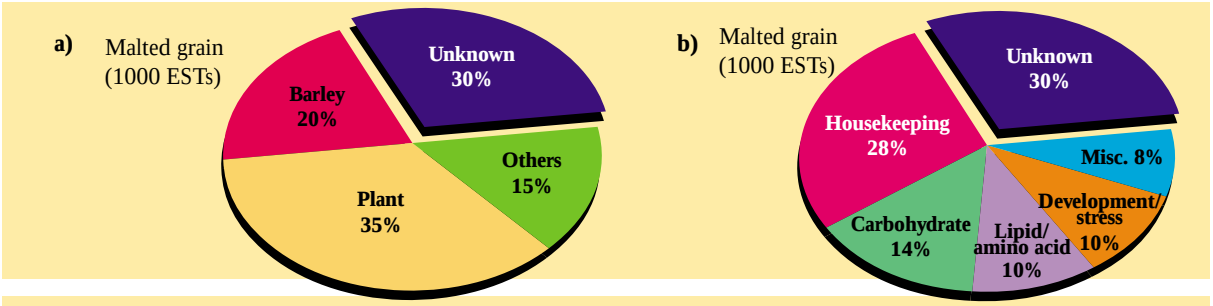

Figure 2 EST composition. a) 70% of the malted barley ESTs show significant homology to existing database sequences, primarily plant sequences, while 30% show no significant homologies. b) The ESTs to which a putative function can be assigned by homology have been classified according to that function: most deal with cellular housekeeping and organ level responses to the malting, but a significant proportion is involved in determining the intensive carbohydrate, lipid and amino acid metabolism activated during this process.

presented in several ways, as illustrated in Figure 2. Full interpretation of this data presents a daunting task in bioinformatics. Individual sequences have founded novel investigations in our own and collaborators' labs. These have goals relating to a much wider area than malting quality. They include (as examples): a determination of the prevalence of microsatellites and single nucleotide polymorphisms in the expressed portion of the barley genome and their exploitation in diversity studies and association genetics; the cloning of disease resistance gene 'islands'; the characterisation and activity of barley retrotransposable elements; and the development of novel selectable markers for barley transformation. The assembled sequence information also offers a preliminary insight into various metabolic processes contributing to malting quality in the germinating grain during malting. We can begin to assess these at the level of a metabolic pathway rather than, as previously, as single enzymatic conversions. This approach will be greatly enhanced by further EST sequencing in malting tissues and extended by the collection of EST information from the developing grain - the sequencing of 40,000 ESTs will be undertaken in the Genomics Unit over the next 3 years.

The above data represent a snapshot of gene expression in the barley grain at one stage of malting. A further way to add value to this information is to put it in a temporal and spatial context. This can be achieved by depositing the sequences on microarrays and interrogating these with complex probes encompassing the range of genes expressed at specific times in the malting process or from specific tissues of the malting grain. A good versus poor malting quality comparison can also be included. These parallel gene expression studies are now in progress, while the pace of technological advance in this area is breathtaking. Data derived from them, combined with genetic, phenotypic and bioinformatic analyses, will bear on the selection of genes, or gene sets, for manipulation towards the improvement of malting quality and other complex traits. Accelerated breeding or transgenic routes can be used as appropriate to achieve the desired improvements. For many genes, stable transformation will offer the most definitive determination of gene function. Progress in barley transformation has been greatly increased by our experience with wheat but priorities remain in increasing efficiency and minimising genotypic dependency. Just as the development of transformation technology in wheat has benefited barley, much of what we learn about gene expression in barley will complement research on wheat and other cereals.

Prospectives The new millennium heralds a new era in biological research in which we can take a more integrated view of molecular, cellular and whole plant processes. The wealth of new information from genomics will impose significant challenges in bioinformatics if the maximum value is to be derived from it, in combination with existing genetic and phenotypic information. If this enterprise is successful, we can expect much increased definition in the selection of targets to effect desired phenotypic changes. These will be facilitated by the precision engineering of genomes involving the introduction of highly targeted changes to specific (sets of) genes with no collateral modification. In addition, candidate genes emerging from such studies will provide new opportunities to connect sequence diversity to phenotype and provide more informative ways of describing plant genomes. Coupling the power of an integrated science approach to tackle complex biological problems is an exciting endeavour that will require connections between discovery and hypothesis driven research.

New potato cultivars

Golden Millennium is a new crisping cultivar National Listed in 1999. It has been selected within a targeted breeding programme funded by Golden Wonder. It is a product of a cross between the cultivar Brodick and an SCRI parent with processing characteristics - 14025 A 3. It is a maincrop, producing round/oval tubers with a light yellow flesh colour and pale pink eye. Golden Millennium has good dry matter, storage and processing characteristics. It also has good levels of resistance to gangrene, common scab, blackleg, virus Y, damage and has partial resistance to the golden potato cyst nematode (*G. rostochiensis*, Ro l pathotype).

Golden Millenium

Harborough Harvest is another new crisping cultivar National Listed in 1999. It was also selected from within the targeted breeding programme funded by Golden Wonder. It is a product of a cross between the SCRI cultivars Brodick and Eden. Harborough Harvest produces round white tubers with a cream coloured flesh. It has excellent dry matter, storage and processing characteristics for the crisping industry. It also has good levels of resistance to tuber blight, blackleg, leafroll virus and is resistant to the golden potato cyst nematode (G. *rostochiensis*, Ro 1 pathotype).

Harborough Harvest

Montrose is the first cultivar to be selected from a targeted breeding programme funded by McCain (UK) and aimed at their specific end uses. It is the product of a cross between two SCRI processing cultivars (Brodick and Eden) and was National Listed in 1999. It is a maincrop producing long white, blue eyed tubers with a light yellow flesh colour. It has good dry matter, storage and processing characteristics which make it particularly suitable for the chipping industry. It exhibits good levels of resistance to late blight, virus Y and leafroll virus.

Montrose

	Golden Millenium	**Harbourough Harvest**	**Montrose**
Origin :	Brodick x 14025 A 3	Brodick x Eden	Brodick x Eden
Year of cross :	1982	1987	1987
Maturity	Maincrop	Maincrop	Maincrop
Wart	Resistant	Resistant	Resistant
Late blight (f)	5	5	5
Late blight (t)	5	6	6
Gangrene	7	8	7
Common scab	8	3	3
Powdery scab	3	4	2
Virus:			
PVY	8	5	7
PLRV	5	6	6
PCN:			
G. rost.	5	8	Susc.
G. pall.	Susc.	Susc.	Susc.
Blackleg	9	5	4

Plant biochemistry and cell biology

Howard V. Davies & Karl J. Oparka

Institute restructuring has resulted in the formation of four scientific research divisions. The Department of Cellular and Environmental Physiology, which has evolved into the Division of Biochemistry and Cell Biology, houses two research units – Plant Biochemistry and Cell Biology. Following the retirement of Dr W.W. Christie M.B.E., as Head of the Chemistry Department, the Chemistry Group has been incorporated into the Plant Biochemistry Unit. These structural changes, together with a revised SCRI Corporate Plan and science strategy, will form the basis of new research alliances and programmes, further integrating our project portfolio.

Plant Biochemistry

Metabolism and developmental processes The functions of two novel plant α-glucosidases genes, both cloned at SCRI, have been studied in detail using transgenic potato plants as experimental tools. Clear-cut evidence demonstrates that one of the α–glucosidases is required for glycoprotein processing. Under field conditions, this gene is essential for normal plant development. The second α–glucosidase gene contributes relatively little to total enzyme activity and there appears to be little impact on the plant of down-regulating its expression. In light of this evidence, the role of this class of enzyme in carbohydrate metabolism must now be re-assessed. Also in potato, biochemical markers of dormancy break in tuber apical buds have been identified. The markers have been exploited to define the stages of meristem activation to be used in a gene discovery programme with the objective of dissecting the signal transduction processes involved in dormancy break. Differentially expressed genes are being identified and microarray technology will be employed to assess the sequence of events that occur at the molecular level.

Tuber formation is characterised by a switch from apoplastic to symplastic unloading of assimilates in the sub-apical stolon region. The results are enhanced sink potential of stolon tips and marked changes in the compartmentalisation of sucrose. The latter may

be directly responsible for the co-ordinated up-regulation of genes involved in storage metabolism and in the marked changes in invertase activities during tuberisation. Data indicate that the mechanisms responsible for the induction of symplastic unloading during tuber formation are central to the regulation of resource allocation and sink-source relationships in potato. Also highly relevant to tuber development are the findings that over-expressing S-adenosylmethionine decarboxylase in tubers modifies both tuber numbers and size grade distributions (larger number of smaller tubers produced).

Detailed analysis of the role of the urease enzyme in nitrogen metabolism in potato is underway. Urea can be used as a foliar crop spray to meet nitrogen requirements whilst preventing extensive losses to, and leaching from, the soil. However, urea accumulation in the leaf can produce leaf scorch and reduce photosynthetic efficiency. This programme aims to modify endogenous urease activity and to determine the impact on nitrogen metabolism. The potato urease gene has been cloned and a range of transgenic plants has been produced to probe the function of this gene. The urease accessory proteins, essential for optimal urease function, are also under study and several novel genes in this class have been cloned. Functional analyses are underway involving transgenic potato plants as experimental tools.

Variability in the rheological properties of potato starch has been examined. Potato starch granules are distinct from cereal starch granules in that they contain covalently-bound phosphate ester groups that effect the properties of the starches, particularly their viscosity. The peak viscosity and viscosity profile are dependent on the potato genotypes from which the starches have been isolated. In general, the higher the phosphate ester content, the higher the peak viscosity at equivalent concentrations. The viscosities of starch granules are also affected by the presence of ions in the medium. Both monovalent cations, such as sodium, and divalent cations, such as calcium, have an effect but the effects are different. The peak viscosity is higher for sodium than for calcium. The temperature programme used in the Rapid Visco-Analyser has a profound effect on the viscosity profile. These differences can have a major effect on the viscosity of starch granules derived from starches that have an inherently low viscosity, especially those that have a low phosphate ester content. Using a maximum temperature of 65°C, that still allows the gelatinisation temperature to be reached, the viscosity profiles of these starches do not give a peak viscosity in the early stages of the profile and the highest viscosity coincides with the final viscosity.

From the developments outlined above it is evident that the Division continues to play a role in developing the potential of GM technologies applied to crop plants. EU funding has been obtained to assess the impact of genetic transformation on potato tuber composition and the potential for unintended effects on nutritional and anti-nutritional components. The EU consortium will use state-of-the-art technologies (microarrays, proteomics and metabolomics) to assess the extent to which metabolic processes are perturbed.

Antioxidants and free radical processes Antioxidants are a diverse set of molecules, which are able to donate hydrogen atoms to reactive free radicals, negating the damage that these cause through interaction with cellular components. This work is currently conducted in the core program of the Unit, along with a SERAD Flexible Fund grant and a grant from the EU Northern Periphery Programme. The aims of the programme include the identification of nutritionally-relevant antioxidants in soft fruit, their bio-availability and accumulation in tissues and the correlation of uptake with biomarkers of disease risk. It is hoped that the results will provide the initial information required to improve the antioxidant content of red berry fruits, by conventional or biotechnological means. The ultimate aim is the establishment of recommended intake of antioxidants for optimal health.

An important dietary antioxidant is vitamin C (ascorbic acid). The biosynthesis of L-ascorbic acid (L-AA) by yeast (*Saccharomyces cerevisiae*) has been examined. Under 'natural' conditions (i.e. when grown in the presence of D-hexoses), *S. cerevisiae* cells synthesise D-erythroascorbic acid (D-EAA) but not L-AA. D-EAA is a five carbon L-AA analogue with similar redox properties and is thought to perform similar antioxidant functions in yeast to those performed by L-AA in other eukaryotes. However, yeast cells can be induced to synthesise L-AA when grown in the presence of L-galactose (L-Gal), L-galactono-1,4-lactone (L-GL) and L-gulono-1,4-lactone (L-GuL). Studies with radiolabelled substrates showed that yeast cells lack the pathway of L-AA biosynthesis present in plants but can be made to synthesise L-AA via the pathway naturally used for D-EAA biosynthesis. This finding has potential biotechnological applications.

A multi-disciplinary EU-FAIR project, involving the

Division of Biochemistry & Cell Biology and the Division of Pathology, continues to increase our understanding of the oxidative stress imposed upon host plant tissues by the necrotrophic fungal pathogen, *Botrytis cinerea*. EPR spectroscopy and LC-MS quantitation of key analytes indicate that the pathogen brings about pronounced changes in the redox state of the surrounding tissues. The implications of this shift to more oxidising conditions are poorly understood at present, although redox state is increasingly becoming recognised as having a major influence upon gene expression.

Lipids, waxes and lectins The complex lipids of blackcurrant leaves have been examined for the first time and contain α–linolenic acid, hexadecatrienoic acid and stearidonic acid. This would appear to be the first report of these three acids being found together in the complex lipids of leaves from the same plant. All previously studied plants have fallen into one of three groups according to the presence of α–linolenic acid only (18:3 plants), α–linolenic and hexadecatrienoic acids (16:3 plants), or α–linolenic and stearidonic acids (18:4 plants).

The physiochemical nature and physiological function of plant and insect cuticular waxes varies between different parts of the organism. For example, in several brassica species studied, waxes from lower (abaxial) leaf surfaces had proportionally more wax esters, secondary alcohols hydroxy ketones and aldehydes, whereas the upper (adaxial) leaf surface had more ketones and amyrins. Such differences are of importance to herbivorous insects such as aphids, which feed on the lower surface and may require specific chemical cues to identify the correct host plant. Aphids in turn have their own characteristic suite of cuticular chemicals. These include aphid-specific triacyglyerols (see review article) and complex mixtures of alkanes. The distribution of individual members of both classes of compound appears to be species-specific, differences having been found between raspberry aphid and pea aphid. In addition, alkanes from raspberry aphid were found to differ in composition between the surface and interior of the cuticle.

Studies on plant waxes have also been extended to include the characterisation of flower waxes. Initially, these have been concentrated on faba bean flowers, where two new classes of epicuticular wax esters based on phytol and cinnamyl alcohol have been identified. The high relative concentration of cinnamyl alcohol esters would appear to reflect the high concentration of phenyl propenoids previously reported in the volatiles released by these flowers.

Large scale screening of 50 species of plants has identified 14 species that produce mannose-specific lectins. These have monomeric molecular weights of 11-13 kD, which suggests that they are part of the superfamily of mannose-specific lectins. Detailed glycosidic linkage analysis has been undertaken using simple and complex oligomannosaccharides. This showed that, in general, the lectins displayed a preference for α(1-3) and/or α(1-6) glycosidic linkages. However, one of the lectins, (from *Beta vulgaris*) did bind α(1-2) linkages. The lectins have been screened for Human Immunodeficiency Virus (HIV) and Simian Immunodeficiency Virus (SIV) binding ability using ELISA systems. In general, HIV and SIV binding activities co-eluted during HPLC separations. However, binding specificities were separable in some species. *In vivo* testing showed that selected mannose specific lectins were very effective at inhibiting Feline Immunodeficiency Virus (FIV) infection and syncitia formation. The mannose specific lectin (*Hippeastrum* Hybrid Agglutinin [HHA]) from *Amaryllis* was the most effective, reducing FIV viral production by 85% at a lectin concentration of 160 ng/ml. This out-performs the current pharmaceutical candidate compound, a cyclolactone derivative.

Cell Biology

Plasmodesmata Recent research on the structure/function relations of plasmodesmata, the small pores that interconnect higher plant cells, has shown that discrete classes of plasmodesmata behave differently with respect to their capacity to traffic macromolecules. Simple plasmodesmata can traffic relatively large proteins while branched plasmodesmata allow the passage of only relatively small solutes. Simple plasmodesmata predominate in sink (unloading) tissues, suggesting that the increase in molecular size exclusion limit of these plasmodesmata may accommodate the rapid fluxes of proteins and solutes required for growth in rapidly expanding sink tissues. Significantly, viral movement proteins (MPs) target only branched plasmodesmata and do not associate with the simple plasmodesmata in sink tissues. This unique feature of MPs has been used to study the progression of plasmodesmal development in leaf tissues undergoing the sink-source transition. Using transgenic tobacco expressing a MP-green fluorescent protein fusion, it was possible to follow the distribution of simple and branched plasmodesmata non-invasively during leaf development. The results revealed an enormous loss of simple plasmodesmata during the

sink-source transition, suggesting that this class of plasmodesmata has a transitory existence in sink tissues. In contrast, branched plasmodesmata persist throughout leaf development and predominate in source tissues. The formation of branched plasmodesmata occurs asynchronously in different cell layers and is correlated with the duration of cell division in a given leaf tissue. It would appear that the relative distribution of simple versus branched plasmodesmata in a given tissue governs the ability to traffic macromolecules, including systemic RNA signals, between cells.

Virus movement and gene silencing Basic studies have continued on the cell-cell and long-distance movement of plant viruses. Previous studies concentrated on the movement of a range of viruses that infect dicotyledons. Recently, attention was turned to monocotyledonous-infecting viruses. In collaboration with Biosource Genetics Corporation, the movement of *Barley stripe mosaic virus* (BSMV) expressing green fluorescent protein has been examined. These results confirmed previous studies on dicotyledonous species and established that only major leaf veins are used in the unloading of solutes and viruses. In contrast to previous published reports, the data show that barley utilises a symplastic phloem unloading mechanism.

A well-reported feature of virus infection is the inability of viruses to invade meristematic tissues. Using a range of GFP-tagged viruses, the ability to invade shoot and root meristems was studied non-invasively using confocal microscopy. In the case of Tobacco Mosaic Virus (TMV), it was shown that this virus is able to infect lateral root meristems. However, following the activation of the lateral root meristems, a wave of viral suppression occurs that is initiated in the meristem and progresses basipetally along the root. A detailed report on this topic appears on page 132. In contrast to the above observations, it was found that Tobacco Rattle Virus (TRV) effectively invaded root meristems and persisted in dividing cells of the meristem. This feature makes TRV a particularly valuable vector in virus-induced gene silencing (VIGS) studies in which a range of meristem-specific genes can be silenced using virus-based vectors. Using transgenic *Arabidopsis* plants expressing meristem-specific GFP constructs, it was shown that TRV expressing a truncated GFP sequence was capable of initiating GFP silencing throughout the shoot apical meristem.

The TMV-induced hypersensitive response Using a combination of molecular and cell biology, the events underpinning the hypersensitive response (HR) have been studied using TMV tagged with the green fluorescent protein. These studies have revealed some of the very early events associated with the HR and have identified several novel physiological effects induced during the HR. A detailed report on this study appears on page 136.

SCRI-Biosource collaboration The first year of the above collaborative research project has been completed. Basic studies underpinning virus movement have been combined with more applied projects examining the potential of viral-based vectors in the production of novel proteins. To date, considerable progress has been made in the construction of stable viral vectors for therapeutics and vaccines.

Plants and aphids: the chemical ecology of infestation

T. Shepherd, G.W. Robertson, D.W. Griffiths & A.N.E. Birch

Aphids can cause serious damage to a variety of economically-important host plants, either by the direct effects of feeding or by the transmission of pathogenic viruses. Host plant selection involves a complex sequence of events, which include selection in response to visual and olfactory cues, landing on a plant, testing of the leaf surface, probing and penetration by the stylet to locate the phloem tissues, and testing of the phloem contents. Selection of a suitable host plant is followed by feeding and reproduction. In the absence of the appropriate stimuli, the sequence may be interrupted at any stage, and ultimately the insect may leave. Behavioural analysis indicates that, for many of the Aphidae, the nature of the leaf surface is an important determinant factor. Chemicals found within plant cuticular waxes are thought to have a direct involvement in host selection and in many cases are insect-host specific (Table 1). Optimal leaf surface physiochemical characteristics for successful colonisation by aphids include good surface adhesion and minimal impediment to movement, probing and stylet penetration. These traits are often associated with glossy (glabrous) phenotypes, which usually have less wax, a simpler wax microstructure, and altered chemical composition when compared to the normal waxy (glaucous) phenotypes[1].

The raspberry plant – raspberry aphid system At SCRI, we have a long-standing interest in factors that confer resistance to insect infestation, particularly of the *Rubus* genus to the large raspberry aphid, *Amphorophora idaei*, the only vector of significance for several viruses that infect raspberry in Europe[2-4]. Preliminary studies indicated that raspberry leaf wax plays an important role in determining resistance and susceptibility to the insect[5]. We have now conducted a more rigorous comparison between the *A. idaei*-resistant cultivar, Autumn Bliss, which contains the major resistance gene A_{10}, and the *A. idaei*-susceptible cultivar, Malling Jewel[6]. To correlate aphid behaviour with leaf chemistry accurately, bioassays were performed with *A. ideai*, which densely populates Malling Jewel but not Autumn Bliss, immedi-

Plant Species	Aphid or Related Insect	Behaviour Effected	Determinant Factor (R = Resistance or inhibition; S = suscepibility or Stimulation)
Cabbage (*Brassica oleracea* L.)	*Brevicoryne brassicae* L	Feeding	Increased surface wax levels (R)
Sorghum (*Sorghum bicolor* L.)	Green bug, *Schizaphis graminum* (Rondani)	Feeding	Increased surface wax levels (R)
Sorghum	Various aphids	Feeding	Higher levels of α- and β-amyrin (R)
Winter wheat (*Triticum aestivum* L., glossy phenotypes)	English grain aphid, *Sitobion avenae* (F.)	Feeding	Increased surface wax levels (R); Lower levels of secondary alcohols, ketones, diketones and hydroxy ketones (R)
Alfalfa (*Medicago sativa* L.)	Spotted alfalfa aphids, *Therioaphis maculata* (Buckton)	Feeding	Increased wax levels on young leaves (R); Reduced wax levels on mature leaves (S); Triacontanol (C_{30}) in wax (R); Higher abundance of wax esters (R)
Various	Alate green peach-potato aphids, *Myzus persicae* (Sulzer)	Settling	Short chain fatty acids (C_8-C_{13}) (R), Fatty acids of chain length > C_{16} (S)
	M. persicae	Settling/ Crop damage	Application of dodecanoic acid (C_{12}) to leaves (R)
Vicia faba L (host).	Pea aphid *Acyrthosiphon pisum* (Harris)	Probing and feeding	Alkanes from wax (main components C_{27}, C_{29}, C_{31} C_{33}) (S).
Brassica spp (non-host)	*A. pisum*		Alkanes from wax (mainly C_{29}) (R)
V. faba	*A. pisum*	Movement to lower leaf surface (feeding site)	Alkanes (S)

Table 1 Factors influencing behaviour of aphids on host and non-host plants.

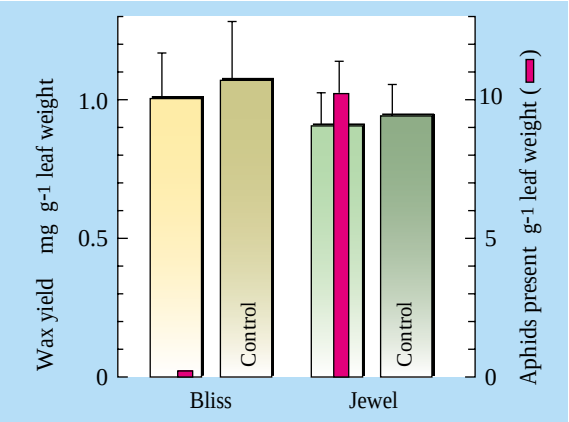

Figure 1 Yields of wax recovered from leaves of red raspberry cultivars Autumn Bliss and Malling Jewel and bioassay results for resistance to *A. idaei*. Values are means and standard deviations for four replicates.

ately prior to collection and chemical analysis of the epicuticular wax (Fig. 1). This confirmed that, at the time of sampling, Autumn Bliss was strongly aphid-resistant and Malling Jewel was highly aphid-susceptible. An aphid-free control group of plants, not subject to aphid bioassay, was analysed to test for any aphid-induced effect on wax composition. The similarity of wax yields from both species suggests that wax thickness was not itself significant. Resistance or susceptibility was more likely related to chemical differences in wax composition.

Wax composition - an invitation to the aphid? Chemical analysis by capillary gas chromatography-mass spectrometry (GC-MS) revealed a broad spectrum of wax components, of which primary alcohols and long-chain esters were most abundant. Sections of the GC-MS Total Ion Chromatogram (TIC) traces obtained for Bliss and Jewel are shown in Figures 2A and 2B. These illustrate the major compositional differences between the waxes.

Studies of other plant-insect systems show that most classes of wax component have potential for biological activity, which may be related to factors such as their abundance in the wax and the distribution of individual compounds, including homologues and positional isomers. Several correlations were made between the observed variation in raspberry wax composition and resistance/susceptibility to *A. idaei*, and are summarised in terms of the major chemical classes as follows.

Fatty acids, primary alcohols and alkanes Resistance may be associated with a narrower chain length distribution for acids and alkanes and a greater abundance of long alcohols in Autumn Bliss, and susceptibility with a wider distribution of acids and alkanes and increased abundance of short alcohols in Malling Jewel.

Alkyl esters Biological activity towards *A. idaei* was unlikely to be related directly to the amounts of alkyl esters present in the wax, which were similar for both raspberry genotypes. However, as the predominant constituent, esters influence wax structure and morphology. For example, esters with long acid-short alcohol combinations were more abundant in Malling

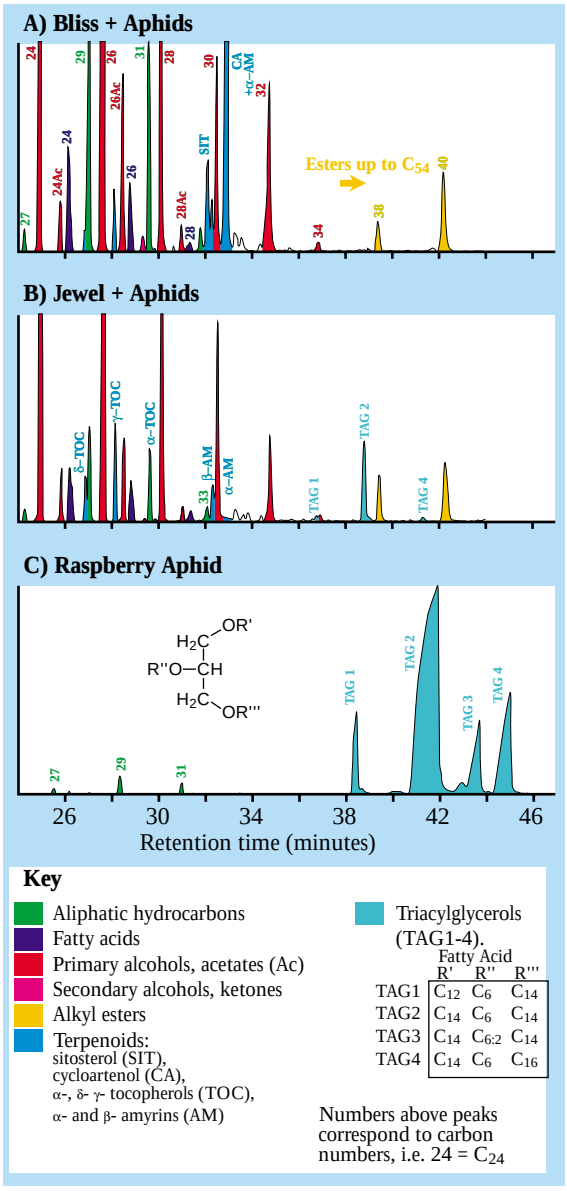

Figure 2 Main components of cuticular waxes from raspberry cultivars Autumn Bliss (A) and Malling Jewel (B) and raspberry aphid (C).

Jewel, and this may effect the distribution and morphology of other wax components and the way in which the aphid perceives specific compounds. Synergism between leaf surface chemicals has been postulated for a number of plant-insect interactions[1].

Secondary alcohols and ketones Susceptibility may be related to the presence of the symmetrical C_{29} secondary alcohol and the equivalent C_{29} ketone, both minor components in wax from Malling Jewel, whereas they were absent from Bliss.

Triterpenoids and terpene esters The greatest difference between the raspberry cultivars was manifest in the distribution of certain terpenoids. Resistance to *A. idaei* may be associated with the much higher levels of sterols, particularly cycloartenol and its alkyl esters, found in wax from Autumn Bliss. There was no correlation between general levels of tocopherols, amyrins and amyrin esters and resistance to *A. idaei*, although there were differences in the distribution of individual members of each class. However, resistance may be associated with the higher abundance of α–amyrin and its alkyl esters in Bliss, since α–amyryl palmitate from suberin wax of the sandal tree, *Santalum album*, is known to affect development of several lepidoptera species[7]. Low levels of sterols in Malling Jewel may be indicative of impaired synthesis from squalene, whereas transformation of squalene to amyrins appears to proceed with equal facility in both genotypes. The high cycloartenol/sterol ratio observed for Autumn Bliss may also indicate reduced transformation of cycloartenol to sterols, since cycloartenol is not usually found in plant leaf waxes.

All these factors identified as possible characteristics of resistance to *A. idaei* in raspberry, appear to be collectively similar to those identified in numerous other plant-aphid studies (Table 1).

Triacylglycerols – the aphid's calling card Small amounts of an unusual group of triacylglycerols were found exclusively in wax from the susceptible genotype, Malling Jewel, that had been subject to bioassay with *A. idaei* (Fig. 2B). These have a short C_6 acid moiety at C-2 of the glycerol backbone and differ from those found in internal plant lipids which usually have three long chain fatty acids. We found the same triacylglycerols as the major surface lipids of *A. idaei*. (Fig. 2C), and clearly the occurrence of these triacylglycerols in the plant wax was due solely to the presence of aphids. Previous reports of similar compounds as wax constituents from various grasses and Canada thistle, *Cirsium arvense* L.[8], can probably now more correctly be attributed to the presence of aphids, since the source plants were grown outdoors and were sampled when flowering, a time when the probability of aphid infestation was high.

These 'marker' triacylglycerols, also found in other species of aphid, occur internally and also externally in defensive secretions produced by the insect's cornicle glands[9]. Interestingly, we found that their characteristic chemical signature was retained by exuviae shed during the insect's growth and development. Leaf surface triacylglycerols are most likely then to arise from the presence of insect exuviae and the direct incorporation of cornicle fluid into leaf wax. The 'marker' compounds were found on leaves of field-grown Malling Jewel, but not Autumn Bliss, at a time when aphids were ubiquitous in the environment of both plants. On widening the study, they were also detected on field-grown plants of other species, including brassica and potato, which were colonised by other aphid species and not *A. idaei*. These findings suggest that measurement of the relative levels of aphid-specific triacylglycerols in plant waxes would provide a chemical index of the degree of aphid-infestation, and hence susceptibility, and should be a useful tool for the field screening of plants for aphid-resistance.

Indeed, these aphids appear to have left their 'chemical fingerprints' all over the scene of the crime, or as Sherlock Holmes put it, "Elementary my dear Watson".

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Mannose-specific plant lectins from plants as diagnostics, vaccines and tools for the elucidation of viral infection mechanisms in animals

E. Davidson, J.M.S. Forrest, I.M. Morrison & D. Stewart

Lectins are proteins which bind carbohydrates both specifically and reversibly. One class of these proteins, mannose-specific lectins (MSLs), binds mannose (Man) residues. Mannose is an abundant cell surface sugar present in glycoconjugates that are implicated in a wide range of important receptor-mediated cellular processes. The MSLs, therefore, are potentially useful tools in research, with a wide scope of potential applications. Daffodil (*Narcissus pseudonarcissus* agglutinin, NPA) and snowdrop (*Galanthus nivalis* agglutinin, GNA) lectins were the first shown to exhibit this exclusive mannose specificity. Additionally, they demonstrated a more complex specificity, than for example Con A, in that they distinguished Man-Man linkages, with NPA preferring Man α(1-6) Man and GNA favouring Man α(1-3) Man linkages (Fig. 1).

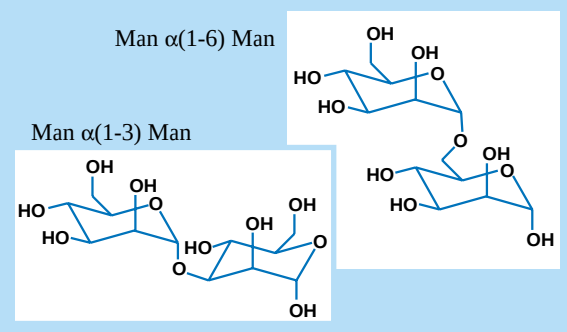

Figure 1 Man α(1-3)Man and Manα(1-6)Man linkages.

MSLs have been found to bind to the mannosylated region of the envelope glycoproteins of various retroviruses such as human (HIV), simian (SIV) and feline (FIV) immunodeficiency viruses. These glycoproteins are the site of interaction with the cell surface molecule CD4, the co-receptor for HIV virus. HIV binds to CD4 *via* an interaction between the first domain of CD4 and a discontinuous region of the HIV-1 outer envelope glycoprotein gp120. Similar interactions have been implicated in SIV and FIV pathogenesis. Binding of selected MSLs to the envelope glycoprotein of HIV viruses has resulted in the potent and selective inhibition of infection *in vitro*[1].

The MSLs were initially thought to be limited to only a few species but our studies have shown that they are present, albeit often at very low levels, in a wide variety of cultivated agricultural and horticultural crops.

Linkage specificity Although all the MSLs were purified on a mannose-affinity column and were shown to agglutinate rabbit red blood cells, thereby indicating their specificity for mannose, their detailed specificity remained undefined. This was remedied by employing quantitative precipitation and precipitation-inhibition assays. Highly branched mannan from the yeast *S. cerevisiae* was used to quantitatively precipitate the lectin. Selected oligomannans, with defined mannose-mannose linkages, were purified to homogeneity by reverse-phase HPLC and used to inhibit the precipitation of the MSL/mannan system. The concentration of oligomannan required to obtain a 50% inhibition of precipitation was determined and a relative inhibitory potency (RIP) ranking was established with D-mannose ranked at 1 (Table 1).

Both NPA and GNA exhibited their reported linkage preferences of α(1-6) and α(1-3), respectively. The presence of a reducing terminal did not make a significant difference to their relative inhibitory potency. For example, in the assays of GNA, methylation of the reducing terminus of

Lectin	Snowdrop (GNA)	Daffodil (NPA)	Amaryllis (HHA)	Ramson (AUA)	Shallot (AAA)
M. Wt. (kD)	12.5	12.5	12.5	12.5	12.5
D-Man	1.0	1.0	1.0	1.0	1.0
α-D-Man p-OMe	1.8	1.7	1.3	1.7	1.2
β-D-Man p-OMe	0.1	0.3	0.7	0.3	0.6
Man β(1-2) Man	<0.1	<0.1	<0.1	<0.1	<0.1
Man β(1-2) Man β(1-2) Man	<0.1	<0.1	<0.1	<0.1	<0.1
Man β(1-4) Man	<0.1	<0.1	<0.1	<0.1	<0.1
Man β(1-4) Man β(1-4) Man	<0.1	<0.1	<0.1	<0.1	<0.1
Man α(1-2) Man	2.2	2.9	3.2	3.6	3.4
Man α(1-3) Man	12.1	3.8	3.7	5.3	6.1
Man α(1-4) Man	1.1	1.7	1.4	1.3	1.9
Man α(1-6) Man	3.2	5.0	6.0	4.1	17.0
Man α(1-3) Man α-OMe	11.7	4.8	10.5	10.2	10.9
Man α(1-6) Man α-OMe	3.5	6.0	12.3	5.1	9.0
Man α(1-6) Glc	2.3	2.0	4.4	4.2	1.9
Man α(1-6) Man α(1-6) Man	2.5	6.7	22.7	7.0	9.0
Man α(1-2) Man α(1-3) / Man α(1-2) Man α(1-6) }Man-R	2.1	0.9	4.4	2.2	2.0
Man α(1-4) / Man α(1-2) }Man-OMe	1.9	2.3	1.7	2.4	2.2
Man α(1-3) / Man α(1-6) }Man-OMe	22.1	5.2	14.2	11.3	17.0
M α(1-6) / M α(1-3) }M α(1-6) / M α(1-3) } M β(1-4) GlcR R = NAc β(1-4) Glc NAC-Asn	28.0	30.1	33.2	30.0	27.1
M α(1-6) / M α(1-3) }M α(1-6) / Man α(1-2) Man α(1-3) }M β(1-4) GlcR R = NAc β(1-4) Glc NAC-Asn	30.1	28.3	47.3	35.0	21.8
Linkage preference	α(1-3)	α(1-6)	α(1-6)>(1-3)	α(1-3)>(1-6)	α(1-3)>(1-6)

Table 1 The mannose binding preferences of selected mannose-specific lectins. The concentrations of oligomannan (mM) required to obtain a 50% inhibition of precipitation was determined and a relative inhibitory potency (RIP) ranking was established with D-mannose ranked at 1.

Man α(1-3) Man only changed the RIP from 12.1 to 11.7. Similarly, for NPA the methylation of Man α(1-6) Man changed the RIP from 5.0 to 6.0.

The linkage preference was less clearly defined for the other lectins. The amaryllis MSL, (*Hippeastrum hybrid* agglutin; HHA) exhibited a slight preference of α(1-6) over α(1-3) linkages. The most significant difference was the preferential binding of non-terminal Man-Man linkages. For example, methylation of the reducing terminus of Man α(1-3) Man and Man α(1-6) Man changed the RIP values from 3.7 and 6.0 to 10.5 and 12.3, respectively. This preference for internal linkages is reflected in the high RIP values (33.2 and 47.3) obtained with the complex oligomannosaccharides.

The ramson lectin (*Allium ursinum* agglutin; AUA) displayed a linkage preference similar to snowdrop (GNA) but with a greater affinity for the internal α(1-3) linkage. Methylation of the reducing terminal of Man α(1-3) Man resulted in an almost 100% increase in RIP value for AUA.

The shallot lectin (*Allium ascalonicum* agglutin; AAA) exhibits unusual linkage specificities with the RIP values suggesting that terminal α(1-6) and internal α(1-3) linkages were favoured. Significantly, the RIP values for the complex oligomannosaccharides were the lowest of the MSLs shown, possibly indicating that this lectin favours smaller substrates.

Isolectin distribution and activity *In planta*, the lectins are often the product of more than one gene leading to the production of iso-lectins, proteins which vary slightly in their amino acid composition and hence net charge. This can lead to variation in

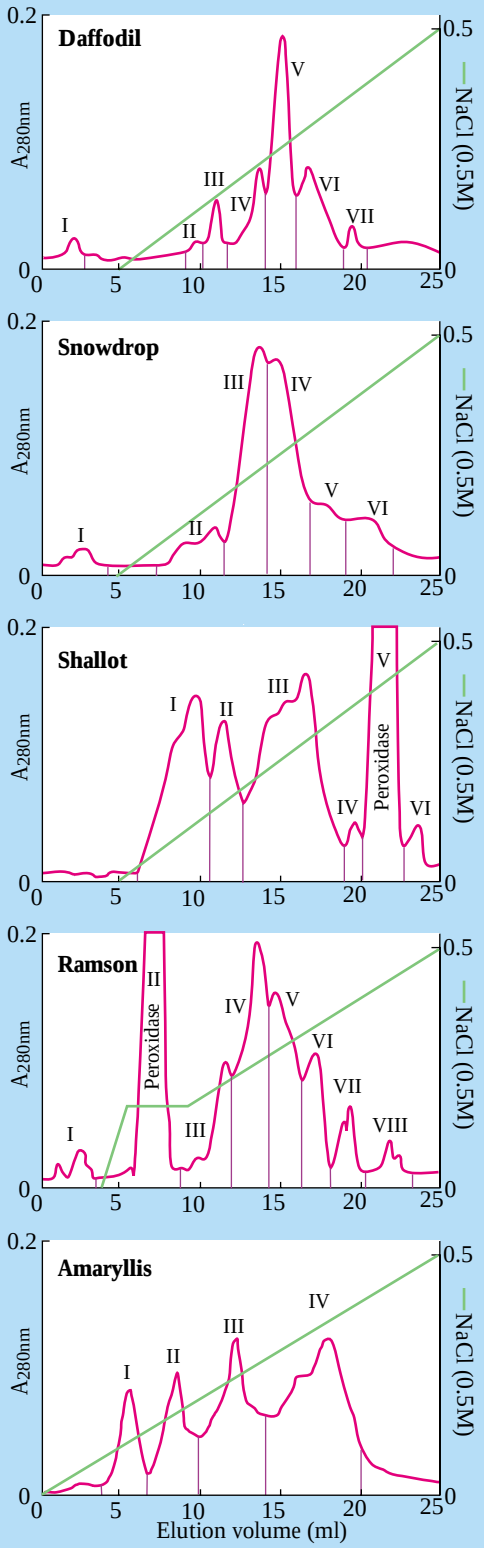

Figure 2 HPLC chromatographs of selected mannose-specific lectins.

Lectin	Agg.	gp120	gp148
Daffodil			
I	-	-	-
II	-	-	-
III	+	+	+
IV	+	+	+
V	++	++	++
VI	+	+	+
VII	-	-	-
Ramson			
I	-	-	-
II	-	-	-
III	+	-	-
IV	++	++	++
V	++	++	+
VI	+	+	-
VII	+	+	-
VIII	-	-	-
Snowdrop			
I	-	-	-
II	-	-	-
III	++	++	++
IV	++	+	++
V	+	+	+
VI	-	-	-
Shallot			
I	++	+	++
II	++	+	+
III	++	++	+
IV	-	-	-
V	-	-	-
Amaryllis			
I	+	+ +	+ +
II	+	++	++
III	+	++	++
IV	++	+	+

+, ++ mild and intense binding/agglutination
- no binding/agglutination

Table 2 The (rabbit) red blood cell agglutinating (Agg.) and HIV(gp 120) and SIV (gp 148) binding abilities of the fractions obtained from the IEHPLC of selected mannose-specific lectins.

the 'saccharide'-binding site. To study this, selected MSLs were subject to ion exchange HPLC to separate any isolectins and test them for agglutination and HIV and SIV binding activities (Fig. 2).

The iso-lectin profiles were distinct giving a disparate range of binding activities. However, there were no cases of immunodeficiency virus binding activity without agglutination activity, which supports the premise that the isolates are actually binding to the immunodeficiency viruses *via* the complex mannan side chains.

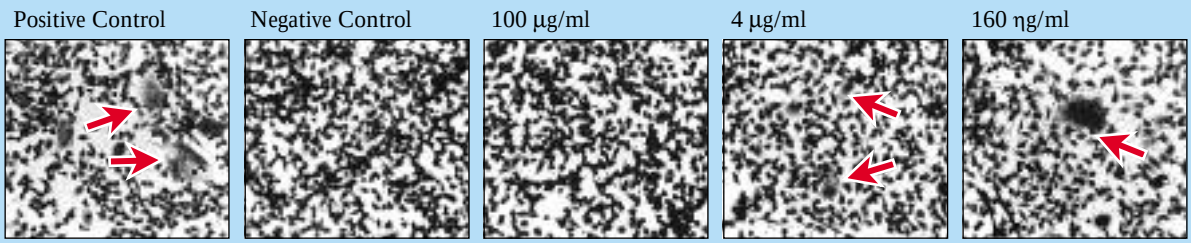

Figure 3 Inhibition of FIV infection by selected MSLs. Arrows indicate syncytia formation.

Some of the MSL fractions displayed distinct HIV and SIV binding. The chromatogram of the shallot, AAA, showed that optimal HIV and SIV binding activities were separated and found in fractions III and I, respectively. This separation of activities suggests that the substrate specificities of the isolectins are different, a fact highlighted during analysis of the linkage specificities (Table 1).

In vivo anti-immunodeficiency virus (IV) activity In conjunction with Drs Brian Willett and Margaret Hosie (Faculty of Veterinary Medicine, Retrovirus Research Laboratory, University of Glasgow), selected MSLs (Table 1) were used to study the efficacy of such lectins at inhibiting the *in vivo* infection of FIV, an HIV model system. These all exhibited, to varying extents, a dose-dependent inhibition of FIV infection. Only the infected cells exhibited the expected syncytia formation. The lectin itself did not induce syncytia formation. The dose-dependency of lectin inhibition was reflected in the reduced incidence of infection-related syncyitia formation accompanying an increase in lectin concentration (Fig. 3).

An ELISA based on production of the FIV protein p24, showed that HHA proved to be the most potent inhibitor of FIV infection (Fig. 4). This gave an 85% reduction in viral p24 production at a lectin concentration of 160 ng/ml. GNA and NPA also performed well, exhibiting 76% and 69% inhibition, respectively, at this concentration. These results reflect previous findings for HIV[1] and highlight the benefits of using the FIV animal model system.

Conclusion Plant-derived mannose specific lectins are clearly more prevalent than was first thought. Although simply classed as mannose specific, there are a broad range of linkage specificities and affinities within this class of lectins. It is evident, from HPLC analysis alone, that the majority of the existing and novel MSLs exist as a collection of iso-lectins and that these exhibit distinct and often varied specificities and affinities towards simple and complex substrates.

The ability of the MSLs to bind immunodeficiency viruses means that they represent an academic and economic opportunity, hitherto untapped. Academically, they will allow the part played by the mannosylated regions of IV in the infection process to be elucidated. Economically, they have the potential for development as ELISA-based IV-detection systems or, by employing anti-idiotypic antibody methodology, they offer the chance to produce IV vaccines.

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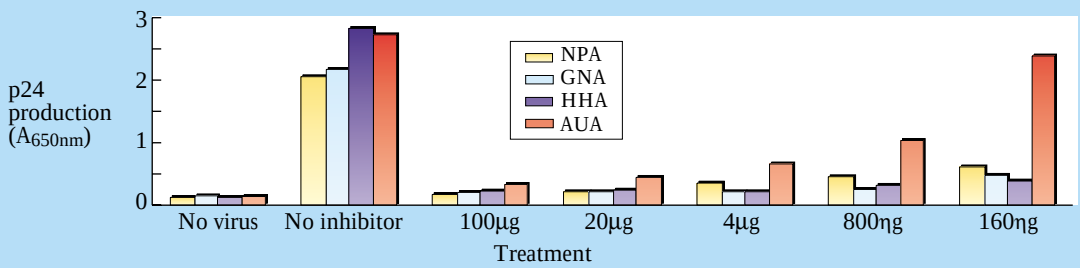

Figure 4 Inhibition of FIV infection by MSLs from daffodil (NPA), snowdrop (GNA), amaryllis (HHA) and ramson (AUA). Inhibition of virus replication was reflected in reduced production of the viral protein p24.

Oxidases participate in the formation of wood

A. Richardson, N. Deighton, J.A. Duncan & G.J. McDougall

The process of wood formation Wood is the major renewable resource of timber for building, pulp for papermaking and is the main source of fuel in developing countries[1]. Trees also fix millions of tonnes of carbon dioxide into wood, which locks up this 'greenhouse' gas and influences global temperature control.

New wood is formed by an extraordinary differentiation process. Trees increase in girth by depositing annual rings of wood during spring and summer (Fig. 1). A transverse section from a branch of Sitka spruce in May (Fig. 1, insert), illustrates the various stages of the differentiation process. New wood cells arise from the division of meristematic cells in the vascular cambium (VC), which lies between the old wood and the bark or phloem tissues. The undifferentiated new wood cells are initially spherical and thin-walled, and appear to be near-identical to the cambial cells. After expansion and elongation (E), the new wood cells deposit a thick cellulose-rich secondary wall inside their original walls that becomes encrusted and waterproofed with the plastic-like polymer, lignin (SCL). The secondary cell wall is the main component of fixed carbon in wood and its formation represents a huge investment of photosynthetic products. During maturation (M), the cells lose their cellular contents and die. The fully differentiated wood cells are hollow, interconnected tubes with reinforced and waterproofed walls that function to transport water from the roots to the leaves.

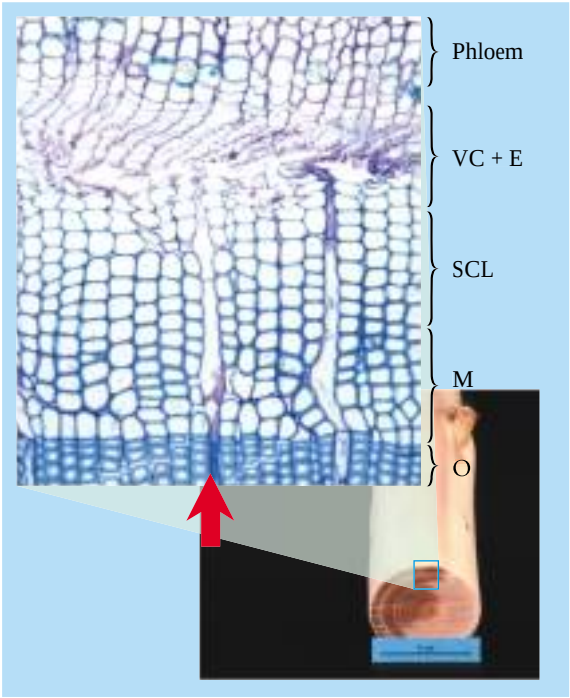

Figure 1 New wood formation in Sitka spruce. A branch of Sitka spruce showing the old wood, new wood and phloem and bark. The expanded insert shows the phloem, vascular cambium (VC) and enlarging wood cells, developing wood cells with lignified secondary cell walls (SCL) and mature wood cells (M). The arrow denotes the position of the vascular cambium.

Oxidase activity co-localises with lignifying wood cells Oxidase activity can be detected in the differentiating wood cells of the gymnosperm, Leyland cypress (Fig. 2a), by specific staining with a colour-generating reagent. Oxidase activity is localised in the differentiating wood cells that have begun to deposit their secondary cell walls but it is absent from the old wood, the vascular cambium and the phloem cells. On closer inspection, it can be seen that the pattern of oxidase activity effectively co-localises with the presence of lignin in differentiating wood cells (compare Figs 2a & 2b). Oxidase

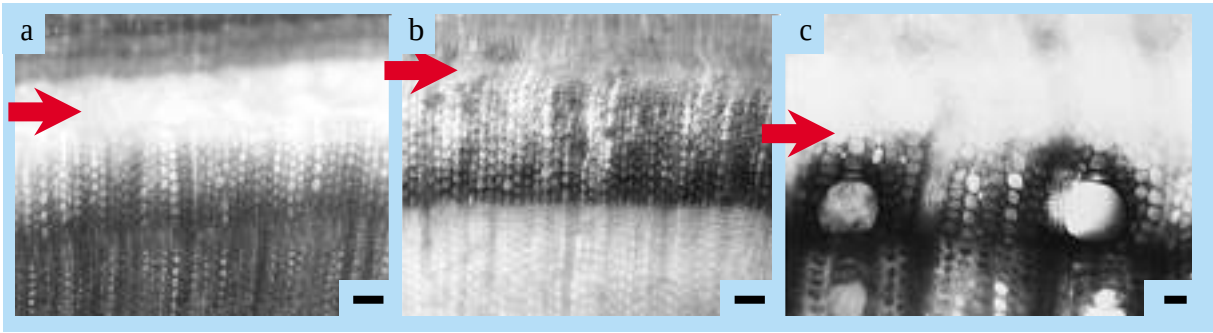

Figure 2 Oxidase activity and lignin deposition in developing wood cells. Figure 2a shows the oxidase activity present in developing wood cells of Leyland cypress. Figure 2b shows the extent of lignification of these wood cells. Figure 2c shows the presence of oxidase activity in the lignifying developing wood cells of sycamore. Arrows denote the position of the vascular cambium. All bars represent 25 µm.

activity is also specifically expressed in the lignifying cells of the differentiating wood of the angiosperm, sycamore (Fig. 2c). Indeed, oxidase activity showed the same pattern of expression in the lignifying, differentiating wood of a taxonomically diverse range of tree species. It has been found in the most ancient species of the gymnosperms, *Ginkgo biloba*, and the oldest family of the angiosperms, the Magnolias, as well as other representative species from all the main gymnosperm and angiosperm families. The antiquity and ubiquity of oxidase activity in tree species suggests that it may have a conserved role in wood formation in trees.

The role of oxidase activity in wood formation Oxidase activity has been postulated to be involved in the final step of the biosynthesis of lignin[2], catalysing the oxidation and polymerisation of lignin monomers, monolignols, to lignin in the walls of the wood cells (Fig. 3). Peroxidases acting with hydrogen peroxide can also catalyse this reaction and there has been some discussion about the relative roles of the two enzymes. Two observations strongly support a role for oxidases in lignin polymerisation. Activity-staining studies suggested that oxidase activity was localised in the walls of lignifying wood cells (Fig. 2) and biochemical studies[3,4] confirmed that oxidase activity was closely associated with the cell walls of the differentiating wood cells, the site of lignin formation. Secondly, oxidases extracted from the differentiating wood of Sitka spruce (and other species) oxidise monolignols *in vitro*[5] to form lignin-like polymers. In addition, oxidases produced the same three dimeric intermediates as peroxidases and hydrogen peroxide from the monolignol, coniferyl alcohol (Fig. 4). Both enzymatic systems produced the dimers in the same proportions, which indicated that the reactions proceeded through the same intermediates and produced similar products. Therefore, oxidases are equally able to carry out this biologically important reaction as peroxidases.

Purification and identification of an oxidase from a conifer Oxidase activity was extracted from the cell walls of the differentiating wood of Sitka spruce and purified by sequential lectin affinity, immobilised metal affinity and gel permeation chromatography steps (Fig. 4, lanes A-D). The purification was characterised by an increase in the specific oxidase activity

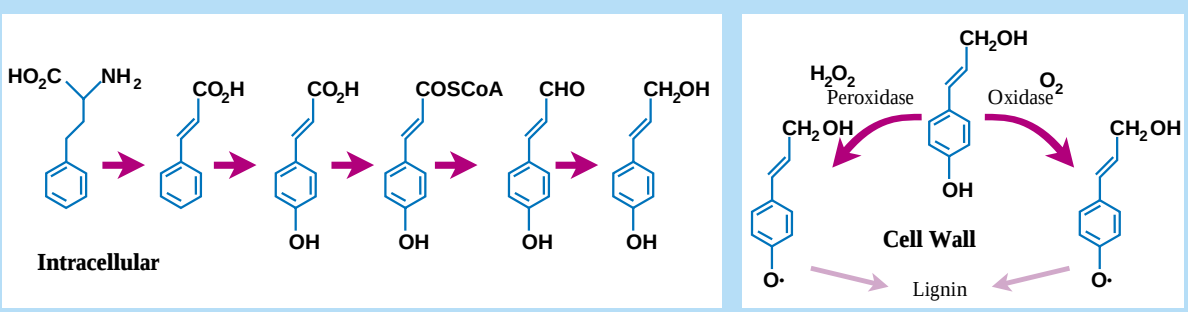

Figure 3 Oxidases in lignin biosynthesis. Oxidases and peroxidases have both been postulated to be involved in the final step of lignin biosynthesis, the oxidation and polymerisation of monolignols into lignin.

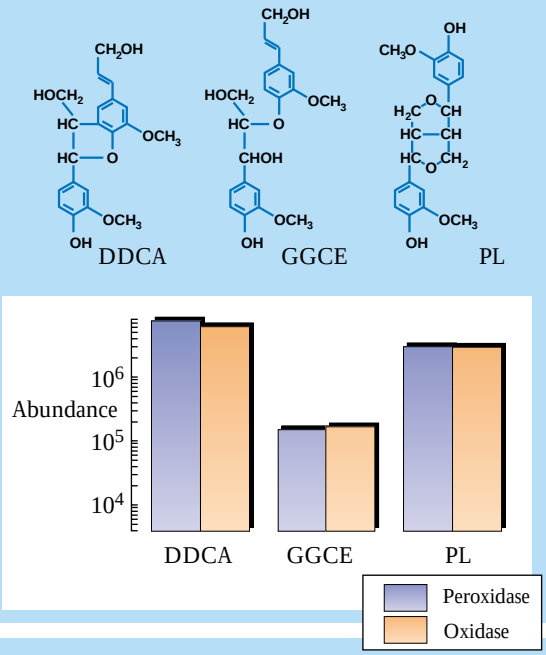

Figure 4 Products of coniferyl alcohol oxidation by oxidases and peroxidases
The oxidation of coniferyl alcohol (a) by oxidases or peroxidases produced the same three dimeric intermediates I, II and III.

and a decrease in the number of polypeptides in the samples after each step. One polypeptide with a relative molecular mass of 80 kDa became enriched as the specific oxidase activity increased (see Fig. 4, arrow) and was the main candidate for the oxidase activity. This polypeptide was selected for protein sequencing and it yielded an amino-terminal protein sequence that had homology to plant laccase-type oxidases. This was the first protein sequence of a laccase-type oxidase from a conifer.

Conclusions Oxidase activity is specifically expressed in the differentiating wood of a taxonomically diverse range of tree species and may be involved ubiquitously in wood formation. These enzymes can oxidise and polymerise monolignols and are expressed in the correct subcellular locale of lignifying cells to be involved in monolignol oxidation. Our studies strongly suggest

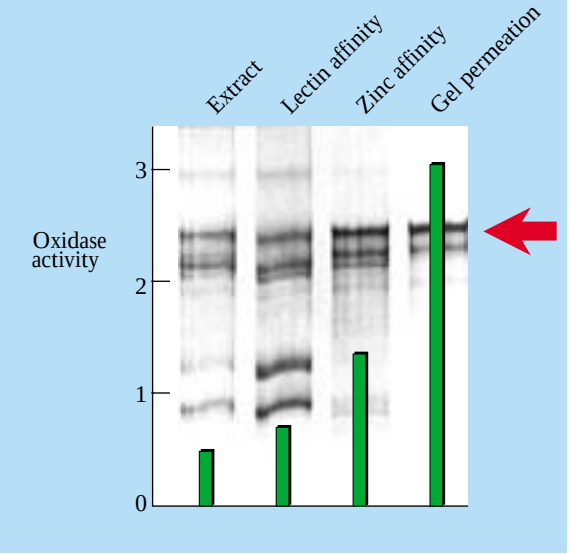

Figure 5 Purification of an oxidase from the developing wood of Sitka spruce. The oxidase activity was enriched by these sequential purification procedures. The polypeptide at 80 kDa (denoted by arrow) was selected for protein sequencing.

that the oxidase activity associated with the lignifying wood of conifers is mainly due to the expression of laccase-type enzymes. The apparent duplication of roles between oxidases and the peroxidases in lignin polymerisation may be a consequence of the importance of lignin biosynthesis in wood formation and tree growth but it is possible that the two enzymes have separate roles in lignin deposition.

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The silence of the RAMs – viral suppression in Root Apical Meristems

T.A. Valentine, M. Dorward & K.J. Oparka

Systemic plant viruses possess the capacity to move both cell-to-cell (local movement) and also over long distances in the vascular system (systemic movement). Both local and systemic components of the viral movement process appear to be under the control of one or more viral gene products synthesised during the infection process. As a general rule, systemic viruses follow the pathway of assimilate translocation in the plant[1], moving from source tissues to sink tissues. To date, however, the systemic movement of viruses has been studied almost exclusively in leaves, and almost nothing is known of the pattern of virus infection in roots, despite the fact that several viruses invade the root systems of many commercially important crop species.

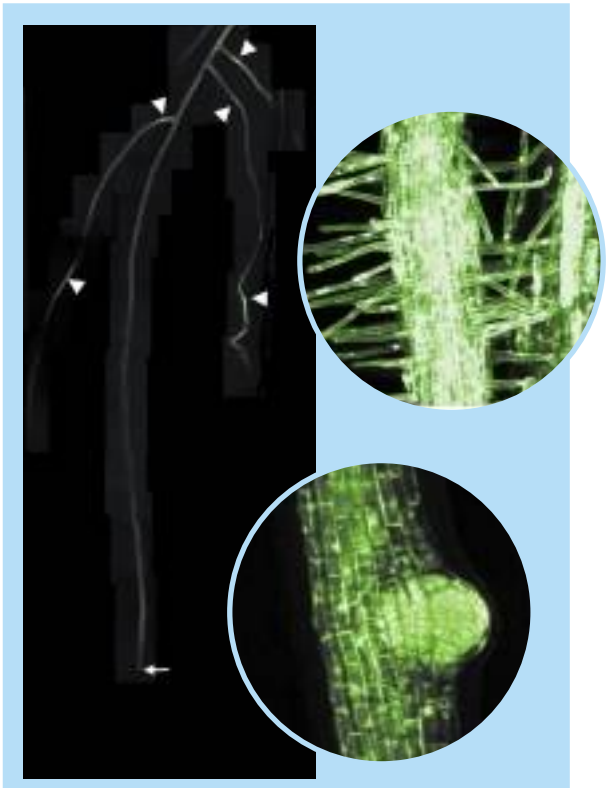

Figure 1 Distribution of TMV.GFP in *N.Benthamiana* root as indicated by GFP. Triangles indicate the first unloading points of TMV from the phloem, which are visible as green flecks. Arrow indicates primary root apex. Upper insert: GFP expression in a root highly infected with TMV.GFP. Lower insert: Developing lateral root primordium highly infected with TMV.GFP.

It appears that, in both roots and shoots, the apical meristem is largely devoid of virus infection. The inability of viruses to invade apical meristems has been exploited commercially and forms the basis of meristem-tip culture, a method by which virus-free clones can be obtained by growing excised shoot tips in tissue culture[2]. In general, a zone close to the apical meristem (usually within 0.1mm) is free of replicating virus. Why do meristems fail to support virus replication? One possibility is that a barrier to infection is established by cells located some distance from the functional meristem, although such a putative barrier has yet to be established[2]. A second hypothesis for the ability of meristems to escape infection is that a mechanism for viral gene silencing occurs in meristematic cells that targets the viral RNA for degradation, allowing newly differentiated tissue to escape infection. There is now substantial evidence for a strong parallel between the phenomenon of post-transcriptional gene silencing (PTGS), in which transgenes are silenced by a sequence-specific RNA degradation process, and the RNA-mediated defence that occurs against plant viruses[3]. In an ongoing collaboration with Large Scale Biology Corporation, we have examined the capacity of a range of systemic plant viruses, tagged with the green fluorescent protein (GFP), to invade the root system of the host plant *Nicotiana benthamiana*. In particular, we were interested in the ability of virus to enter and replicate within the root apical meristem (RAM). The results presented here relate specifically to the movement of tobacco mosaic virus (TMV).

Viral invasion of root systems Plants infected with TMV.GFP were imaged non-invasively within petri dishes using a confocal laser scanning microscope (CLSM). In this way, root systems could be imaged over several consecutive days without affecting their development. Fluorescence within the primary root system, indicating TMV replication, was visible at 5-10 days post-inoculation as intermittent 'streaks' arising from the phloem of the vascular cylinder (Fig. 1). With time these streaks spread longitudinally, and also radially outward into the cortex and root hairs (Fig. 1, inset). Contrary to our expectations, the lateral root primordia that formed within infected roots became highly fluorescent, and were visible as bright plaques of cells within the root stele. As the lateral pri-

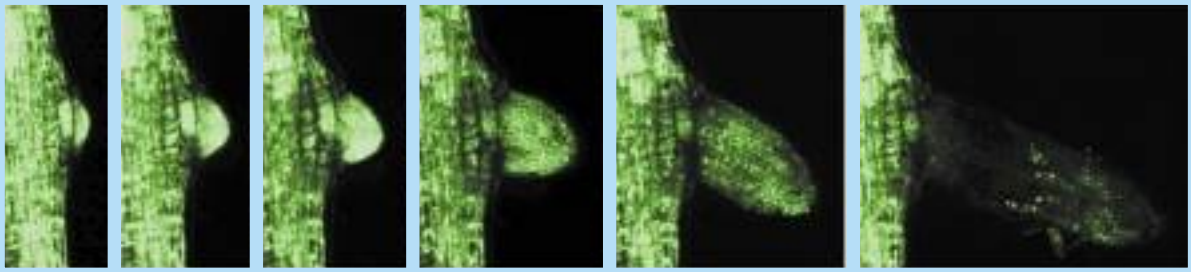

Figure 2 Developmental sequence of a lateral root primordium into a fully functional lateral root. Initially the primordium is highly fluorescent due to infection of cells with TMV.GFP. As the root develops, areas of reduced fluorescence appear near the apex. These areas of viral suppression gradually spread throughout the lateral root. The primary root does not diminish in fluorescence.

mordium emerged, the cells within it remained highly fluorescent due to the presence of replicating virus (Fig. 1, inset).

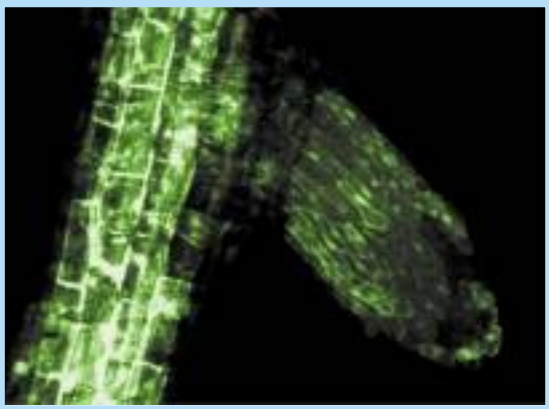

Figure 3 Lateral root beginning to undergo viral suppression. Note the dark non-fluorescent region at the tip of the root.

Viral suppression in emerging lateral roots As the lateral roots began to elongate, they gradually lost fluorescence, despite the maintenance of unaltered levels of fluorescence in the primary root. We imaged, non-invasively, the emergence of over 30 infected lateral roots, and all of these showed an identical pattern of viral suppression. A typical sequence is shown in Figure 2. By the time the lateral roots had reached an approximate length of 200 µm, a distinct zone lacking GFP fluorescence was observed close to the RAM (Fig. 3). With time this 'cone' of suppression spread basipetally along the lateral root up to the point of its connection with the infected primary root. In all suppressed roots, a sharp demarcation was observed between the base of the lateral root and its connection with the primary root. Primary roots did not decrease in fluorescence, indicating that the signal for viral suppression did not spread into mature infected cells of the primary root system.

Figure 4 Removal of communication with the shoot. To investigate whether a signal for viral suppression was being transported to developing lateral roots from the shoot, primary roots were cut just above the developing lateral roots prior to the onset of viral suppression (dotted line). Lateral roots continued to undergo viral suppression after this treatment.

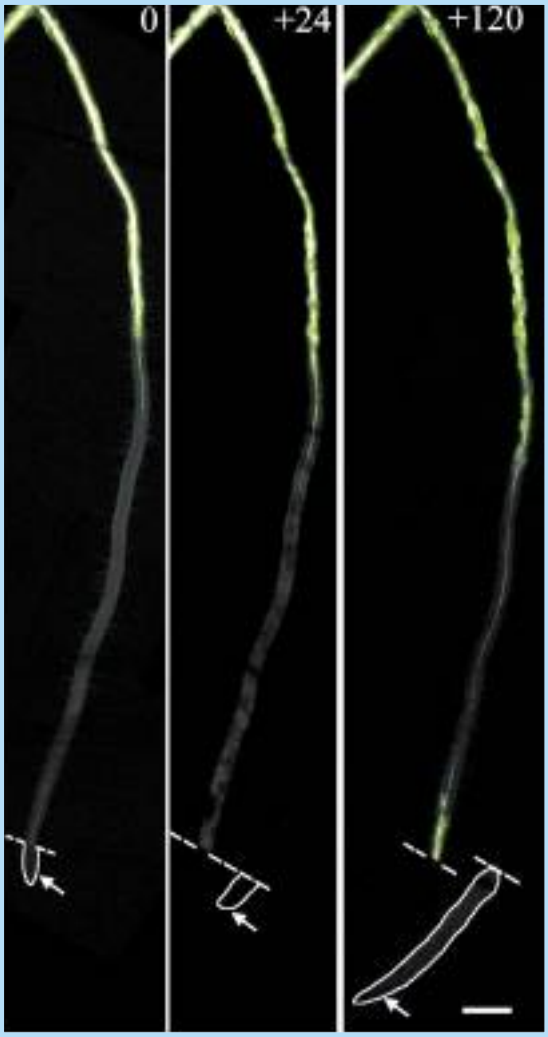

Figure 5 Removal of communication with the root meristem allows virus to replicate up to the excision point. The cut meristem continues to grow but remains uninfected.

The signal for viral suppression originates in the lateral RAM In order to examine the origin of the viral suppression signal, we removed the source tissues of seedlings by severing the primary root directly above the first detectable lateral root initials (Fig. 4). The roots were then observed for a further 3-4 days. During this time, the lateral roots continued to emerge and underwent a progression of viral suppression identical to that observed in intact, infected plants (Fig. 4). These results suggested that the signal for viral suppression did not arise in source tissues, and we concluded that a phloem-mobile signal from the shoot was unlikely to have been the trigger for viral suppression. In a second series of experiments, we surgically removed the apical regions of infected roots, and monitored these roots for a further 4 days. During this time, replicating virus moved into the non-fluorescent cells behind the RAM, up to the point of meristem excision (Fig. 5). However, no virus infection was established in cells apical to the excision point (Fig. 5). Collectively, these data suggested that the onset of viral suppression in the subapical region of the root was brought about by a RAM-derived signal that moved basipetally.

What is the mechanism of viral suppression? We are investigating further the mechanism of viral suppression in developing root systems. Although many of our observations are consistent with a PTGS-like mechanism, we have not shown, unequivocally, that viral suppression results directly from a gene-silencing mechanism in which the viral RNA was detected and

Figure 6 Potential for virus induced gene silencing: (a) Lateral root of a plant expressing GFP from the 35S promoter. (b) Root of a plant expressing GFP from the 35S promoter, infected with TMV.GFP. The lateral roots suppress both the viral GFP and the integrated 35S GFP.

degraded by a host surveillance system[4]. It remains possible that viral replication was impeded by more indirect mechanisms, such as the degradation of one or more viral proteins, or competition of viral RNA with host RNA for translational machinery in meristematic cells. However, we now have evidence that the signal moves through plasmodesmata, and is impeded from entering the primary root due to a symplastic barrier (functional loss of cell-cell communication) at the junction between the lateral and primary root systems (data not shown).

Utility of viral vectors in silencing host-root genes Contrary to published dogma, the above studies have shown that at least some systemic viruses are able to enter the RAM. Regardless of the mechanism of viral suppression, the ability of these viruses to replicate within the RAM, albeit transiently, prompted us to explore the possibility of silencing host-root genes using a virus-vector system. This approach involves the insertion of a host gene sequence into the virus vector and the subsequent silencing of this host gene by a PTGS-like mechanism[4]. This phenomenon, known as virus-induced gene silencing (VIGS), has been described elsewhere for shoot systems[4] and may be a useful tool in plant genomics studies. To examine the potential for VIGS in roots, we utilised transgenic *N. benthamiana* plants that expressed GFP constitutively under a 35S promoter[5]. In these plants, the GFP was conspicuous in the lateral RAM (Fig. 6a). We then inoculated these plants with a TMV vector carrying the complementary *gfp* sequence and examined the plants for subsequent loss of the endogenous GFP signal. At 20 days post inoculation, fluorescence was completely absent in many emerging lateral roots, indicating that silencing of the endogenous *gfp* had occurred (Fig. 6b). Uninfected root systems, or root systems infected with TMV lacking the *gfp* gene, failed to show silencing (data not shown). These observations indicate the potential for analysing root-gene functions using systemic viral vectors.

A developmental link between viral suppression and meristem activation? Why does viral suppression in lateral roots occur some time after the lateral root has emerged? Recent studies on *Arabidopsis* provide a clue. Lateral roots are initiated by anticlinal cell divisions in the pericycle, a single layer of cells found immediately within the root endodermis. One of the first events in lateral-root formation is the onset of anticlinal divisions of individual pericycle cells as these undergo re-entry into the cell cycle[6]. We found that the pericycle initials that give rise to lateral primordia became heavily infected with virus during this early stage in the formation of the lateral root primordium. Malamy and Benfey (1997)[6] have found that, during the emergence phase, the number of cells in the primordium epidermis and root cap remain constant, suggesting that growth during the emergence stage is achieved largely through cell expansion within the primordium. After emergence, the number of cells begins to increase, and new cells appear near the apex. The root then starts to grow via new cell divisions at the apex, concomitant with the formation of a functional lateral RAM. This stage is referred to as 'meristem activation', and occurs when the emerging lateral root has achived a length of approximately 200 µm[6]. This is precisely the point at which we observed viral suppression in the emerging lateral root, suggesting that the signal for viral suppression was initiated in the newly formed lateral RAM. A clear challenge for the future will be to isolate and characterise the host mobile signal(s) that give rise to viral suppression in root systems and to dissect their molecular mode of action.

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Life at the edge – imaging the hypersensitive response induced by TMV

K.M. Wright & S. Santa Cruz

The hypersensitive response (HR) is a mechanism by which resistant plants can defend themselves against infection by viral, bacterial, fungal and nematode pathogens. It is frequently characterised by the rapid death of a limited number of cells near the invading pathogen. This sacrifice of cells blocks further progression of infection and results in the formation of necrotic lesions (Fig. 1).

Figure 1 Necrotic lesions on *Nicotiana edwardsonii* resulting from the hypersensitive response to TMV.

Gene-for-gene resistance Triggering of the HR is usually a highly specific event, which depends on a matching specificity between a disease resistance gene in the plant and an avirulence gene in the pathogen. We have investigated the HR induced in a species of tobacco, *Nicotiana edwardsonii*, that carries the *N* gene for resistance to *Tobacco mosaic virus* (TMV). This *N* gene-mediated response is temperature dependent, the plant only responding defensively at temperatures below 27°C. This characteristic allows plants to be inoculated and maintained at temperatures at which the resistance response is inoperative, prior to lowering the temperature to initiate and synchronise the induction of the HR.

Visualising the virus Until recently, the viral infection sites could not be localised non-invasively before the appearance of visible symptoms. However, by using TMV expressing the green fluorescent protein (GFP), we are now able to localise precisely virus-infected cells in relation to the host response. Under UV illumination, the infected cells fluoresce green and the infection sites can be seen as green dots against the purple colour of the leaves (Fig. 2).

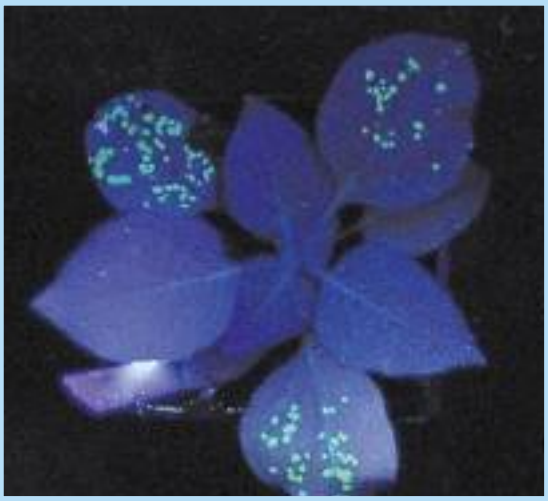

Figure 2 TMV infection sites fluorescing green under UV illumination.

We investigated the course of development of the HR in *Nicotiana edwardsonii* plants using four approaches.

1. By eye or under bright-field illumination using a stereomicroscope.
2. Under blue light using a Confocal Laser Scanning Microscope (CLSM) at low magnification.
3. Using the CLSM at higher magnification.
4. Following treatment of the leaf with a red dye, Texas Red, to trace the supply of water to the leaf. Dye uptake was achieved by dipping the leaf petiole in a solution of Texas Red, which travels with the water into and around the leaf via the xylem.

Xylem Restriction In all experiments, plants were inoculated with TMV-GFP and maintained at 32°C for 48 hours to allow virus infection and growth. The plants were then transferred to 20°C to initiate the hypersensitive response. Using these methods, the first effect of the HR was seen in the supply of water to the infection site. Under normal conditions, the labelling of water with Texas Red shows the water in all areas

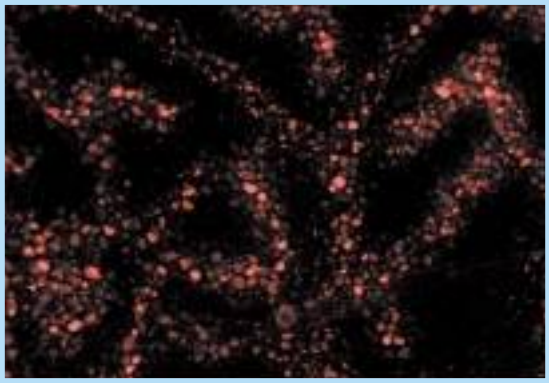

Figure 3 Accumulation of Texas Red in the vacuoles of cells surrounding the xylem.

of the leaf, with the dye accumulating in the vacuoles of individual cells surrounding the xylem, hence the red dots in Figure 3. However, at about 11 hours after the temperature shift, the HR led to a restriction in the supply of water to the infection site, resulting in no red marking of the infected area (Fig. 4a-c).

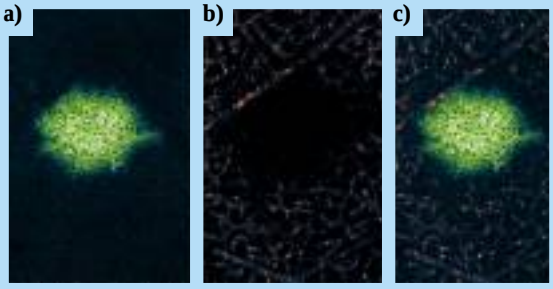

Figure 4 Restriction in water supply 11 hours after HR induction indicated by failure of Texas Red to label the TMV infection site.

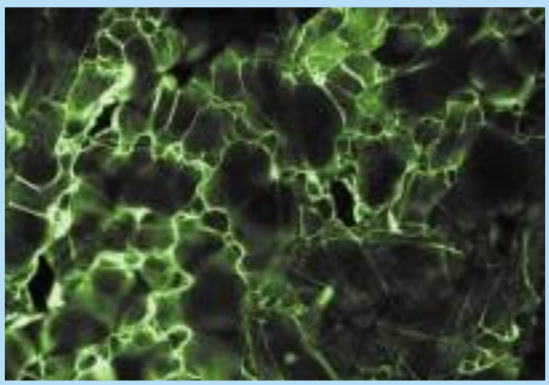

Figure 5 Healthy epidermal cells 11 hours after HR induction.

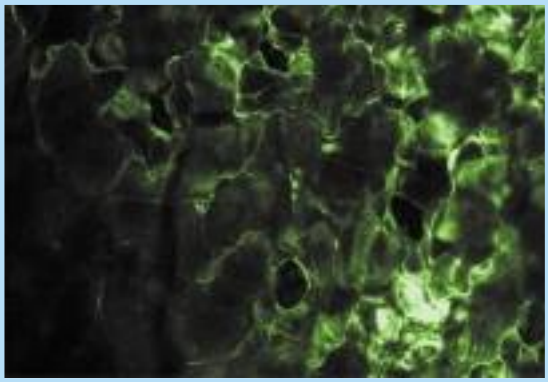

Figure 6 Epidermal cells collapsing onto the underlying mesophyll layer 13 hours after induction.

Cell collapse High magnification of the upper layer of the leaf, the epidermis, revealed that the cells have a rounded, jig-saw-like appearance (Fig. 5). However, after 13 hours, these cells started to collapse onto the underlying mesophyll cells (Fig. 6). This coincided with the first visible signs of collapse seen by eye or under bright-field illumination. The collapsing cells become more obvious over the following 2 hours (Fig. 7). In addition, by 15 hours, the mesophyll cells at the

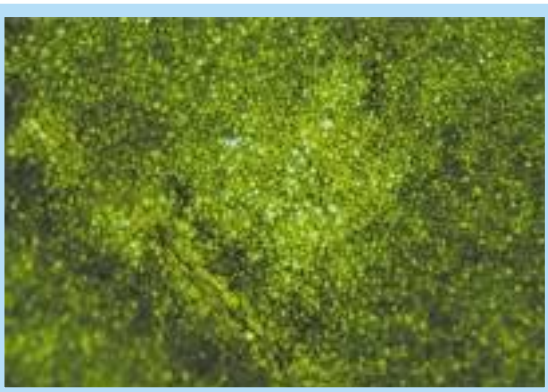

Figure 7 Bright field image of a collapsing infection site 15 hours after HR induction.

centre of the infection site were also seen to be collapsing (Fig. 8), resulting in a dark patch at its centre (Fig. 9).

Cell death and lesion formation It is clear that many changes take place within the cells to generate the HR. We therefore attempted to identify some of the less visible effects that precede cell collapse. To do this, the leaves were infiltrated with Evan's Blue, a dye that is only able to permeate damaged membranes. Up to 8 hours after HR-induction, all the cells within the infection site excluded the dye (Fig. 10). However,

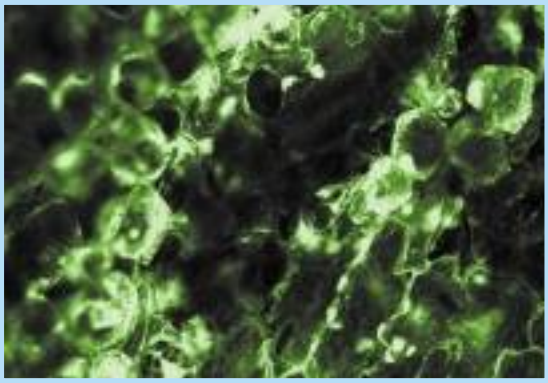

Figure 8 Collapsing mesophyll cells 15 hours after HR induction.

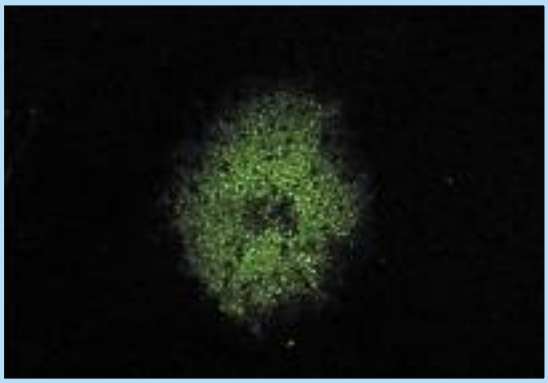

Figure 9 Low magnification CLSM image of a TMV infection site showing collapse of the central cells.

Figure 10 Bright field image of a TMV infection site treated with Evan's blue 8 hours after HR induction showing no staining of cells.

at 9 hours, a subset of infected cells was stained (Fig. 11) indicating damage to their limiting membranes, which leads to cell death.

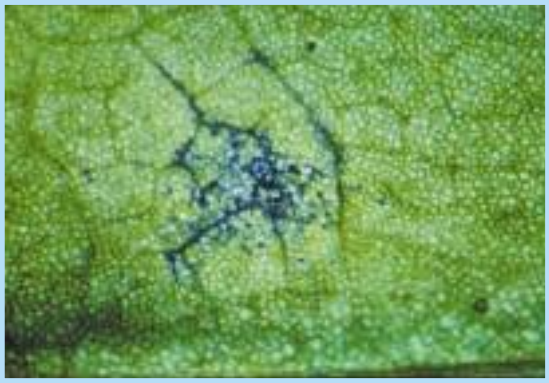

Figure 11 Bright field image of an Evan's blue stained TMV infection site 9 hours after HR induction.

It was also possible to identify a time when the cellular processes leading to cell death become irreversible. Following an initial temperature shift to 20°C, plants were maintained for progressively longer periods before transfer back to 32°C. After a further 24 hours, the leaves were treated with Evan's Blue. As shown in Fig. 12 irreversible progression to membrane damage began from approximately 5 hours. However, irreversible commitment to HR lesion formation was not established until 10 hours or later. This demonstrates that cell death does not necessarily lead to formation of visible lesions. It is possible that, whilst a threshold number of damaged cells may be necessary for lesion formation, other factors, such as water supply, may also be involved.

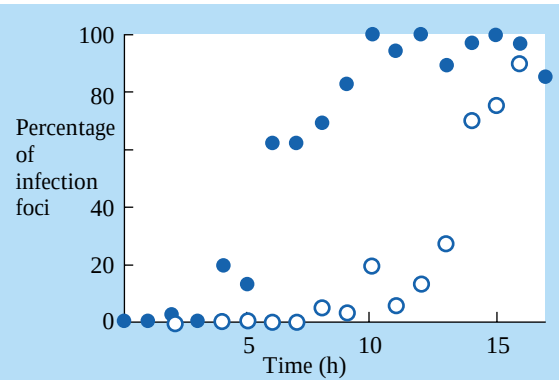

Figure 12 The percentage of infection foci (identified by GFP fluorescence) stained with Evan's blue (closed symbols) and the percentage that developed visible HR lesions (open symbols) after a temperature shift to 20°C for the indicated time followed by maintenance at 32°C for a further 24 hours.

Life at the edge After 17 hours, HR lesion formation progresses with further cell death and collapse, and

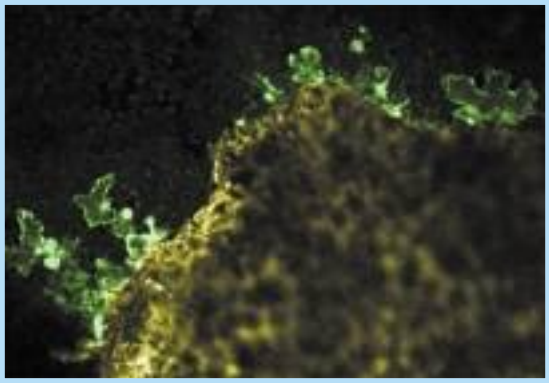

Figure 13 Single TMV-GFP infected cells at the edge of a necrotic lesion 52 hours after induction of HR.

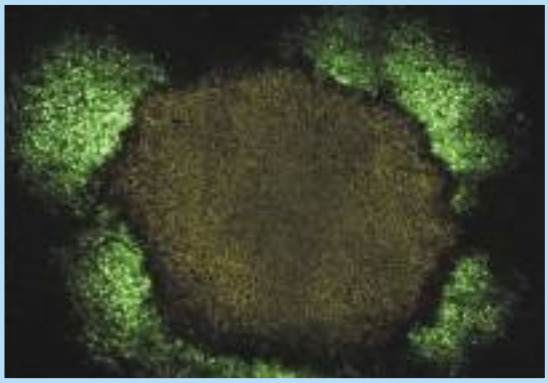

Figure 14 Secondary TMV-GFP infection sites resulting from single cells, following transfer to 32°C for a further 65 hours.

desiccation of the dead area. However, close examination shows that, from about 52 hours onwards, isolated cells become visible at the lesion periphery, persisting for up to 120 hours (Fig. 13). These cells appear to have avoided the initial phase of cell death, presumably because they were only recently infected during the first phase of cell collapse, so that the low level of viral elicitor was insufficient to trigger the HR. It remains to be determined why the virus cannot spread from these cells at low temperature but the inhibition of movement is clearly reversible. If these plants are transferred to high temperature, these cells form the source of virus for development of secondary infection foci (Fig. 14).

Future prospects Using this experimental system, it has been possible to identify the time scale of a number of important changes associated with the HR generated against TMV infection which have previously been obscured due to the inability to identify infected cells. Whilst it is beyond the scope of this report to discuss the biochemical changes and signals which are proposed to be associated with this HR, it should now be possible to link, more accurately, the timing and progression of these changes with the appearance of visible symptoms.

Pathology

Peter Palukaitis, Hugh Barker & James M. Duncan

The broad directions of work within the former Departments of Nematology, Virology, and Fungal and Bacterial Plant Pathology, have been described in previous Annual Reports. Although most of the elements within the three departments have now been merged into the Unit of Mycology, Bacteriology and Nematology, and the Unit of Virology, the themes remain the same. These are (1) to understand at the molecular level the biology of the pathogens and their interaction with their hosts (and vectors), especially with regard to host resistance, (2) to delimit the extent of pathogenic variation, integrating the results into wider epidemiological studies, and (3) to exploit those results in the production of rapid, sensitive and specific diagnostics, and, wherever possible, control strategies. Many of the fundamental questions are similar for each group of pathogens, although the approaches employed to study them must be affected by their different biology. A principal goal of the new Units is to integrate the different experience and knowledge of staff gained about bacteria, fungi, nematodes, or viruses into teams that can tackle important questions about host specificity, host resistance and evasion of host defence mechanisms, which are common to plant pathogens.

Diagnostics and epidemiology The standardisation and harmonisation of pathogen tests in Europe is an important issue in terms of open borders and free trade. Work is progressing to assist this important goal. The diagnostics developed for the potato tuber blemish pathogens (Ann. Rep. 1998/99, 136-138) have been extended for use with real time quantitative PCR (Taqman™), thereby making possible the rapid processing of large numbers of samples and the development of PCR diagnostics (PCRDs) for fungal tuber rots in store. All of the above PCRDs are based on the internal transcribed spacer (ITS) regions of rDNA.

Other PCRDs have been developed for distinguishing *Meloidogyne chitwoodi* and *M. fallax* from other root knot nematodes. *M. chitwoodi* and *M. fallax* are important pathogens of potato that are present in the USA and the Netherlands, from where they have spread to several other EU countries but not yet the UK.

Recent surveys in Scotland, in the Nordic countries and in Poland have shown that only about 60% of isolates of *Erwinia carotovora* subsp. *atroseptica (Eca)*, the causal agent of potato blackleg disease, belong to

serogroup I, vs. >90%, as previously believed. This has led to extensive studies on the population structure of *Eca* using AFLP and phage sensitivities. Different strains can now be tracked in the farm environment and sources of contamination identified. Closely linked is the development of new PCRDs for *Erwinia*: one for detecting all *Erwinia* spp. in potato tissue cultures and one specifically for *Eca*. The latter can quantify the number of *Eca* present in samples of potato peel. The gene for green fluorescent protein (GFP) has also been introduced into *Eca* and has been used to trace the movement of the bacterium in and on potato plants in the laboratory (Fig. 1). Early results have emphasised the close similarity of *Eca* in its aetiology to other vascular wilt pathogens. A spin-off of the phage typing of *Eca* is the development of phage typing kits for strain determination in clinical bacteria, especially for the very important pathogen *Escherichia coli* O157.

The interactive, PC-based, integrated control model for the white potato cyst nematode (PCN; *Globodera pallida*), mentioned in last year's Annual Report, has been shown to be very sensitive to small variations in the effectiveness of nematicides and PCN decline rates during the growing of rotational crops. In addition, the model has demonstrated the need for major changes in the protocols for sampling of PCN, including sampling post-harvest as well as pre-planting.

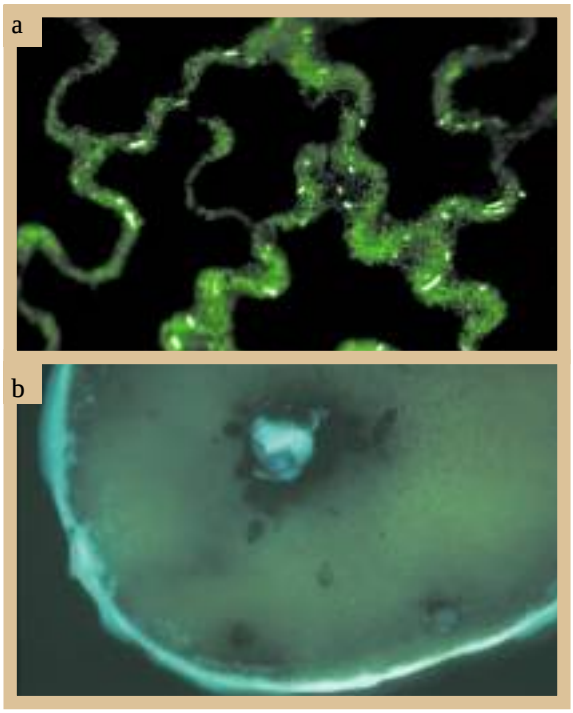

Figure 1 Fluorescence produced after infection of potato by *Eca* expressing the GFP. The bacteria (fluorescence) are seen in the intercellular spaces of an infected leaf (a) and concentrated under the epidermis and centre of pith in a potato tuber (b).

A detection method has been developed for *Tobacco rattle virus* (TRV) RNA by RT-PCR in extracts from potato tubers without interference of PCR inhibitors. TRV was shown to be distributed uniformly throughout the tubers of symptomlessly infected potato cv. Wilja. Tests on seedlings grown from botanical seed produced on Wilja plants, systemically infected with TRV, produced no evidence for seed transmission.

Resistance and control Since most of the work in Pathology is focused on potato as a host, wild *Solanum* species have been and will continue to be valuable sources of resistance to all potato pests and diseases.

In a study of *Solanum* mainly from Mexico, Bolivia, Argentina and Peru, 40 outbreeding and 28 inbreeding accessions were found to be uniformly resistant to the PCN *G. pallida* (races Pa 2/3) with a further 17 segregating for resistance. Eleven of these accessions which together represent 34 different species of wild potato, are new sources of resistance to *G. pallida.*

High levels of resistance to fungal and bacterial diseases (powdery scab, dry rot, blackleg and soft rot) have also been identified in clones of a long-day-adapted *Solanum tuberosum* group Phureja population. Exploitable levels of resistance to these diseases are often absent in *S. tuberosum* group Tuberosum. Selected clones of *S. tuberosum* group Phureja have been hybridised with each other, and with *S. tuberosum* group Tuberosum germplasm.

Recently, PLRV resistance has been assessed in various diploid species of potato including *S. raphanifolium* and *S. tuberosum* group Phureja. Some accessions of *S. tuberosum* group Phureja contained genotypes that had very strong resistance to PLRV accumulation, whereas other genotypes in the same accession were susceptible to PLRV. When resistance tests were made on *S. raphanifolium*, a proportion of genotypes in some accessions remained virus free after graft inoculation.

Molecular biology of pests, pathogens and host-pathogen interactions In collaboration with Dutch partners, one thousand ESTs from second stage juveniles of *G. rostochiensis* have been sequenced. *In situ* hybridisation and other techniques have been used to

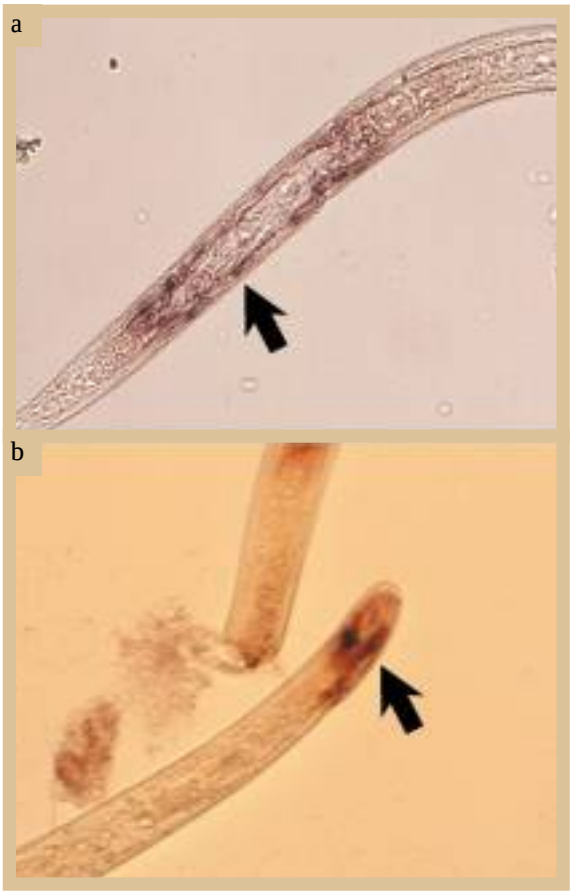

Figure 2 *In situ* hybridisation of digoxigenin labelled probe to fragments of *Globodera rostochiensis* second stage juveniles. Genes encoding secreted proteins expressed in the hypodermis (a) and the amphids (b) are shown. A positive reaction is indicated by a purple/brown deposit (arrows).

characterise some of their functions. (Fig. 2). Antioxidant proteins have been identified in secretions of *Globodera rostochiensis*. A secreted pectate lyase has also been identified in *G. rostochiensis* – the first gene of this type from any animal and the first demonstration of non-symbiotic degradation of plant cell walls by any animal.

The Ty3-*gypsy* group of retrotransposons has been found in *Globodera* and *Meloidogyne*; a first report for plant pathogenic nematodes. Sequence and phylogenetic analyses have shown that the reverse transcriptase sequences are different between the genera but highly homogeneous within each individual species.

Large insert DNA Bacterial Artificial Chromosome libraries have been generated from *E. carotovora* subsp. *carotovora*, and *P. infestans*. The *P. infestans* library is being used for map-based cloning of avirulence genes (*i.e.* genes that elicit the hypersensitive resistance response in potato). The *Erwinia* genomes are being reconstructed *in vitro*, for comparison with the closely related *E. coli*, in the search for novel sequences implicated in pathogenicity and host-range. An entire '*hrp*' cluster, of the type implicated in pathogenicity in other plant pathogenic bacteria, has been discovered in *Eca*.

The technique of suppression-subtraction hybridisation (SSH) has been used in the isolation of plant response genes activated by both *Eca* and *P. infestans* (Ann. Rep. 1998/99, 133-135). Signalling pathways distinct to the hypersensitive response, and to general elicitor-based activation, have emerged. The SSH system has also allowed *P. infestans* genes expressed specifically during early stages of late-blight infection to be isolated.

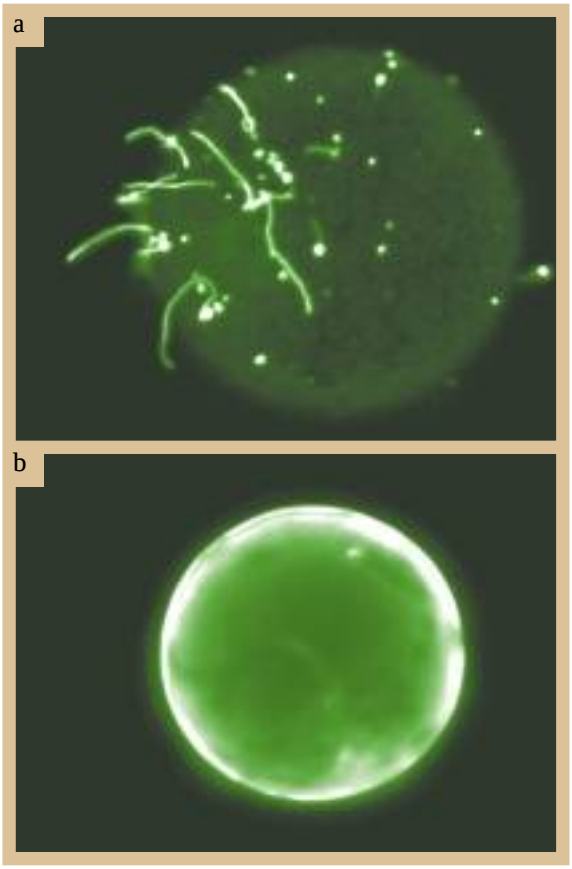

Figure 3 Confocal microscopy showing the presence of fluorescence due to the presence of CMV 3a-GFP. Wild-type 3a-GFP forms tubules on the surface of infected tobacco protoplasts (a). A 3a movement protein mutant fused to GFP does not form tubules, but rather shows fluorescence limited to the surface of the infected protoplast (b).

Tobacco plants transformed with a full-length copy of the genome of PLRV, contain very few infected cells (Ann. Rep. 1998/99, 142-145). When such plants were crossed with transgenic plants expressing the P1/HC-Pro genes from *Tobacco etch virus* (TEV), progeny plants contained about 10-fold more virus than those expressing the PLRV genome alone, and the number of infected cells increased substantially. HC-Pro produced by potyviruses such as TEV are known to suppress post-transcriptional gene silencing. This result suggests that the presence of the P1/HC-Pro genes from TEV may suppress some, but not all post-transcriptional gene silencing that occurs in transgenic plants containing the full length PLRV genome.

Bean yellow vein banding virus (BYVBV) is an umbravirus, isolated from faba beans in England, and reported to use the luteovirus *Pea enation mosaic virus* 1 (PEMV1) as its helper for aphid transmission. The nucleotide sequence of a PCR amplified region of BYVBV ORF3/4 was 97% identical to that of the equivalent region of the unbravirus PEMV2; the predicted sequences of the BYVBV ORF3 and ORF4 proteins were 93% and 97% identical, respectively, to those of the PEMV2 proteins. Thus, while BYVBV is a strain of PEMV2, natural infections of PEMV2 independent of PEMV1 have not previously been reported.

Various aspects of the interactions between *Cucumber mosaic virus* (CMV) and its hosts have been examined. CMV was shown to be able to form tubules protruding from the surface of infected protoplasts (Fig. 3a). These tubules were formed by the 30 kDa movement protein (MP) of CMV, and were observed if the MP was fused to the GFP. These types of tubules have been seen before with other viruses, and it was believed that they might provide a role in cell-to-cell movement. A MP mutant of CMV was generated that could not promote movement between epidermal cells, but could promote movement within infected plants, between other cell types. Tubules were not generated on the surface of infected mesophyll protoplasts from this mutant MP fused to GFP (Fig. 3b), indicating that tubule formation is not essential for movement of CMV between mesophyll cells.

Specific articles follow concerning aspects of the increasing importance of recombinant antibodies in diagnostics, nematode secretions and PCN pathogenesis, the population diversity of *P. infestans*, the development of PLRV-GFP, and the long-distance movement and transmission of plant viruses.

Long-distance movement of viral RNAs in the absence of coat protein

H. Barker, K.D. McGeachy, D.J. Robinson, E.V. Ryabov & M.E. Taliansky

Communication between cells is a fundamental process underlying many aspects of plant growth and development and the effects of viral pathogens on plants. It is believed that not only low molecular weight metabolites but also macromolecules, including mRNA, can move from cell to cell through plasmodesmata and systemically *via* the phloem system. Plant viruses move cell-to-cell and over long distances by exploiting and modifying these pre-existing endogenous pathways for macromolecular movement. For some time, it has been known that viruses produce specialized virus-encoded movement proteins (MP). Some viruses encode single MPs that modify plasmodesmata and facilitate transport of the viral nucleic acid, together with the MP itself, through the modified channel. Others contain a set of genes called the 'triple gene block', which encodes three proteins that, together with the viral coat protein (CP), are proposed to function coordinately to transport viral RNA through plasmodesmata. Less is known about the molecular details of long-distance movement, although there is evidence of the need for specific virus factors, different from those involved in cell-to-cell movement. With only a few exceptions, CP is essential for efficient long-distance transport of plant viruses.

Recent work has revealed some interesting information about the long-distance movement of two very different viruses, *Groundnut rosette virus* (GRV) and *Potato mop-top virus* (PMTV). GRV does not encode a CP, but nevertheless accumulates and spreads systemically within infected plants. PMTV encodes a triple gene block, and although it accumulates and moves systemically in *Nicotiana benthamiana*, its movement and systemic spread in potato seems poor. Often PMTV does not invade every stem on an individual potato plant and a proportion of progeny tubers produced on an infected plant are virus-free. Furthermore, the virus may be passed to only 20% of plants grown from infected tubers.

Characterizing PMTV behaviour in resistant transgenic plants The genome of PMTV comprises three single-stranded positive-sense RNA molecules; RNA 1 (6.5 kb) encodes the replicase functions, RNA 2 (3 kb) encodes the triple gene block proteins and a cysteine-rich protein of unknown function, and RNA 3 (2.3 kb) encodes the coat protein (CP) along with a possible readthrough protein (thought to be involved with vector transmission) which is expressed by leaky termination at the CP gene stop codon.

Challenge inoculum of PMTV does not multiply to produce symptoms or infective virus particles in transgenic *N. benthamiana* that express a translatable version of the PMTV CP gene[1]. In experiments made to understand the mechanisms underlying this resistance, RNA extracts were prepared from transgenic plants that had been inoculated with PMTV particles and analysed by Northern blotting using specific probes to PMTV RNAs 1, 2 and 3. Surprisingly, it was found that RNAs 1 and 2 accumulated in inoculated and systemically infected tissues to levels that were similar to those found in PMTV-infected wild-type plants[2]. However, neither genomic RNA 3 (approx. 2300 nucleotides) nor virus particles were found in the transgenic plants, although small amounts of CP transgene RNA transcript (650-700 nucleotides) were detected.

Accumulation and systemic movement of PMTV RNAs 1 and 2 in non-transgenic plants RNA extracts, prepared from transgenic plants containing RNAs 1 and 2, were inoculated to non-transgenic *N. benthamiana* and *N. clevelandii*. Total RNA extracts from systemically infected leaves of both species contained RNA 1 and RNA 2, which

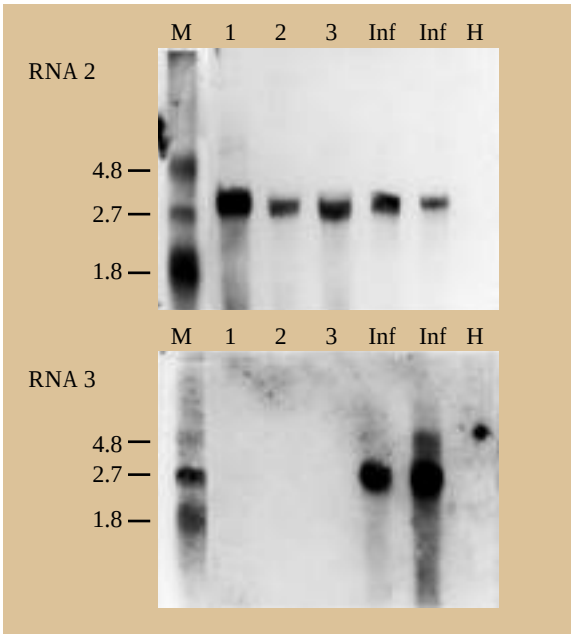

Figure 1 Northern blot analysis of systemic leaf RNA extracts from: three WT *N. benthamiana* plants (lanes marked 1-3) inoculated 22 days previously with RNA extracts from WT RNA 2 +/RNA 3 – plants; two infected WT *N. benthamiana* plants (lanes marked 'Inf'); a virus-free healthy plant of *N. benthamiana* (lane marked 'H'). RNA was run on two gels and blots were treated with specific probes to RNA 2 and RNA 3. The positions of genomic length PMTV RNA 2 and RNA 3 (3×10^3 and 2.3×10^3 respectively) are shown. Marker RNA is in lane 'M'.

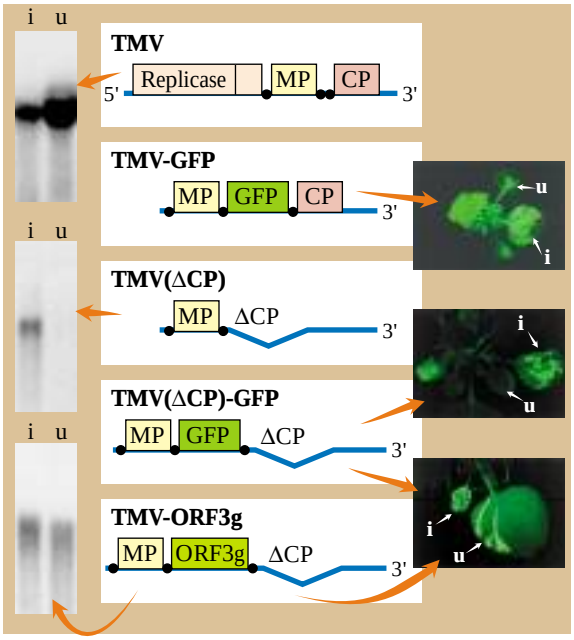

Figure 2 Long-distance movement in *Nicotiana benthamiana* of TMV derivatives. Left panel: Northern blot detection of viral RNA in inoculated (i) and upper uninoculated (u) leaves of plants inoculated with (left to right) unmodified TMV, TMV(ΔCP), and TMV(ORF3g). Right panels: fluorescence due to GFP expression in plants inoculated with (top to bottom) TMV-GFP, TMV(ΔCP)-GFP and TMV(ΔCP)-GFP+TMV(ORF3g).

had replicated in the absence of RNA 3. Because RNA 3 was absent from the infected plants, no coat protein or virus particles were produced. Symptoms did not develop. These data (illustrated in Figure 1) provide evidence that long-distance spread of infective PMTV RNA can occur in the absence of CP or virions.

Systemic spread of TMV(ORF3g), a hybrid TMV in which GRV ORF3 replaces the CP gene The RNA genome of GRV contains four open reading frames (ORFs), none of which encodes a CP. The two ORFs nearest the 3' end of the RNA (ORF3 and ORF4) overlap each other in different reading frames. ORF4 encodes a 28 K cell-to-cell MP which exhibits high similarity with MPs of several other viruses, which localises to plasmodesmata and which can functionally replace MPs of such unrelated viruses as *Cucumber mosaic virus* (CMV) or *Potato virus X*[3, 4]. Database searches with amino acid sequences of the 27 K ORF3 protein detected no significant similarities to any other known viral or non-viral proteins[5]. To analyze the function of the ORF3 protein, we employed a gene replacement strategy to generate hybrids between *Tobacco mosaic virus* (TMV) and GRV[6]. CP is not required for cell-to-cell movement of TMV but is essential for its long-distance movement. The CP gene of TMV was deleted and replaced by ORF3 of GRV to give the hybrid TMV(ORF3g) (Fig. 2). A TMV mutant with the CP gene deleted, TMV(ΔCP), was used as a control (Fig. 2).

Both viruses were inoculated to *N. benthamiana* plants, and total nucleic acids extracted from inoculated and upper uninoculated leaves. Tests for infectivity on the hypersensitive host, *N. tabacum* cv. Xanthi NN, and Northern blot analysis, both confirmed that TMV(ORF3g) RNA spread systemically in *N. benthamiana* plants. In contrast, TMV(ΔCP) spread from cell to cell in inoculated leaves but, as expected, did not move systemically. Dot blot hybridization experiments detected TMV(ORF3g) RNA in mesophyll protoplasts isolated from uninoculated systemically-infected leaves, demonstrating that it is able not only to move from inoculated to uninoculated leaves, but also to exit from the vascular system in uninoculated leaves.

Complementation of the long-distance movement defect of the TMV CP deletion mutant by TMV(ORF3g) Green fluorescent protein (GFP) is often used as a non-invasive reporter to monitor virus infections. However, attempts to generate infectious TMV derivatives expressing both GRV ORF3 protein and GFP were unsuccessful. Therefore, complementation of the long distance movement defect of TMV(ΔCP) by GRV ORF3 protein was tested using a TMV derivative in which the CP gene was replaced by the gene encoding GFP. As expected, this virus, TMV(ΔCP)-GFP (Fig. 2), was able to develop fluorescent foci 3 days post-inoculation in inoculated leaves but did not cause any systemic infection. However, when TMV(ΔCP)-GFP was co-inoculated with TMV(ORF3g) onto *N. benthamiana*, the majority of the doubly-infected plants showed systemic symptoms characteristic of TMV(ORF3g) and developed green fluorescent zones in both inoculated and uninoculated leaves, implying systemic spread of TMV(ΔCP)-GFP in the presence of TMV(ORF3g). The first indication of entry of TMV(ΔCP)-GFP into an uninoculated leaf in this case, was the appearance of fluorescent flecks along veins on the lamina, indicating that the virus was being unloaded at discrete points. Subsequently, some leaf veins became more clearly delineated by continuous fluorescence, and, with time, the mesophyll tissues neighbouring the flecks also became labelled. Confocal laser scanning microscopy confirmed these observations and showed that up to 90% of mesophyll cells in the fluorescent area were infected with TMV(ΔCP)-GFP. The time of appearance of GFP fluorescence (about 8 days post-inoculation) and the pattern of virus unloading in uninoculated leaves observed in mixed TMV(ΔCP)-GFP + TMV(ORF3g) infections were similar to those observed for TMV-GFP (i.e. 'normal' TMV with the GFP gene added (Fig. 2)) and corresponded to the usual manner of vascular-associated long-distance virus movement described for other viruses.

These results show that the GRV ORF3 protein can mediate the long-distance movement of RNA of the unrelated virus, TMV, both *in cis* in the form of TMV(ORF3g) and *in trans* in the form of TMV(ΔCP)-GFP. Thus, it seems likely that the role of the ORF3 protein in GRV is to mediate systemic movement of GRV RNA.

Conclusion CP is essential for efficient and rapid long-distance transport of most viruses. There are a few exceptions in which CP is dispensable for systemic infection. Members of the genus *Umbravirus*, such as GRV, represent a special situation because they do not encode a CP, but accumulate and spread systemically very efficiently within infected plants. Functional analysis of GRV ORF3 protein suggests that it is a long-distance RNA movement factor that can act *in cis* or *in trans*. In chimeric TMV, it can functionally replace CP which is critical for long-distance spread of TMV. PMTV is an example of a small group of viruses which possess a CP, but in which systemic movement is not dependent on CP expression or virion formation.

Thus, although PMTV and GRV are very different viruses, their RNA genomes share the ability to enter the vasculature and move long distances within the plant. The triple gene block proteins of PMTV and the ORF3 protein of GRV could represent a class of long-distance RNA movement factors.

Several other plant virus proteins, such as the 2b protein encoded by CMV or the HC-Pro protein encoded by potyviruses, have also been shown to be involved in systemic virus spread. These proteins can suppress post-transcriptional gene silencing and it is suggested that they act by blocking a potential host-defence mechanism (akin to gene silencing) that restricts systemic spread rather than by promoting the process of long-distance movement itself. In accordance with this suggestion, the CMV 2b or *Potato virus Y* (potyvirus) HC-Pro proteins were unable to replace functionally TMV CP[6]. Thus, these proteins are distinct from the class of long-distance movement factors represented by the GRV ORF3 protein and the PMTV triple gene block proteins.

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Fluorescent tagging of *Potato leafroll virus*

M.A. Mayo, M.E. Taliansky, K. Nurkiyanova[1], E. Ryabov, G.H. Duncan, T. Canto, S.M. Gray[2] & U. Commandeur[3]

Potato leafroll virus (PLRV) is an important pathogen of potato crops. The disease it elicits can cause significant crop losses and is a constraint in the production of high quality virus-free seed potato crops. PLRV is one of several viruses in the family *Luteoviridae* that cause significant diseases in a variety of crops world-wide. One of the defining characteristics of viruses in this family is that they are transmitted by aphids in a circulative, persistent fashion. In recent years, research at SCRI has focused on critical events in the transmission cycle of PLRV in its aphid vector(s), both for their intrinsic scientific interest and in a search for an "Achilles heel" in virus biology at which control measures could be aimed.

Figure 1

Transmission occurs when virus particles are taken up by aphids feeding on infected plants, absorbed from the gut, transported from the aphid haemolymph (= body fluid) into the aphid accessory salivary glands and then excreted into new plants when the aphid vector feeds. Another characteristic of viruses in the family *Luteoviridae* is that they are relatively specific for certain aphid species, presumably because of molecular interactions at cell boundaries during the elaborate transmission process. Electron microscopy has revealed some details of these processes (*SCRI Ann. Rep.* 1996/1997, 164-167) but what happens between the arrival of an aphid at a new plant and the plant becoming infected by PLRV is something of a 'black hole'. As with many such unexplored areas, new methods of molecular biology are now starting to shine light into this part of the PLRV infection cycle.

In previous Annual Reports, it has been shown how DNA encoding the jellyfish green fluorescent protein (GFP) can be added to virus genomes so that virus multiplication causes host cells to become fluorescent. This has revealed where viruses multiply and details of how they move between infected and healthy cells (*SCRI Ann. Rep.* 1997/1998, 67-70). This fluorescent tagging has now been achieved with PLRV by modifying the virus genome – the first time that this has been done with the genome of a luteovirus.

illustrates the modification principle. DNA that encodes GFP was inserted near the 3' end of open reading frame (ORF) 5 of PLRV. This ORF codes for P5, a minor protein that is thought to lie on the surface of PLRV particles. The effect of the insertion of the GFP gene was to replace the C-terminal 100 amino

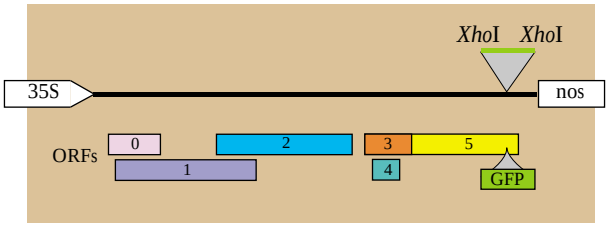

Figure 1 Construction of pPLRV-GFP. cDNA that corresponded in sequence to PLRV RNA was inserted either between the 35S promoter and the nos terminator in a Ti plasmid or downstream of a sequence with T7 RNA polymerase promoter activity. The encoded open reading frames (ORF; numbered 0 to 5) are shown in different colours. ORF 5 was cut with *Xho*I and cDNA that encoded GFP was inserted as shown.

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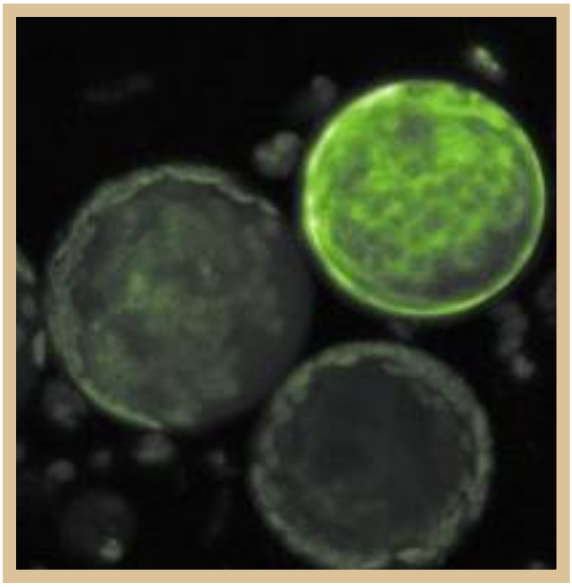

Figure 2 Protoplasts from a sample electroporated with transcript RNA corresponding in sequence to PLRV-GFP. The protoplasts were cultured at c. 20^{O}C for 3 days. The protoplast at the top right was fluorescent, demonstrating infection by PLRV-GFP, those in the bottom left were not infected.

acids of P5 with the amino acid sequence of GFP. It was known from work with mutant strains of PLRV that virus that lacks this part of P5 is able to multiply in infected plants. Plasmid DNA that contained the entire sequence of PLRV RNA, together with the added GFP gene was propagated in bacteria, purified from them and then used as a template for the synthesis of genomic RNA of PLRV-GFP. This was used to electroporate tobacco protoplasts. After culture for 3 days, a number of the electroporated protoplasts were shown by light microscopy to be fluorescent, that is they contained GFP (Fig. 2). When proteins in these protoplasts were examined by immunoblotting, the protein molecules containing P5 were larger than P5 molecules from control protoplasts infected with unmodified PLRV by an amount that corresponded in size to the added GFP. Thus most, or all, of the P5 molecules that were synthesized in protoplasts infected with PLRV-GFP contained extra amino acid sequence, presumably GFP. Examination of extracts by electron microscopy showed that the protoplasts contained PLRV-like particles. These bound to EM grids that had been coated either with antibodies to PLRV particles or with antibodies specific to GFP (Fig. 3a,b). When these trapped particles were incubated with gold-labelled antibodies to GFP, gold was bound to the particles (Fig. 3c). These results show that the PLRV particles carry GFP molecules on their

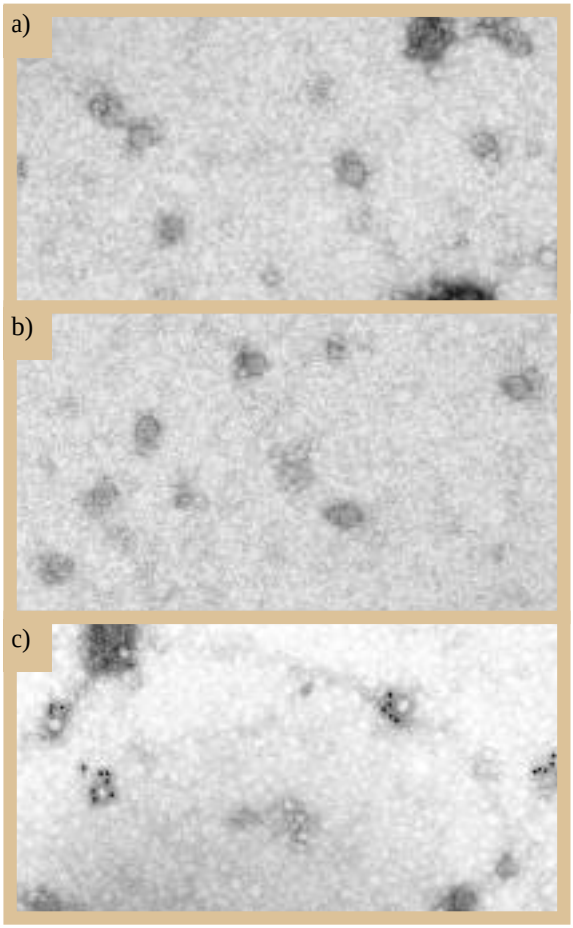

Figure 3 Immunosorbent electron microscopy of extracts of protoplasts infected with PLRV-GFP. (a) particles trapped by antibodies to PLRV particles; (b) particles trapped by antibodies to GFP; (c) particles trapped by antibodies to PLRV particles and then decorated with antibodies to GFP that had been conjugated to gold (black dots).

surfaces, presumably attached to the C-terminal part of the P5 protein carried by a proportion of the major coat protein molecules.

When aphids were fed through membranes on extracts of protoplasts infected by PLRV-GFP for 24 hours and then transferred to test plants, these became infected. Thus, the particles that were carrying GFP on their surfaces were able to pass through the bodies of vector aphids and enter new host plants to initiate infection. When the leaves on which the aphids had fed were examined by light microscopy about 2 weeks after aphid feeding, a small number of cells were fluorescent. These cells were of various types, including epidermal cells and trichomes, but very few such cells were adjacent to other fluorescent cells (Fig. 4). Although PLRV infected these plants systemically,

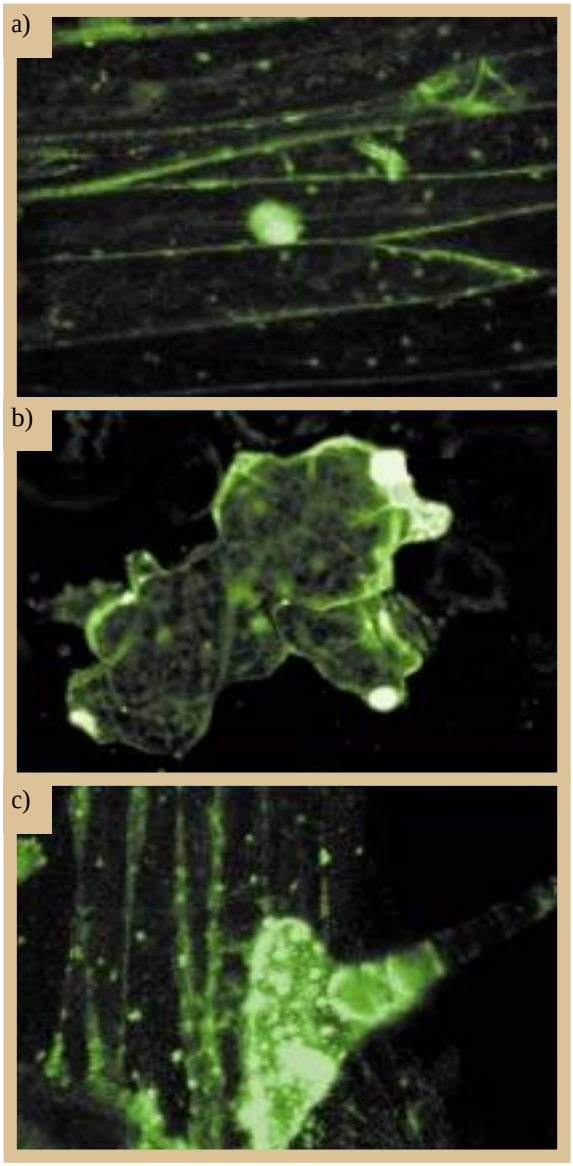

Figure 4 Cells in leaves that had been probed by aphids carrying PLRV-GFP. Some cells of the vascular tissue (a), the epidermis (b), and trichome cells (c) were shown to be infected.

there was no spread of fluorescence. Reverse transcription/PCR with PLRV RNA-specific primers showed that systemically infected tissues of these plants contained P5 genes that had lost some or all of the inserted GFP-coding sequences, presumably because of the deletion that allowed the PLRV to spread.

An alternative method of infecting plants with PLRV in a DNA form is by agroinoculation. In this method, DNA copies of the PLRV genome are inserted into the Ti plasmid and this is introduced into plant cells by injection (agroinoculation) into petioles or infiltration into leaf spaces. As with inoculation by aphids, agroinoculation resulted in infected plants, but none of the infected plants contained PLRV identical to the inoculum PLRV-GFP. All the progeny virus had deleted genomes. Agroinfiltration resulted in cells that were fluorescent because PLRV-GFP had multiplied, but none of the plants became infected.

These results show that the modification of adding the GFP gene to the genome of PLRV results in a genome that cannot move from cell to cell. The oversize RNA was encapsidated in particles that were then transmissible by aphids, and thus were intact, but cell-to-cell movement was not possible. The plants infected by aphids transmitting PLRV-GFP, or by agroinoculation, contained PLRV particles that contained RNA with all or most of the added GFP gene removed by deletion. Thus, some feature of the spread of PLRV infection is prevented if the genome of the virus is oversize.

Nevertheless, what were revealed for the first time by the experiments in which aphids carrying PLRV-GFP fed on plants, were the cells into which viruliferous aphids had introduced PLRV. These cells were of various types, which shows the extent to which aphids probe different leaf cells sufficiently long to transmit PLRV. The system therefore has promise as a means of assessing how the behaviour of aphids relates to the introduction of PLRV into leaf cells.

The status of Scottish late blight populations

D.E.L. Cooke, V. Maughan, P.R.J. Birch, R. Toth, F. Gourlay, S. Carnegie[1] & J.M. Duncan

Like an ominous shadow, *Phytophthora infestans* has followed its principal hosts, potato and tomato, out of the New World and across the globe. This oomycete fungus is the cause of late blight (Fig. 1), an abiding threat to potato growers that requires vigilance and expensive fungicide applications for effective control. The patterns of migration of this pathogen reveal a fascinating history[1] and provide a classic example for the study of fungal population biology and evolution. Its mid-19th century arrival in W. Europe, and the ensuing economic, social and political impact, have been well documented.

P. infestans is a diploid, heterothallic fungus with two mating types (termed A1 and A2), which are found with equal frequency in its Mexican centre of diversity[2]. Within this freely interbreeding population, there is a wealth of genetic diversity. In Europe, however, the initial introduction from the Americas appears to have been of limited size, as the population comprised only the A1 mating type. Moreover, DNA-based genetic analysis has revealed that this population was of a single clonal lineage and represented a classic example of reduced population diversity as a result of passing through a 'genetic bottleneck'. Despite being a clone, the population was capable of adaptation, overcoming the single major R genes for resistance incorporated from wild potato species in the first half and middle of the 20th century. The mechanisms by which such somatic mutations are generated and disseminated are poorly understood in *Phytophthora*.

Figure 1 Typical foliar symptoms of potato blight: a large lesion with a necrotic central zone surrounded by profuse white 'fluffy' sporulation.

In the early 1980's, the status of European blight populations changed markedly. As a result of the dry summer of 1976, Mexican potatoes were imported into Europe bringing with them a much more diverse population of *P. infestans* that comprised A1 and A2 mating types. The 'new' population rapidly displaced the 'old' one, suggested to be as a result of its increased fitness over the existing 'old' clone which had been 'weakened' by 130 years of asexual propagation (Müller's ratchet). A major component in the chemical armoury against blight was also threatened at this time. Overuse of the curative phenylamide fungicide, metalaxyl, resulted in high selection pressure on the fungus and a build up of insensitive isolates markedly reduced the fungicide's efficacy. The arrival of the A2 mating types in the Mexican imports (not discovered until 1981 and not announced until 1984!), added a new dimension to the pathogen's biology. The occurrence of the two mating types raised the possibility that the fungus could undergo its sexual cycle in infected potato plants and form long-lived oospores. These could then be incorporated into the soil with several adverse consequences.

[1] SASA, East Craigs

Figure 2 Geographical spread of sites from which late blight samples were collected.

The presence of a sexual cycle allows a regular re-assortment of its genes. While it has been suggested that such re-assortment may be deleterious as it breaks up 'winning combinations' of genes, in general, evolutionary theory suggests that this would result in a more flexible genetic system, enabling the fungus to adapt more rapidly to man's control efforts. Should this be the case, an accelerated erosion of plant resistance, including both major gene (R-gene) and field resistance resulting from combinations of several to many loci (Quantitative Trait Loci), may occur. Erosion of the resistance of existing (and future) commercial cultivars represents a serious threat to potato production. An accelerated adaptation to existing and new fungicide active ingredients may occur also, threatening one of the most effective means of control. In addition to the, as yet unproven, genetic consequences, there are epidemiological implications of the addition of a durable soil-borne phase into the *P. infestans* life cycle. These hypotheses have yet to be tested fully in W. European systems, though earlier disease outbreaks (observed in Sweden[3]) and a need for longer crop rotations may be an inevitable consequence of a residual soil-borne source of inoculum.

It was against this background that the studies reported here were undertaken at SCRI in collaboration with the Scottish Agricultural Science Agency (SASA), to elucidate the current status of Scottish blight populations and allow predictions of the significance of a changing population to the potato industry (growers, breeders, advisors and scientists alike).

A survey was undertaken by collecting isolates during the 1995, 1996 and 1997 seasons. Samples of blighted plant material were kindly provided by potato inspectors, colleagues in other institutes, and members of the public, as well as being collected by SCRI and SASA staff. In total, 82 sites were sampled, resulting in a collection of 499 *P. infestans* isolates (Fig. 2). Reflecting the varied pattern of potato production in Scotland, a range of sites was sampled: two thirds were commercial farms and the rest were private gardens or allotments. To assess diversity both within and between sites, as many as 15 isolates were collected from each site (the average number of isolates per site was six). The isolates were examined phenotypically for mating type, sensitivity to metalaxyl fungicide and, to a limited extent, virulence against major potato resistance (R) genes. The isolates were also DNA-fingerprinted using the amplified fragment length polymorphism (AFLP) method.

Mating type	Farm sites	Farm isolates	G/A sites	G/A isolates
A1 only	72.7	83.3	56.9	57.8
A2 only	18.9	8.0	7.8	5.1
A1 & A2	8.4	8.7	35.3	37.1

Table 1 Mean percentage of sites and isolates from each of the mating type categories (A1, A2 or a mixture of both mating types) collected between 1995 and 1997. Results are categorised according to site type, farm or garden/allotment (G/A).

The majority (80%) of isolates collected were A1, 19% were A2, and 1% produced oospores without the presence of the opposite mating type (so-called self-fertile types). The type of site from which they came and whether or not that site comprised a single or a mixture of both mating types, can be seen in Table 1. The A1 mating type alone was found on 73% of farm sites, compared with 57% of garden and allotment sites. Since a variable number of isolates was collected at each site, the percentage of isolates belonging to either mating type differs from the percentage of sites where one or both were recovered. Populations of mixed mating types were four times more common in gardens and allotment populations than in farms. Studies in the Netherlands also have shown a similar pattern which is thought to result from amateur growers keeping home-grown seed, co-cultivating tomato and potato, having generally higher disease incidences, and using compost containing peelings from imported potatoes. No clear changes in mating type frequency,

occurrence or distribution were observed over the 3 years, suggesting the populations are relatively stable.

Response to a range of metalaxyl concentrations was scored on the basis of each isolate's ability to grow on amended agar media relative to growth on unamended media. Isolates were classified as resistant, sensitive or intermediate. Of 444 isolates examined, approximately equal percentages were resistant (42%) and sensitive (44%) with 14% showing an intermediate response. In this case, however, a trend was apparent over the course of the study. The number of sensitive isolates was similar each year but the number of intermediates increased as the number of fully resistant isolates decreased. It is interesting that 70% of the intermediate isolates occurred in mixed mating type populations compared with only 23% from single mating type populations. As some reports show, metalaxyl resistance may be governed by a single, semi-dominant gene and heterozygotes have the intermediate phenotype. Thus, the occurrence of high numbers of intermediate types on mixed mating type sites is indicative of sexual recombination. The majority of such mixed populations were from gardens and allotments in which there is a risk of fully resistant A2 isolates developing. Significantly, only four of 82 A2 isolates tested were resistant to metalaxyl.

	Fungicide program		Samples treated with fungicide	
	Untreated	Treated	Phenylamide ingredient	
			-	+
Response to metalaxyl	% Isolates (n=166)	% Isolates (n=133)	% Isolates (n=58)	% Isolates (n=66)
Resistant	27	67	58	76
Sensitive	60	28	29	24
Intermediate	13	5	12	0

Table 2 Percentage of isolates classified as resistant, sensitive, or intermediate in response to metalaxyl. Sites were sub-divided into those untreated and treated with fungicides; the latter being further sub-divided into those treated with or without phenylamides.

An analysis of 299 isolates from 46 samples, for which fungicide application data were available, shows the impact that phenylamides have on *P. infestans* population structure (Table 2). Only 27% of isolates from untreated sites were resistant compared with 67% from fungicide-treated sites, and where phenylamide was a component of the fungicide, this figure rose to 76%. Considering that 96% of A2 isolates were either sensitive or intermediate in their response to metalaxyl, this suggests phenylamides played a significant role in limiting the A2 population size.

The virulence characteristics of representative isolates of a range of 1995 and 1997 samples were assessed in 'whole plant' tests. Considerable variation was noted in virulence phenotypes but no significant differences were observed between the 1995 and 1997 samples. Since collecting virulence data is time-consuming and recent publications[4] have reported a lack of correlation of virulence with epidemiological or molecular characters, it was not pursued further in this study.

In collaboration with colleagues at The University of Wales, Bangor, 31 isolates were examined using restriction fragment length polymorphism analysis (RFLPs) with the moderately repetitive RG57 probe, and eight RG57 fingerprints were identified, hinting at the breadth of genotypic diversity in Scottish populations. In agreement with the phenotypic data, isolates from mixed A1 and A2 sites yielded a mixture of RG57 fingerprints, again suggestive of sexual recombination. Fourteen isolates from nine single mating type farm samples had five different fingerprints but no variation was observed within a sample from a single farm. Thus, single clones probably initiate epidemics on farms but the overall Scottish population consists of many RG57 clones.

AFLPs yielded total genomic DNA fingerprints consisting of around 70 bands per isolate. Using a single primer combination, almost 300 isolates were fingerprinted and the presence/absence data for 15 easily scored polymorphic bands (markers) per isolate were recorded. Considerable molecular diversity was observed, with over 163 (56%) of the isolates having unique marker combinations. A cluster analysis of the fingerprint data from each individual isolate did not resolve any clear grouping of isolates by site, mating type, metalaxyl sensitivity or season. This is consistent with a 'metapopulation model' in which short-lived, local populations are being replaced continually by new genotypes that either migrate from neighbouring populations or are generated through sexual recombination. Averaging the AFLP marker diversity within each site using the computer package POPGENE did, however, reveal a trend. The population was clustered into three groups; in the first group, seven of the ten sites were of A2 only or A1/A2 mixed type, compared with only eight out of 44 sites in the other two major groups. Comparisons of the annual average number of isolates per fingerprint and average number of fingerprints per site do not indicate any trend to increasing

fingerprint complexity *e.g.* as a result of extensive sexual recombination. AFLP fingerprints showed a greater resolution than RFLPs with the RG57 probe.

Conclusions The extensive database generated in this study highlights the complexity and dynamic nature of blight populations in Scotland. The samples from throughout the country were of both mating types, and also included self-fertile isolates. They showed different levels of resistance to the phenylamide fungicides, and a range of virulences was observed.

In agreement with a similar survey in England and Wales, the A2 mating type is present but at a much lower frequency than the A1 type. While the implication of such an imbalance is, for now at least, good news for growers (reduced risk of sexual recombination and thus oospore formation), the reasons are unclear. Unpublished reports of A2 isolates in Scotland as early as 1984 would seem to dispel the theory that it is a recent import and has thus had insufficient time to spread. It is possible that the A2 strains found here are less well adapted to Northern European conditions than the A1 strains. However, 25 and 15% of isolates from commercial potato fields in Norway and Finland[5] respectively were found to be A2's. Since such strains are very likely from the same European population, poor adaptation is also an unlikely reason for the differences in frequency. The results of this survey suggest that the sensitivity of the A2 strains to metalaxyl is the principal factor limiting their numbers. Their prevalence in gardens and allotments is probably a consequence of a combination of poor crop hygiene (discussed above) and the fact that gardeners do not have access to metalaxyl.

Our molecular data suggest that the A2s are of a different genetic background to the majority A1 population. However, the same data and the phenotype and site data (discussed above) also suggest that crossing does occur, albeit infrequently. The eventual consequences of such interbreeding will be a blurring of genotype boundaries. It is also likely that more A2 metalaxyl insensitive isolates will emerge and thus the impact of metalaxyl sensitivity, as a limiting factor on A2 spread, will be reduced. Between 1995 and 1997, the frequency of A2's remained unchanged but ongoing monitoring of mating type and metalaxyl resistance is necessary to check for such population changes.

The risks of oospore formation are clearly greater in gardens and allotments, although mixed populations were also found on commercial farms. Growers, both amateur and professional, should therefore remain assiduous in their monitoring and control strategies and be on the lookout for early infections that may indicate soil-borne inoculum (i.e. oospores). Amateur growers must, as always, avoid using poor quality potato seed and avoid contaminating garden compost with either their own infected material and more importantly that of imported potato material.

The low and stable frequency of isolates fully resistant to metalaxyl suggests Scottish growers are using the phenylamide group of fungicides prudently. However, the rise in the number of isolates of intermediate resistance should be monitored to ensure the longevity of this active ingredient.

The information generated in this project on the current status of Scottish blight populations will help plant health inspectors assess the risk of early epidemic development through oospores. It will also help breeders concerned with the effects of shifts in pathogenicity on the durability of resistant cultivars, as well as guiding policy on fungicide resistance build-up.

The future As well as detailed studies on the host pathogen interaction[6], ongoing work in the Unit of Mycology, Bacteriology and Nematology is targeted at PCR-based detection of oospores in soil, the development of new co-dominant DNA markers (SSR and SNP-based) that can be applied to study the population biology of *P. infestans* in more detail, as well as detailed epidemiological studies on the behaviour of particular isolates in the field.

Acknowledgments

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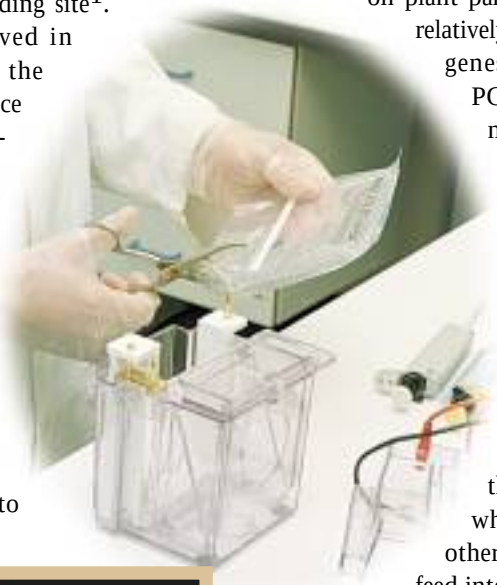
Genes and parasitism in plant pathogenic nematodes

J. Jones, V. Blok, K. Boutsika, A. Paterson, M. Phillips, L. Roberston[1], H. Popeijus[2] & G. Smant[2].

Plant parasitic nematodes cause serious damage to crops in the UK and throughout the world. Our work is aimed at understanding the proteins produced by parasitic nematodes that allow them to parasitise plants. In particular, we are investigating the nature and function of nematode secretions. Secretions of plant parasitic nematodes have been implicated in many aspects of the host-parasite interaction. For example, secreted cellulases are thought to be important in migration of the nematode through the root prior to the induction of the feeding site[1]. Secretions may then be involved in induction and maintenance of the syncytium, although direct evidence for this is still lacking. The nematodes spend a large proportion of their life cycles embedded within an organism possessing a battery of chemical and physical defences, and evidence is emerging that nematodes secrete proteins which help to protect them from these defences[2]. For these reasons, our studies are focused on analysing secretions of the potato cyst nematode (PCN).



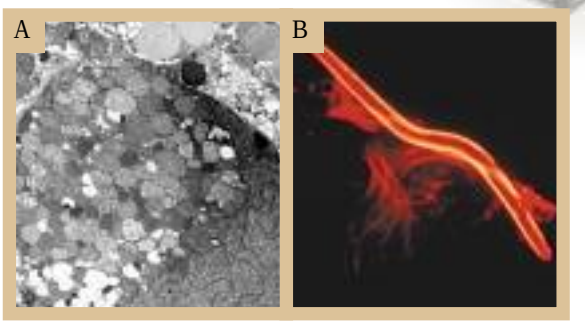


Figure 1 1. Genes encoding secreted proteins are likely to be abundant in a representative cDNA library made from PCN J2s. The gland cells of PCN are large and full of secretory granules (1a) and secreted proteins are produced in abundance from the J2s (1b). In figure 1a some of the secretory granules are arrowed and in figure 1b secreted protein is stained gold/red using an antibody raised against a recombinant PCN secreted protein. Note how the secretory material is present on and shed from the surface of the nematode.

One of the major problems in working with PCN is that, like many parasites, it is an extremely awkward experimental organism. It is an obligate endoparasite which means that many of the most interesting developmental stages are inaccessible and no *in vitro* culture systems exist. Furthermore, PCN is extremely small (to allow it to live inside plant roots) which means that it is not possible to work directly with the tissues of interest (in this case the gland cells). These problems have meant that molecular biological studies on plant parasitic nematodes have progressed relatively slowly. Until recently only three genes encoding secreted proteins of PCN had been identified. An alternative approach was clearly required.

Expressed sequence tag (EST) analysis offers a rapid and cost effective route to the discovery of novel genes. ESTs are single pass sequences of cDNA clones selected at random from a cDNA library. ESTs provide a background of information about the genes expressed in an organism, which can be extremely useful for other molecular biological projects to feed into. Over 25,000 ESTs from nematode parasites of animals are present in databases and have provided a valuable resource for researchers working with these parasites. Although obviously less selective than more direct approaches (such as library screening or differential screening), it is possible to target an EST project at genes expressed at the onset of parasitism which may be important in the host parasite interaction by using a cDNA library constructed from the hatched infectious stage juvenile (J2). Changes occur in J2s during hatching indicative of the transition to a parasitic mode of existence. These changes include activation of transcription in the gland cells as well as behavioural changes and changes in gene expression. It is also feasible that an insight into the molecules secreted by PCN may also be gained using ESTs – mRNAs encoding secreted proteins are likely to be abundant in a representative

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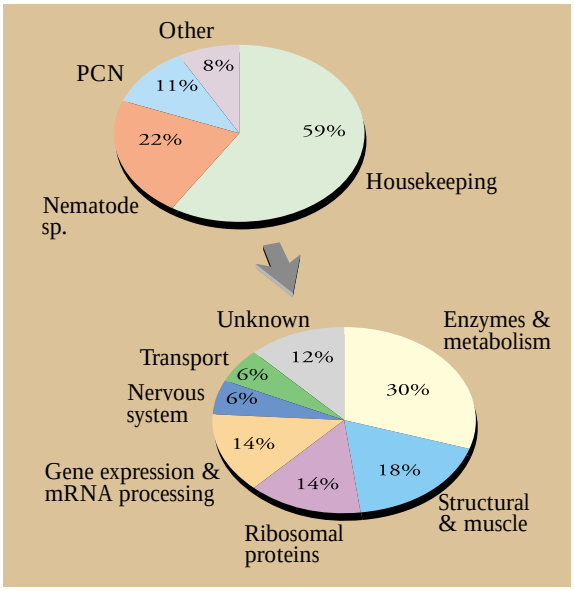

Figure 2 Classification of genes identified from the PCN EST dataset.

cDNA library (as explained in Fig. 1) and therefore likely to form a high proportion of the ESTs. For these reasons, we performed a small scale EST project using cDNA libraries made from J2s of *G. rostochiensis* and *G. pallida*. 1000 sequences were obtained in total. Having achieved our goal of identifying secreted proteins from these nematodes, our work is now aimed at identifying the most important genes and characterising the function of the proteins encoded by these genes. In this article, our work in this area is reviewed.

We first attempted to subdivide the ESTs into meaningful categories. Housekeeping genes were defined as those producing matches in the databases with genes from nematodes and other organisms. Fifty nine per cent of our ESTs fell into this category and were considered as likely to play a role in normal cell or whole body metabolism. Twenty two per cent of the ESTs only produced matches against genes from other nematodes and were thus classified as nematode specific genes. Eleven per cent of the ESTs gave no matches against any genes in any databases, or only produced matches against non-nematode genes (see below). These ESTs were classified as PCN specific genes. The remaining 8% of sequences (other genes) were sequences generated from bacterial or vector DNA fragments, or from sequence reads too short to use for meaningful database searching and were not considered to be of further interest. These figures are summarised in Fig. 2.

The genes classified as housekeeping genes were analysed in more detail. Thirty per cent of the housekeeping genes were enzymes and other proteins involved in basic cellular metabolism, 18% were structural or muscle proteins, 14% were ribosomal proteins and another 14% were proteins involved in the control of gene expression such as transcription factors or splicing proteins. Approximately 6% of the ESTs encoded proteins involved in transport (such as membrane transporters, fatty acid binding proteins) and slightly fewer were categorised as being involved in the functioning of the nervous system. The remainder of the housekeeping genes did not fit easily into any of these categories or have not yet had a precise function defined, despite being present in a wide range of organisms. One of the stated aims of the EST project was to identify genes encoding secreted proteins. It was possible to identify a signal sequence[3] in the predicted amino acid sequences of approximately 10% of the ESTs sequenced. Homologues of proteins known to be secreted by animal parasites and by plant parasitic nematodes were identified.

Having isolated a variety of genes, the next challenge is to identify those likely to be important in the host parasite interaction and to try to assign function to these, and the other, genes. It is relatively simple to assign a function to some genes; particularly those classified as housekeeping genes. These genes often produce matches against genes whose function has been previously defined from another organism. However, there are examples of genes in this category which may be adapted for a role in the parasitic process and which may therefore require further analysis. One example of this type of gene is thioredoxin peroxidase. Thioredoxin peroxidase normally functions as an enzyme which metabolises hydroperoxides generated internally by an organism's metabolic processes. However, our previous work had identified the protein encoded by this gene as being present on the surface of PCN J2s after hatching (See Figure 1b). Further work showed that this protein specifically breaks down hydrogen peroxide. Recent work has demonstrated that hydrogen peroxide is produced by plants as part of the defence response against nematode attack[4]. It seems likely therefore that, as in animal parasitic nematodes, thioredoxin peroxidase is secreted from PCN so that it can protect the nematode from host defence responses.

Many of the genes in the EST dataset produce matches only against other nematode genes for which no functional information is available, or do not

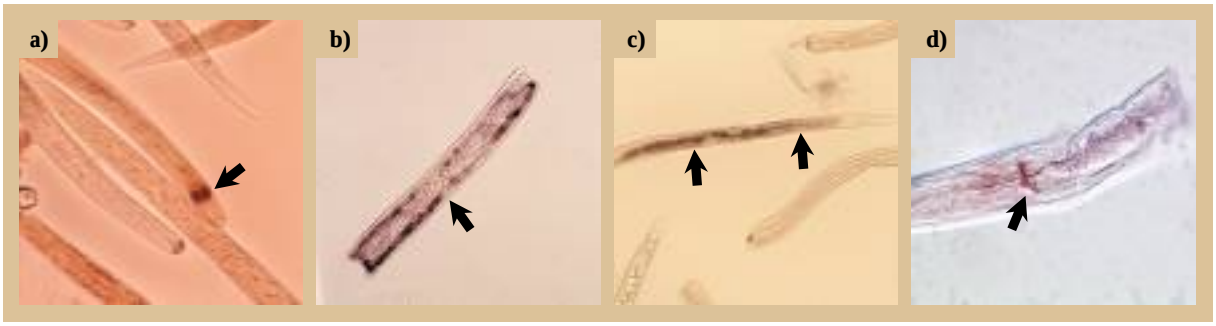

Figure 3 *In situ* hybridisation of DNA probes to *G. rostochiensis* J2s. A purple/brown deposit indicates a positive reaction (arrows). Genes expressed in the amphids (3a), the hypodermis (3b), the gut (3c) and the nervous system (3d) are present in the EST dataset.

match any genes in any of the databases. Naturally, predicting the function of these genes is a rather more difficult proposition. Computer predictions can, in some cases, be used to identify genes of potential interest on which it might be productive to focus further studies. An examination of the spatial expression patterns of a gene can often provide information about a potential function for the gene product. Performing such analysis on a relatively large scale is therefore one method of screening large numbers of sequences of unknown function in order to determine which may be worthy of further analysis. We are currently using this method of analysis to focus our studies on proteins encoded by genes in our PCN EST dataset that may be important in the host parasite interaction of PCN. First the ESTs are analysed to determine which could encode proteins with a predicted signal sequence. We then use *in situ* hybridisation to examine spatial expression patterns of the chosen genes. *In situ* hybridisation is performed by using non-radioactively labelled DNA probes that are amplified from the clones used to generate the original ESTs. J2s of PCN are fixed, cut into 3 to 4 pieces (to allow probe to enter) and incubated with labelled probe. Following extensive washing to remove unbound or non-specifically bound probe, the remaining probe, hybridised to its corresponding mRNA, is detected using a secondary antibody coupled to alkaline phosphatase. A colorimetric reaction allows the spatial distribution of the mRNA in the nematode to be visualised. Our studies are targeted at genes expressed in tissues from which they could be secreted from the nematode to the external environment, such as the oesophageal gland cells, hypodermis and amphidial sense organs. Using *in situ* hybridisation has enabled us to find genes encoding secreted proteins expressed in many of these tissues (Fig. 3).

Once genes of interest are identified, detailed functional analysis on the proteins they encode can be undertaken. Where a clear biochemical function is indicated (e.g. as for the thioredoxin peroxidase or other enzymes), assays can be performed with recombinant proteins. In many cases this is not possible and it may often be desirable to examine the function of a gene product *in vivo* rather than in a test tube. We are currently trying to develop systems to allow this sort of analysis in plant parasitic nematodes. In particular, we are attempting to develop methods of introducing double stranded RNA (dsRNA) into J2s of PCN. dsRNA has been shown to trigger sequence specific gene silencing in a wide range of organisms, including the extensively studied nematode *Caenorhabditis elegans*[5]. Considerable obstacles to using dsRNA inhibition (dsRNAi) in plant parasitic nematodes remain – in particular it is proving extremely difficult to introduce the dsRNA into the nematodes. If it is possible to overcome such problems, the potential offered by using dsRNAi in combination with the techniques described above is enormous. It will be feasible to characterise the functions of proteins with critical roles in the parasitic process of plant parasitic nematodes. As well as having scientific merit, this exercise will have practical benefits in allowing identification of important parasite proteins against which novel control methods can be targeted.

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Plants, soils and environment

Geoff Squire & Karl Ritz

The new structure of the Institute establishes the PSE Division as a major international research group specialising in the science of populations and element fluxes in arable fields. Its main practical aim is to understand and design multi-purpose arable ecosystems that satisfy requirements of food production, environmental safety and wildlife habitat.

The central theme of research is how the number and type of organisms, their interactions and their spatial and temporal arrangement, give rise to the desired arable systems. The new grouping combines expertise in a wide range of taxonomic and functional groups, notably the primary producers in the mandate crops and arable flora, the omnivores, herbivores and predators among nematodes, insects and mites, and the microbial communities of the soil. Scales of study range over six orders of magnitude from the fine soil pores, through the plant, patch, and field to regional dispersion. Organisms and scales are all examined by methods that seek convergence of approaches within the Division and Institute.

The science base in the Division feeds a portfolio of policy-led research, mainly in areas of environmental risk. From an independent stance, we advise government, industry and the public on GM crops, biodiversity, nitrate pollution in water and other matters of ecological and economic importance. The Division has continued the rapid expansion of externally funded work established by its component groups, recently winning contracts in soil-root interactions (BBSRC) and ecological risk (DETR). Our understanding of variability in populations and environment has been taken to the market place with computerised decision-support systems - notably the Management Advisory Package for Potatoes (MAPP) and (with nematologists) the Potato Cyst Nematode Model.

The aim of the Division in the next year is to weld the specialisms in its two Units – Soil Plant Dynamics and Vegetation Systems - through shared experiments and innovative mathematical and statistical tehniques. Collaboration will be increased with organisations whose remit includes land use and advisory work.

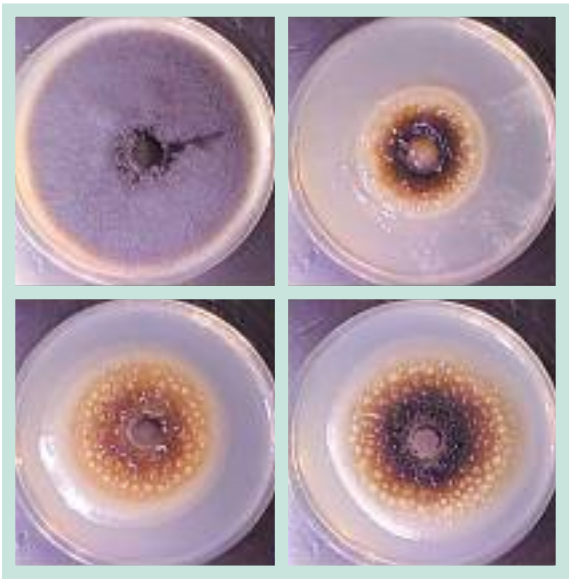
Unit of Soil Plant Dynamics

Soil is a difficult and intractable subject for scientific study at the fine scale, yet new experimental and theoretical approaches have enabled great progress in link-

ing biological, physical and chemical aspects of soils. We begin with some examples of fine-scale interactions, then show how the diversity of soil organisms affects the integrity and leakiness of soils at a wide range of scales.

Volatile-mediated microbial interactions Soil microbes produce volatile organic compounds (VOCs) that affect the way other microbes function. All of a random selection of over 100 soil bacteria produced VOCs that either stimulated, by up to 35%, or inhibited, by up to 60%, the growth rate of at least one fungal species. Four volatile compounds, 2-propanone, 2-methyl-1-butanol, heptanal and octanal were shown to be effective in the inhibition of basidiomycete growth at concentrations down to ppm levels. Further work showed that altering the amino acid content or concentration in the growth media resulted in significant changes in the VOC output. It might be feasible therefore to manipulate soil systems if the effect of VOCs on the interactions between organisms was better understood. The results are already finding a potential application in the biological control of fungi in order to prevent wood decay and the production of spoilage compounds such as sap-stains (with the University of Abertay Dundee and funding from the EU).

Spatial organisation of soil communities The spatial distribution of soil organisms and communities has a strong influence on how they function. At a microscale, organisms may be physically protected by residing in pores that are too small to allow access by larger predators, while an organism's position in the soil fabric relative to the pore network affects the movement of solutes and gases around it. Pioneering studies in the Unit are now enabling us to see soil microbial communities in their natural positions. The technique, described in an accompanying article, involves infiltration of soil cores, staining and thin sectioning to produce maps of biological material preserved within the solid phases and pore network of the original soil.



Effect of VOCs derived from different bacteria on growth and morphologyof the fungus *Sclerophoma pityophilia*. Top left plate is the control.

Communities are also being mapped at much larger size scales. For the first time in the British Isles, the distribution, shape and size of potato cyst nematode (PCN) foci in a previously designated seed potato field were quantified. Six years after the previous seed potato crop, foci of nematode populations were distributed in either a north/south or east/west direction, suggesting secondary spread had occurred due to cultivation in these directions. An individual focus measured approximately 30m in diameter. Future work will aim at confirming the above findings in other fields and devising optimum sampling strategies to detect early infestations of PCN in seed potato land. Similarly, the spatial distributions of both the New Zealand flatworm and earthworms within the same field have also been studied for the first time in the UK. Flatworms were confined to within 70-80 metres of the perimeter of the field where they had a statistically significant negative effect on the earthworm population. Future work will follow the progress of the infection in that field, and investigate the impact of temperature, soil moisture and availability of earthworms on the population dynamics of the flatworm.

Root caps fuel the rhizosphere Decaying roots and shoots provide the greatest input of carbon to soil, but the loss of substances from living root material is far from insignificant. The sloughing of border cells and exudation of mucilage from root tips make it easier for roots to penetrate compacted soils. Border cells can remain living in the rhizosphere for a week or more, and act as a source of carbon compounds to soil microorganisms. They might also act as 'decoys' for plant pathogens. We quantified the rate of sloughing of root cap cells in sand that was compacted to create mechanical impedance to root growth. This is the first time that border cell production has been quantified in a realistically abrasive medium. The number of root cap cells sloughed into sand increased twelve-fold as a result of compaction - from 60 to more than 700 per mm of root extension.

The whole of the cap surface area was covered with detached cells in compacted sand, compared with about 7% of the surface area in loose sand. This lubricating layer of border cells and mucilage still accounted for less than 10% of the total carbon deposited in the rhizosphere by living roots, the majority of carbon being contained in mucilage exudates elsewhere in the root system. The work was performed in a collaborative project between SCRI, IACR Long Ashton, and Nagoya University, Japan, and is continuing with the aid of a Royal Society Collaborative Research Grant and the addition of Tokyo University to the group.

Soil community structure and resilience The resilience of an ecosystem is a measure of whether and how fast it returns to its initial state after being disturbed. Measurements of the resilience of soil processes to persistent and transient stresses have confirmed the potential usefulness of resilience as an assay for 'soil health'. More specifically, a measure of resilience enables us examine how a microbial community's composition affects the processes that go on in soils. Comparisons were made of industrially polluted and non-polluted soils, and of intensively and organically managed agricultural soils. Standard biological indicators (protozoan populations, which are sensitive to environmental disturbance) and substrate utilisation kinetics (how well the soils use different substrates) could not distinguish the compromised soils. In contrast, resilience was a promising discriminator of the biological status of the soils. The accompanying article summarises recent developments. We are now examining the links between soil physical and biological resilience, the comparative resilience of soils to different stresses, and the effects of microbial diversity on resilience (NERC funding, in collaboration with the University of Aberdeen).

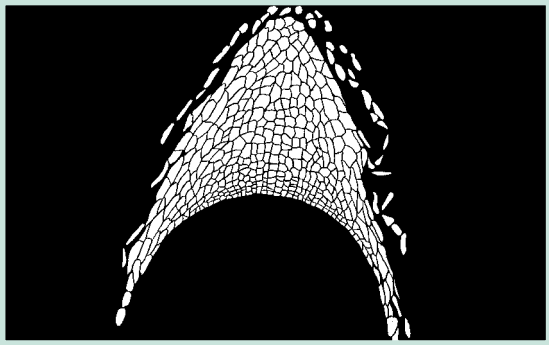
Soil structural integrity Arable soils need specific properties if they are to maintain their stability under repeated disturbance. Their fine structure should be undamaged when wetted, yet they should drain freely and not develop large cracks when dry. We are studying the biological processes that influence the ability of soils to buffer water absorption by their fine structure (water repellency) and so maintain cohesion under stress. As in other studies on soil, progress has been limited by being unable to measure at a fine enough scale. Now, a probe developed at SCRI is capable of measuring water repellency at the scale of a few millimeters. The measurement technique is based on soil transport properties that can be used directly to assess water flow in structured soil. The probe has shown that many British soils have low water repellency, and that the level of repellency diminishes if soil is disturbed and depleted of nutrients by intensive cultivation. A model study of soil structural genesis in the rhizosphere has shown that enhanced aggregation is a product of not only stronger interparticle bonds but also of higher levels of water repellency caused by root mucilage. Although cracking is one of the major processes in the structural genesis of soil, the physical conditions required for cracking to occur are poorly understood. We have adopted state-of-the-art approaches from fundamental fracture mechanics research to describe ductile crack growth in soil. This approach is now being used to assess how the biological properties of soil will affect cracking and hence soil structural genesis.

Thin section of soil showing fungi proliferating through a crack in soil.

Nitrogen cycling in arable soils Soil nitrogen is present as several different compounds, both inorganic (ammonium, nitrate and nitrite) and organic. Most chemical transformations in the N cycle are carried out by the action of soil organisms in a complex interdependent network of biological processes. A key compound in the N cycle is nitrate, which is important as a supply of N to plants and as a pollutant in ground and surface waters. Soil nitrate can derive from fertilizer, but large quantities are also generated by the oxidation of ammonium, a process carried out predominantly by autotrophic soil bacteria. An important uncertainty is the large spatial and temporal variation in the rate at which nitrate is produced. Possible explanations have included variation in the distribution of bacterial nitrifier populations, in the sources of NH_4^+-N or in the substrates that the bacteria live on. We have examined these possibilities using an experimental protocol - the augmented nitrification assay - devised at SCRI. Briefly, a number of different carbon and

nitrogen compounds were added to soils during short term incubations in the laboratory at concentrations ranging from those that prevail in root exudates (very low concentration) to those previously determined as optimal for heterotrophic respiration. In many instances, the addition of a C-containing compound at a low concentration, including pure amino acids, resulted in significant increases in potential nitrification, often more so than corresponding effects at high concentration. These concentrations suggest a mode of action operating at a 'signal' rather than 'substrate' level. The findings suggest that stages within the nitrogen cycle are not simply determined by gross amounts of microbes, sources of N or substrates, but may be more interdependent and interlinked by subtle interactions than previously thought. The study indicates the potential for manipulation of biogeochemical nitrogen transformations by changing cultural and management practices.

Further detailed knowledge of N-cycling events at fine temporal resolution on a field scale was obtained from use of the natural abundance levels of the stable isotopes of N ($\delta^{15}N$). Most chemical and biochemical transformations of N are acompanied by changes in the isotopic composition of both the substrate N and its product N. Work at SCRI aims to characterise the processes isotopically and use the resulting knowledge to model N transformations and pathways at a high temporal resolution in the field. The first step in achieving this has been to devise new chemical methods for isolating the target N pools from soil solutions, existing methods being inadequate for use with $\delta^{15}N$. We have developed a compound-specific technique that allows us to determine the $\delta^{15}N$ of nitrate in soil solution, and in eutrophic and marine waters. The new method has been used with success in arable soils for 2 years and is revealing much new information about the generation of nitrate in these soils and the periods in which the system purges itself of excess nitrate through gaseous loss of N. The results of this work have potentially profound implications for the efficient use of fertiliser and the sustainability of agriculture.



Border cells detaching from maize root cap.

Plant-soil interactions at the intra-specific level The basis for plant biodiversity within species resides in the functional life form and the genotype. The long-term goal of work in this area is to connect functional performance, through for instance a physiological indicator, to molecular information on a plant's genome. Progress was made with the realisation that the natural abundances of the stable isotopes of N ($\delta^{15}N$) and C ($\delta^{13}C$) could be used as indicators of plants' physiologies that discriminated their performances in nature. $\delta^{15}N$ and $\delta^{13}C$ are used alongside traditional plant physiological techniques, $\delta^{15}N$ pointing to variations in the plants' N relationships, and $\delta^{13}C$ to variations in the plants' C, water and N relations as well as indicating anatomical and structural differences. We can now assess and interpret variations in $\delta^{15}N$ and $\delta^{13}C$ against a growing body of knowledge, much of which was pioneered at SCRI. We have now shown that while $\delta^{13}C$ mainly reports on the conditions under which the plant gains and sequesters C, including the type of N nutrition, $\delta^{15}N$ mainly reports on the conditions under which plants lose N. Most of the N loss, and much of the C loss, is through the roots to the soil. As described above, such losses are a rich source of nutrition sustaining soil microbial communities. New work will continue to emphasise the functional diversity in the barley genome and investigate the chemical types and isotopic signatures of N lost from wild plant genotypes and lifeforms, collected from English chalk grasslands and investigated in collaboration with researchers at Sheffield University.

Moving up a scale: soil heterogeneity in relation to transport processes and root uptake The movement of pollutants to water is one of several issues that have to be considered on a scale much beyond the single field. Water quality is a topic of great public concern, and subject to increasingly strict environmental regulations. The concentrations of pollutants, including fertilizers, pesticides, and microbial organisms, in stream water are determined largely by farming practices and by the adsorption and desorption of chemicals as rain water percolates through the soil. Computer simulations are often used for predicting pollutant concentrations in stream water, but assume that soil has largely homogeneous transport properties – they ignore the fact that soil structure channels

much of the flow through small fractions of the soil pore space. Therefore, to gain a more realistic appreciation of this complex subject, SCRI, with MLURI and Aberdeen University, are studying the effect of soil structure on solute transport processes at scales ranging from the individual soil pore (1 μm or smaller) to that of a sub-catchment (1 km^2). The approach is to use detailed mathematical theory to generate hypotheses that can be tested experimentally at scales of the soil core, lysimeter, and field plot. So far, mechanistic models have been constructed of liquid flow and solute transport through complex three-dimensional fractal structures, which have transport properties that can be related to those of real soils, at the core scale. Input and test data are provided by experimental work at the project's contrasting test-sites at a low-input, upland sub-catchment near Aboyne, and an intensive lowland farm in the Ythan catchment. Geostatistical analyses of soil physical and chemical properties are used to identify the pattern of spatial variability at scales of 10 cm to 1 km. The advanced theoretical science in the project shows excellent promise for application to many problems, such as the relations between root uptake and soil structure, and the impact of soil structure on pollutant flow in soils.

Unit of Vegetation Systems

The Unit examines the links between plant process, the structure and composition of vegetation, and the invertebrates that feed off and live in it. Given the great range of potential animal and plant subjects, the work seeks generic methods that can be applied to the Institute's mandate vegetation systems and more widely. Its broader practical aim is to help devise more efficient, integrated strategies for managing weeds and insect pests, which impose by far the costliest burden on crop protection.

The relation between trait and community Agricultural plant communities are repeatedly challenged by new crop varieties, by new weeds and by a range of management interventions, all of which act through the physiological traits that link a plant with its environment. Our main aim is to understand the links between trait and community so as to answer questions of two general types: how does an introduced trait, in a new crop cultivar for example, influence the composition of the arable community; and what combinations of traits and individuals can coexist in communities constrained by climate or management? The central theme taken to study these links is the expression of physiological trait space within real physical space. Trait space is the many-dimensional, abstract space formed by the axes of botanical characters. While plants can be defined in this abstract space, they compete and otherwise interact through a physical, heterogeneous resource in a field. Progress has been made in defining trait space *ex situ* for both arable seedbanks and species-rich grassland (the latter with BioSS, MLURI and SAC in a SERAD FF project). The arable seedbank has been examined as sources of both weeds and floristic diversity, for which characteristics of germination and dormancy have received particular attention, since they determine seasonal growth cycles and longevity. A review of rate-temperature curves and secondary dormancy in the flora has been undertaken using data supplied mostly by external collaborators, the main finding being that germination in crops, weeds and wild species could be characterised by the same few parameters. Attention was then directed at germination and dormancy in feral oilseed rape, which is now an important member of the seedbank and a recipient of gene flow from new crop varieties. Seed lots and populations of oilseed rape were examined by statistical and molecular techniques that quantified the genetically based variability in germination.

Examination of the botanical trait space becomes exceedingly complicated as populations advance through their life cycle. As a way of understanding this, we have developed unique models that link plant traits and communities. The most important advance is a spatially explicit model based on real physiological processes rather than the simple decision rules that form the basis of most alternative approaches. Methods have been found to incorporate data on trait space and, from there, to show that wide-scale ecological metrics such as the species-abundance relation might exist on a small scale within populations. This conceptual framework is a particularly important achievement, since it enables us to transfer ideas between populations and ecosystems, to set up hypotheses that can be tested quantitatively in the field and ultimately, to incorporate further trophic layers. The developments are described in one of the accompanying articles.

Tri-trophic systems Biotechnology has the potential to provide new forms of pest control. It is possible to modify plants to express certain antifeedants which will either retard insect pest development or have a more harmful effect on the pest. Higher yield and the use of fewer chemicals might result. There are, however, potential side effects in that the pest might adapt to the toxin, and the toxin might be transmitted

through the food web to cause harm to insects that eat the pest. The potential for both side-effects is being examined by a mathematical approach to plant-pest-predator interactions (MAFF funding). A version of the Lotka-Volterra predator-prey model has been modified to explore the rate of adaptation of a herbivore to a transgenic crop, which has insect resistance determined by an allele at a single locus. The model acounts for the different palatability of the plant genotypes and for factors in the pest's life cycle. The model has been extended to consider the primary producer, herbivore and predator as three layers that interact through the transmission of food and toxin. The rate at which pests and their predators grow and move out from foci depends on the nutritional supply and architectural traits of the vegetation, while analysis of the physiological trade-offs between production and defence in plants provides potentially valuable guidance for plant improvement and habitat creation. The model will henceforth be fully integrated with the Unit's experimental programme, which has now tested prototype experimental systems for barley, oilseed rape and raspberry crops containing weeds and feral plants.

Ecological risk and benefit of GM technology Many of the Unit's skills have been brought to bear on the issue of GM risk assessment. The basis for GM technology is the introduction of a gene into a plant. The gene itself is not going to have any direct consequences on the environment. Instead it is the gene product, in most cases a protein, which is responsible for the direct physical effects of a gene or its phenotype. It is therefore important to understand the potential interactions of the gene products which are being considered for use in GM technology. In 1999, we were involved in a study to determine if the prospective anti-insect, toxin protein from the snow drop (GNA lectin) could interact with human white blood cells. The findings did indeed confirm that a number of human white blood cell glycoproteins (sugars linked to proteins) reacted with the toxin. It is highly likely that these interactions involve surface receptors, which are also part of the process of cell division. However, this research has ended and it has not been possible to identify these human receptors. These binding properties are consistent with the known activities of many other naturally occurring lectins in plants such as peas, beans, onions and leeks. The full consequences of these lectin molecules in diets is still not understood and they are likely to depend on many factors including digestibility, uptake rates and the surface molecules with which they finally interact. The snow drop toxin is not part of any food plant and more independent research is required before it could ever be considered.

Impact of GM technology on farmland biodiversity Research on gene flow and plant demography in arable systems has continued on several fronts. In particular, progress has been made on factors that determine life cycle biology in feral oilseed rape and other weeds. A synthesis of seedbank records showed that feral brassicas (probably oilseed rape) decayed rapidly for a year or two after seed deposition, but thereafter existed at frequencies typically several hundred per square metre of soil down to plough depth. These are small frequencies compared to the seedbanks of many weeds, which can number in the 10,000s per square metre, but confirm persistence over several years. We then developed a life cycle model of feral oilseed rape that is based on physiological responses and driven by events in the arable calendar, such as cultivation and application of herbicide (MAFF funding). Given that many of the input variables are naturally stochastic, the model will be used to generate probability distributions of certain outcomes, such as specified percentages of mixing in seed or seedlings. The work is collaborative with CSL, York, who will help to implement its use in a range of experiments (e.g. the BRIGHT trials) and industrial applications.

The government's Farm Scale Evaluations of GM Crops, carried out jointly with the Centre for Ecology and Hydrology (CEH) and the Institute for Arable Crops Research (IACR), entered the first year of the scale-up phase (funding from DETR/MAFF/SERAD). The experimental protocols devised in the previous year were applied at farm sites to compare the effects of GM and conventional technology on plant and invertebrate diversity. Each partner has responsibility for one of the crops, certain experimental protocols and for measurements at farm sites near to the respective organisation. The work is tightly co-ordinated, the partner organisations having effectively combined their resources to tackle this major issue at a national scale. Nevertheless, these experiments are highly contentious and several experimental sites were damaged by people and groups opposed to them. The work has brought staff at SCRI into direct and regular contact with farmers, elected members of government, the industry and a range of other interests. The results of the evaluations will be complete in 2002 to 2003.

Visualising the spatial organisation of soil microbial communities

K. Ritz, D. Crabb, K. Harris, N. Nunan, K. Wu, J.W. Crawford[1] & I.M. Young[1]

A very wide range of organisms inhabit soil. Larger creatures, such as rabbits, moles, earthworms, slugs and snails are familiar, but there is also a vast range of microbes and micro- and meso- fauna. A teaspoon of soil from under a pasture will typically contain 10^9 bacteria, several hundred metres of fungal hyphae, thousands of protozoa, hundreds of nematodes, dozens of mites, many other insects and spiders, and several metres of plant roots. The biodiversity belowground equals or exceeds that of a tropical rain forest canopy, and, just as there are many unrecorded species in the rainforest, we know very little in detail about the true range of species in the soil.

Soils function as a consequence of the myriad of interactions between these different organisms, but such interactions are strongly affected by the underlying physical structure of the soil. The architecture of the soil is fundamentally a labyrinth, comprising a complex network of blind and connected pores at spatial scales that span orders of magnitude, typically from micrometres to millimetres. It is these pore networks that govern the rate and limits of movement of gases, liquids, solutes, particles and organisms, and form the structural habitat for all life in the soil.

Techniques have been developed at SCRI that enable the visualisation of soil organisms in an intact state, and in their natural positions, in this complex soil matrix. The aim of these methods is to enable the production of 'underground maps' that allow the spatial distribution of microbes, pore networks and solid phases of the soil to be studied. The resultant maps can then be used to inform modelling frameworks that will allow the consequences of such spatial organisation to be understood. The approach is essentially a form of 'soil histology' akin to the sectioning and microscopic observation of plant and animal tissues. However, there are significant difficulties associated with producing thin sections of mineral soils in which biological material is to be preserved and observed. Soils are fragile and will collapse readily when disturbed; they contain extremely hard mineral components such as sand grains which preclude the simple cutting of sections. In contrast, most microbes are very soft – for example many protozoa are little more than naked masses of cytoplasm bound by a thin membrane – and sectioning such material in the proximity of quartz grains is technically demanding. Microbes are very small by definition and so it is difficult to visualise them in the context of much larger features such as pore networks or biological features such as roots. As discussed above, they are also very diverse in terms of the number of different forms and the size scales they encompass. Few microbes have any inherent contrast that would enable them to be visualised against the highly complex background of the

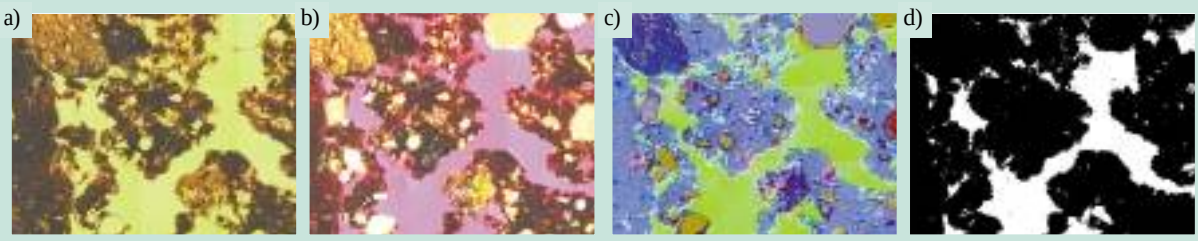


Figure 1 Segmentation of transmission pore networks from thin sections of undisturbed soil. Image width c.3 mm.
(a) Bright field transmitted illumination. The light penetrates both the pores in the soil (now full of resin) and sand grains and other clear mineral components, appearing yellow here. The brown material is organic matter and other minerals appear as various hues.
(b) Same image viewed with polarised light. Pores now appear pink and mineral grains various hues.
(c) False-colour image generated by computer-assisted image processing to extend the range of hues to differentiate the different components of the soil.
(d) Binary map of the pore network in the thin section.

[1] University of Abertay, Dundee.

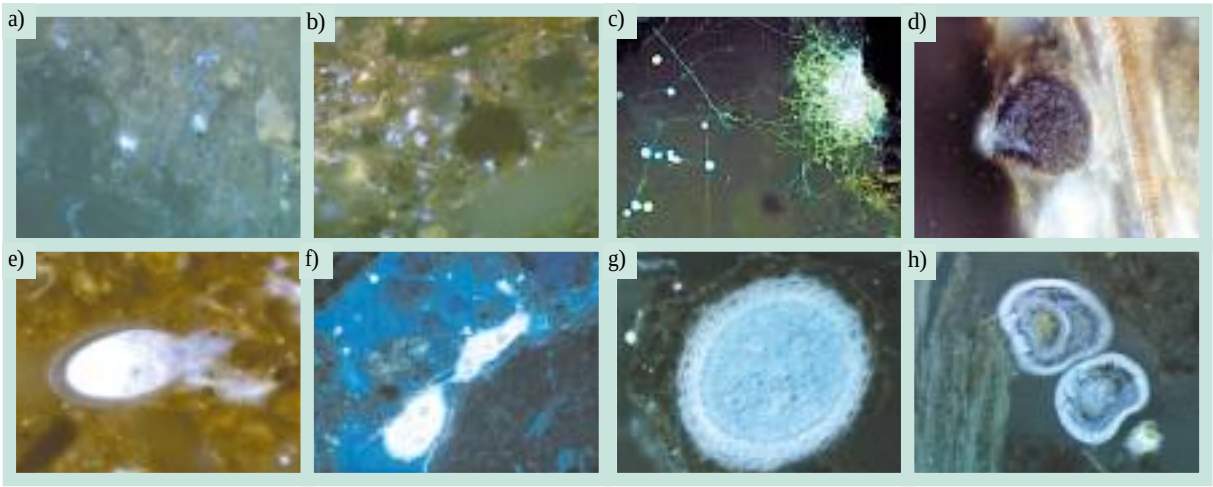
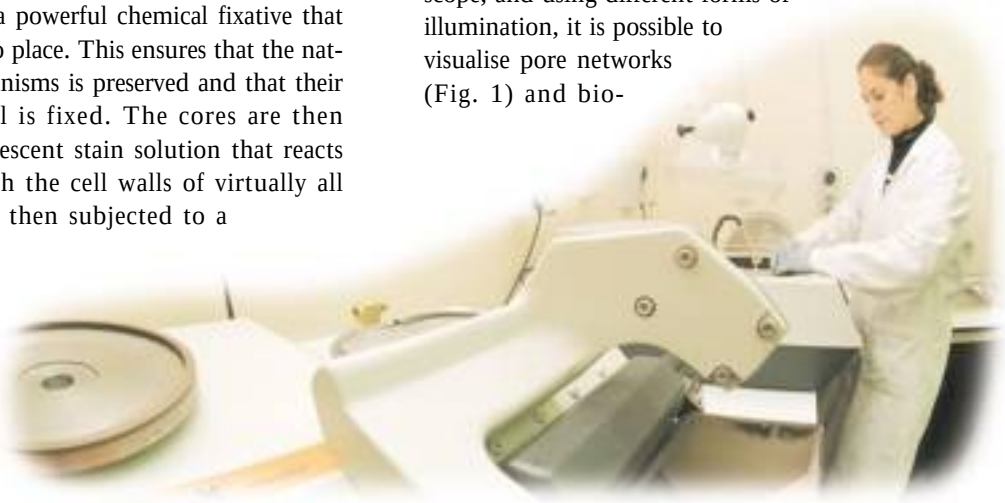


Figure 2 *In situ* visualisation of soil organisms in undisturbed soils as revealed by the biological thin-section technique.
(a) Bacteria (fluorescing blue under UV epifluorescence illumination) in a bulk soil sample from an arable field. Image width c.120µm.
(b) Bacteria in soil from an earthworm cast. Note considerably greater biological activity compared to (a). Image width c.120µm.
(c) Fungal hyphal network growing in pore space. Fluorescent spheres are spore clusters. Image width c.500µm.
(d) Fungal perithecium in decomposing root. Note root vascular tissue on right of image; the perithecium is embedded in the cortex, and the orifice through which spores are ejected opens into a soil pore on the left of the image. Image width c.120µm.
(e) Testate amoeba. Note the cytoplasm extruded from the silicaceous test; this indicates the organism was mobile and actively foraging for bacterial prey before being preserved by the biological thin-section procedure. Image width c.80µm
(f) Naked amoeba penetrating narrow neck between two adjacent pores. Note this is essentially a 2-D thin section, and above the plane of section the pore neck may widen; the organism is a 3-D mass of cytoplasm. Image width c.60µm
(g) Transverse section of root. Note the vascular cylinder is intact and the majority of cortex has disappeared, and that the root is surrounded by a closely-associated soil matrix. Image width c.500µm
(h) Transverse section of enchytreid worm. There are two sections due to the worm looping through the 2-D plane of the section. Note the soil deposited in the lumen of the gut in the upper section. Image width c.200µm.

soil matrix by conventional microscopy. Finally, any resultant images must be amenable to quantification, and to ensure such measurements are representative, a high degree of replication is necessary. The production of such maps therefore requires automated image processing, analysis and quantification procedures.

The techniques devised achieve most of these aims. The production sequence involves removing undisturbed cores of soil from their natural position and infusing them with a powerful chemical fixative that locks all proteins into place. This ensures that the natural state of the organisms is preserved and that their position in the soil is fixed. The cores are then immersed in a fluorescent stain solution that reacts non-specifically with the cell walls of virtually all microbes. They are then subjected to a controlled chemical dehydration in solvent until all water has been removed, followed by impregnation with a low-viscosity polyester resin which subsequently polymerises to produce a solid block of resin-embedded soil. Thick slices (about 5 mm) are cut from these blocks using diamond-coated saws, and adhered to glass microscope slides; thin sections are then produced by a protracted series of cutting and grinding steps with lubricated diamond pastes until the slices are approximately 25 µm thin. The sections are then viewed with a compound microscope, and using different forms of illumination, it is possible to visualise pore networks (Fig. 1) and bio-



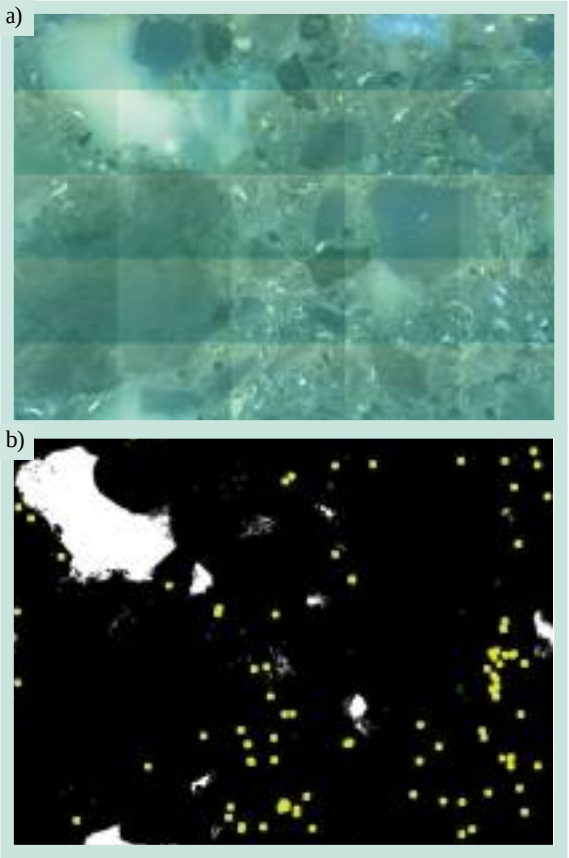


Figure 3 Large-scale imaging of the *in situ* distribution of bacterial cells in undisturbed fallow soil. Image width ≈ 600 µm
(a) Tessellated montage image (total 25 fields-of-view) under UV epifluorescence illumination. Digital file size is 32 Mb. Individual cells are not visible by eye due to their small size.
(b) Overlay map of locations of bacterial cells and transmission pore network produced by automated image processing techniques.

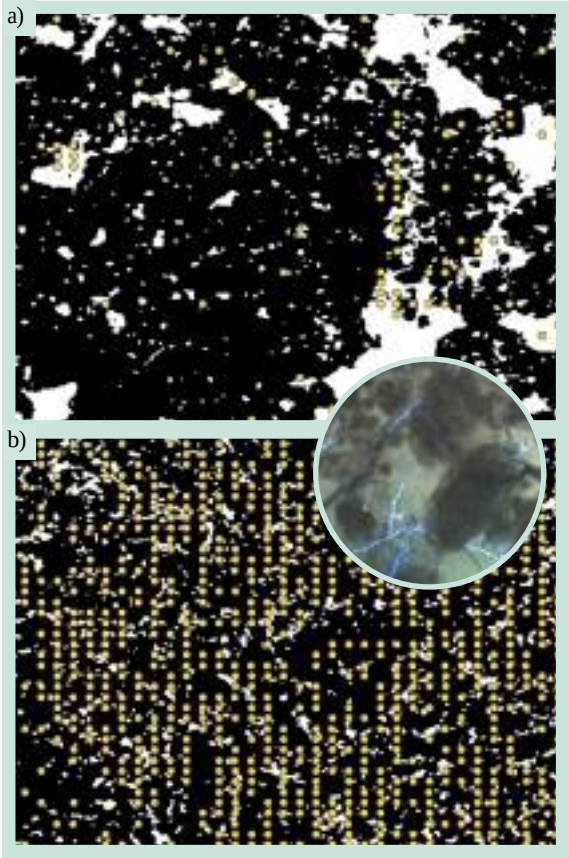


Figure 4 *In situ* spatial distribution of *Rhizoctonia solani* growing in soil at different bulk densities but at the same air-filled porosity of 17 %. Maps produced from analysis of 1400 individual and contiguous images for each section. Inset shows example image where fungus is present. Image width = 25mm.

logical material (Fig. 2). The degree of preservation of biological material is very high, as demonstrated by the intact nature of such delicate structures as fungal mycelia and naked amoebae.

The majority of soil bacteria are approximately 1 µm in diameter . In order to visualise them, high-power microscope objectives are needed which provide a concomitantly small field-of view, of the order of 0.01 mm^2. To increase the scale of visualisation, a computer-controlled microscope stage is used to capture a series of precisely aligned adjacent fields-of-view which are then tessellated to form a contiguous image. The limit to the total area which can be visualised is set by available computer memory; an example of such a tessellated image is shown in Fig. 3a. Although individual bacterial cells in such an image cannot be resolved by eye, the digital information denoting them is present. By use of sophisticated image processing procedures, which involve some 23 individual steps applied to the red green and blue channels of the digital images, the bacterial cells are automatically segmented and their positions mapped (Fig. 3b). By combining such information with the pore images, we have produced the first ever maps of *in situ* bacterial distribution at these sorts of spatial scales. These maps of a fallow soil, produced for a DTI LINK project with Aventis plc, reveal that a very small proportion of the soil volume contains bacterial cells, even though the numbers of cells per gram are huge, and that in such fallow soils a relatively small proportion of bacterial colonies consist of more than a few cells. These maps make it strikingly clear that if, for example, a pesticide were being transported in a heterogeneous manner through such a spatial pattern of cells (which

is almost certain due to the tortuosity of the pore networks), the extent to which the degradation rate may be reduced by the lack of contact of the compound with potential degrader bacteria.

The first large-scale spatial distribution maps of soil fungi have also been produced using these techniques. Fungi can be visualised at lower magnification than bacteria, hence much larger areas of section can be simultaneously visualised using the tessellation procedure, in this case the entire area of 25 x 23 mm sections. In an experiment in collaboration with the University of Cambridge, the effect of soil bulk-density upon the growth of the plant pathogenic fungus *Rhizoctonia solani* was studied. It was shown that the biomass and spatial distribution of fungus were strongly reduced in low bulk density soil compared to higher densities (Fig. 4). There was relatively little proliferation of mycelia in the larger soil pores that prevailed in the low bulk density systems. These spatial patterns of mycelia have important implications for whether the fungus may locate a host root and thus initiate a point of primary infection.

Thin sections provide an essentially 2-dimensional slice taken from a 3-dimensional volume of soil. Extrapolating from 2-D to 3-D can only be achieved practically by mathematical modelling, and this represents the next key step in this unique insight into the organisation of soils that these techniques are enabling.

Characterisation and consequences of soil microbial biodiversity

B. Griffiths, K. Ritz, R. Wheatley, S. Caul & C. Clegg

What is meant by biodiversity? The single most important factor generating reduction in biodiversity is human land use, and agriculture is the most important factor affecting Europe's landscape and biodiversity. Despite this, it is still unclear how biodiversity affects ecosystem functioning, and how ecosystems supporting different levels of biodiversity respond to extreme environmental perturbation. The main conclusion of a recent European Working Group on Research and Biodiversity Report was the urgent need for research to investigate the role of biodiversity in soil processes, or, in other words, to go beyond merely *describing* biodiversity in all its forms and to understand its *consequences*.

Classic concepts of biodiversity tend to revolve around the fundamental taxonomic unit of species. However, operational definitions of biodiversity are not as straightforward as the mass-media would wish. In essence, biodiversity is a concept that aims to rationalise a complex set of factors that encompass the basic genetic, taxonomic, trophic and functional components of communities and their spatio-temporal dynamics, at a variety of scales. The concept also needs to include the number of different biological forms, entities or units from each of these perspectives, their relative abundance and the degree of interconnectedness between them. Food-web diagrams (Fig. 1) illustrate many of these points, and show that life in soils is exceedingly diverse, especially considering that each box encompasses entire organismal groups. If we consider within-group diversity, then things get really interesting. A handful (100g) of forest soil can harbour up to 4,000 bacterial species (genetically distinct units); a sandy agricultural soil can contain over 350 distinct bacterial species, which is of the same order as the total number of plant species in the UK.

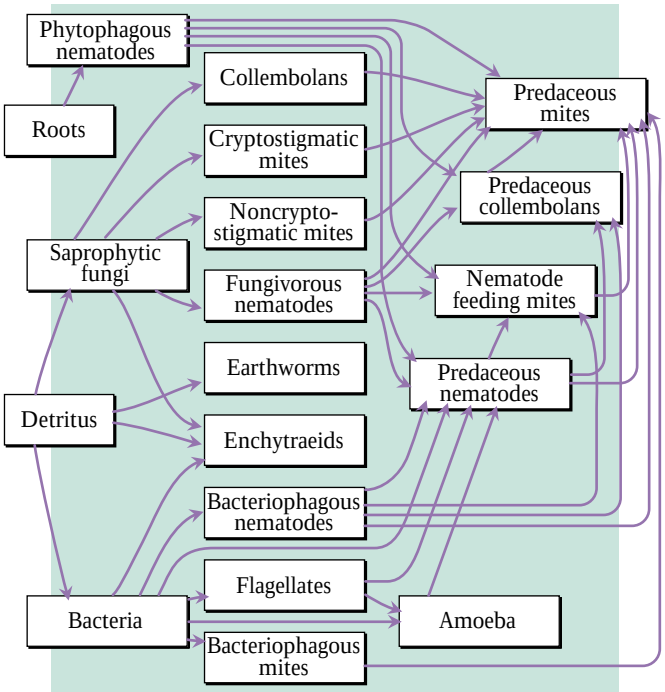


Figure 1 A typical food web from an agricultural soil showing the relationship between the major classes of soil organisms. Organisms in the same trophic group appear in the same column, progressing from the primary substrate supply on the left to the top predator on the right. Figure taken from de Ruiter *et al.* [2].

We need to be aware that biodiversity is entirely a human concept invented to describe complex biological systems. Ecosystems function through the interaction between individual species and communities, and there is no intrinsic mechanistic relationship between biodiversity and ecosystem function.

Characterisation of soil microbial biodiversity Traditional measures of biodiversity rely on species identification, the counting of individuals and knowledge of the ecological role of each of the species. When considering microbial communities, these parameters simply cannot be determined. There are no methods currently available, or likely to be available in the foreseeable future, that can determine the identity, frequency and evenness of all microbial species present. Rather, microbial ecologists are limited by the data that can be obtained and have to devise experimental approaches to overcome the technical shortcomings. The non-cultivability of the majority of microbial species present in soil is now well established, and the analysis of environmental, or whole-community, DNA is being used to overcome this. Environmental DNA can be characterised in a number of ways. For example, its complexity can be measured using reasso-

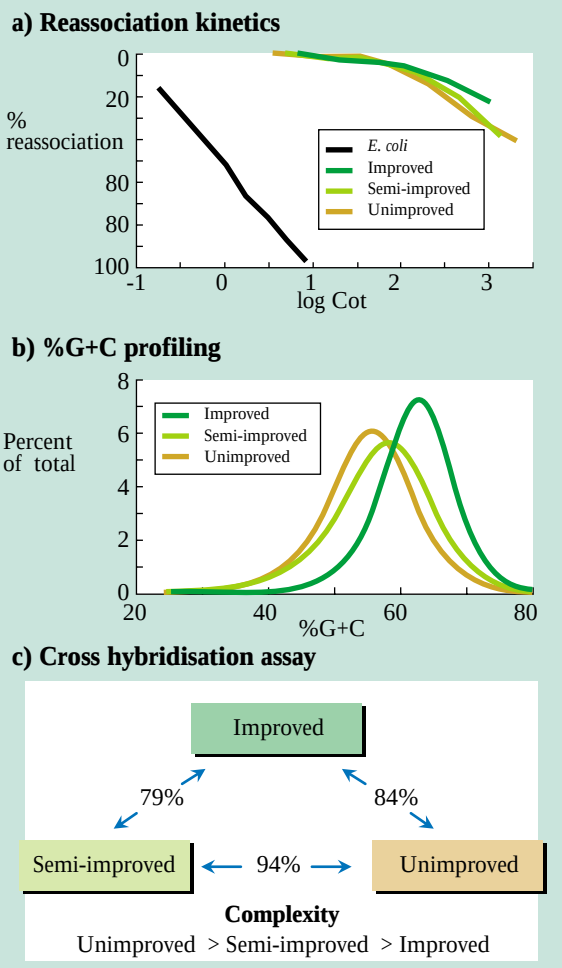


Figure 2 Broad-scale analysis of soil community DNA extracted from upland grasslands receiving different degrees of management. (a) reassociation curves indicating relative complexity of soil communities – the black line shows the rate associated with one bacterial species; note the x-axis is a log scale. (b) %G+C profiles indicating the community composition on the basis of guanine and cytosine bases in the whole soil DNA. (c) cross-hybridisation assay, allowing quantification of degree of similarity between the community DNA, plus another measure of relative complexity. Data obtained within SERAD MICRONET Programme.

ciation kinetics assays, while cross-hybridisation and % guanine+cytosine profiles allow the comparison of DNA from samples to determine how much of the DNA is in common. Some examples of the application of these broad-scale assays to characterise the impact of management systems upon microbial communities in upland grasslands are given in Figure 2. Communities comprised of completely different species could be equally diverse but, as ecological function is more related to species composition than it is to diversity, such characterisation is important.

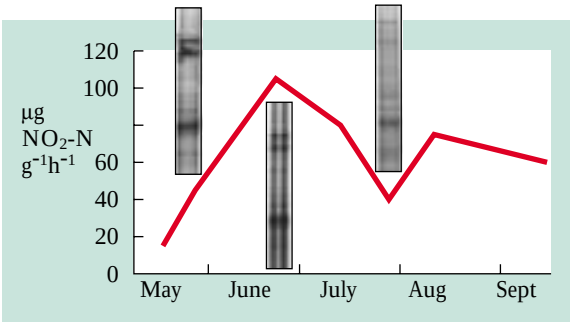


Figure 3 The time-course of potential nitrification through the spring and summer in soil from a crop of field beans at SCRI. The insets show profiles of the microbial community, analysed by DGGE, taken at times of high and low activity.

Polymerase chain reaction (PCR) based techniques for analysing soil DNA allow for a higher-resolution analysis of community structure. PCR product analysis by degrading gel gradient electrophoresis (DGGE) can be used to assess commonality in microbial community structure between samples. Currently, the usefulness of this approach is limited by the number of DNA bands and complexity of the banding patterns obtained. However, the banding patterns obtained from an arable soil throughout the growing season suggest that this is an effective approach for studying soil microbial biodiversity. For example, changes in microbial community structure related to temporal variations in soil processes such as nitrification can be studied (Fig. 3). Here, primers were utilised such that the gel profiles represent the entire bacterial community, but primer design is progressing at a rapid pace and it is now possible to generate profiles representative of specific components of the community (such as actinomycetes, archaebacteria, pseudomonads and fungi). Through the use of RNA technology, it is also

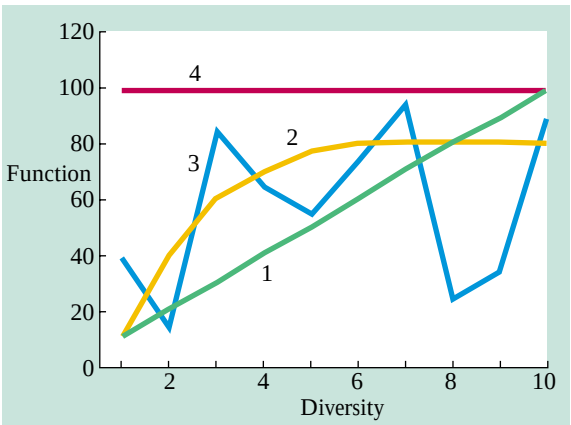


Figure 4 Graphs depicting the theoretical relationships between biodiversity and ecosystem function.

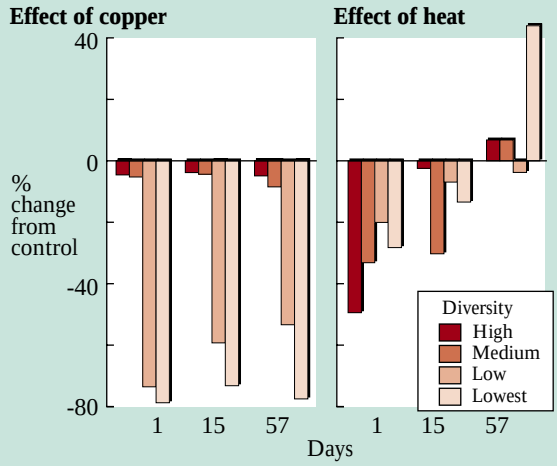


Figure 5 The resilience of fumigated soil differing in biodiversity to the applied stresses of copper or heat. Stress was applied at day 0, and the reduction in decomposition (relative to an unstressed control) determined subsequently. The least diverse soils were very susceptible to copper and decomposition did not recover, while after heat, decomposition recovered fastest in the most diverse soils.

possible to determine which of these components are active in any particular sample.

Theoretical consequences of changes in biodiversity for the functioning of ecosystems Various theories exist of how the functioning of ecosystems could vary as biodiversity changes (Fig. 4). Each species may fulfil a certain role in the environment and so as species are lost, function diminishes (i.e. Curve 1 below). Given the enormous biodiversity of soil microbes, there is undoubtedly some redundancy in function, so that more than one species can fulfil each task and that species can be lost, possibly until some critical threshold value of biodiversity is reached (Curve 2). This is more realistic. However, given the complexity of interactions in soil, the removal of species is likely to affect how other species behave in an entirely unpredictable fashion (e.g. Curve 3). This is the most likely scenario. It is also theoretically possible that loss of species will have no effect on the functioning of the system (Curve 4).

Given that the most likely outcome is Curve 3, it is unlikely that any theoretical framework could be constructed to directly link biodiversity with specific ecosystem functions. It is, however, much more likely that the resilience of the soil microbial community (resilience being its ability to withstand or recover from perturbation) is directly linked to biodiversity.

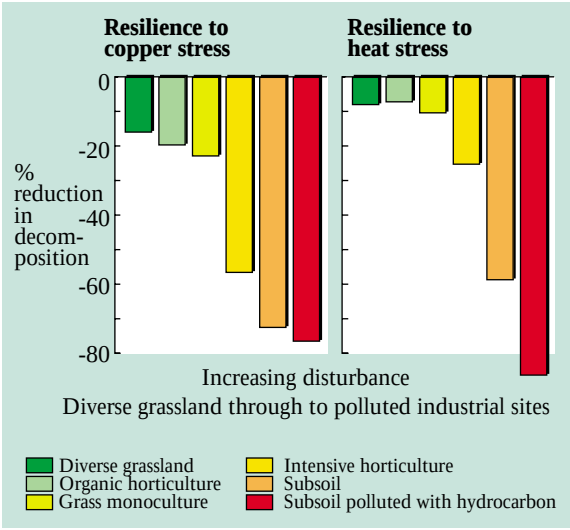


Figure 6 The resilience of soils exposed to increasing degrees of environmental stress to applied copper or heat.

Recent experimental diversity:function studies at SCRI

1. Destructive reduction in biodiversity We were one of the first research groups to use progressive fumigation with chloroform to reduce soil biodiversity experimentally, and to determine the consequences of this for soil processes[1]. Although fumigation reduced soil biodiversity by up to 40%, there were no consistent effects on soil functions. Activities like decomposition, respiration, growth and denitrification were increased in the least diverse soils; while others such as nitrification and methane oxidation were decreased. The most significant finding was that the most diverse soils were more resilient to environmental stresses, in this case experimentally applied copper or heat treatments. This experimental evidence supports the theoretical conclusions given above (Fig. 5).

2. Constructive increases in biodiversity There was some evidence from the chloroform experiment described above that the communities resulting from fumigation were physiologically different from those in unfumigated soil. They might have been selected by the treatment. To overcome this, in further experiments sterile soil was inoculated with microbial communities, differing in biodiversity, that had been extracted from soil. Again there were no consistent effects of biodiversity on soil functions, but also no consistent effects on resilience. It seems likely now that the measure of resilience is an indicator of microbial community stress, such that communities which have been stressed are less able to withstand subsequent stresses. Thus, the communities inoculated into

sterile soil had all been stressed to the same extent and so had equal resilience. In the fumigated soils, although the communities were reduced in biodiversity, they had also been stressed by the fumigation and so differed in resilience.

3. Response of soils naturally differing in biodiversity The functioning and resilience of microbial communities exposed to different degrees of environmental stress, and therefore expected to differ in biodiversity, were also tested. Thus, soils with different aboveground biodiversity (i.e. monoculture *vs* a diverse sward), soils with and without hydrocarbon pollution, and with intensive or organic management, were examined. Protozoan populations, which have been proposed as environmental indicators because of their sensitivity to environmental conditions, were effective at differentiating soils from the same site, but showed little relation to biodiversity between the different soils. The functioning of the soils, measured as the ability to decompose substrates, was not related to biodiversity. In particular, the polluted soil, with a particularly low biodiversity, was more able to decompose a range of substrates than the unpolluted soil. Resilience, on the other hand, was a good measure of previous environmental stress and, therefore, correlated with biodiversity. Given the results of the constructive experiment described above, it is likely that resilience is affected by the previous stresses that the microbial community has been exposed to rather than the biodiversity of that community (Fig. 6).

Conclusions Changes in soil microbial biodiversity *per se* do not impact on the provision of ecosystem services by soil systems. Biodiversity can be altered by many factors, and it is the effects of those factors which are important in determining the outcome for soil processes. The measurement of resilience has been seen to provide useful information about the soil microbial community, and will be studied in more detail in future work. The characterisation of microbial communities from their DNA, and more particularly in terms of RNA to access the active components, is important in understanding what the key components are, how they behave and how this will be affected by the effects of future land use and management changes.

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The individual basis of plant diversity

G.R. Squire, J. Bown[1], J.W. Crawford[1], B. Marshall, G.W. Wright, E. Pachepsky & D.L. Trudgill

The concepts discussed in the preceding article on microbial diversity apply equally to plants, but the advantage of plants as experimental subjects is they can often be defined as individuals, either in their natural locations or as accessions removed and characterised. The same problems with the species as the unit of diversity remain, however[1], and limit our understanding of diversity and function in variable populations. Our approach to this is to treat the individual as the basis of diversity.

Plants are examined in terms of trait 'space' comprising many axes that define an individual's phenotype. At any time, an individual occupies a point in the space, and during its life cycle moves along a unique trajectory through the space. Groups of plants or populations form a collection of trajectories through time, which meander or contort as the individuals interact and are buffeted by pests and environment. The population is therefore an intermeshing net of trajectories. The trajectories have potential to exchange information at reproduction. The biological diversity at any time is described as the distribution of individuals across trait space.

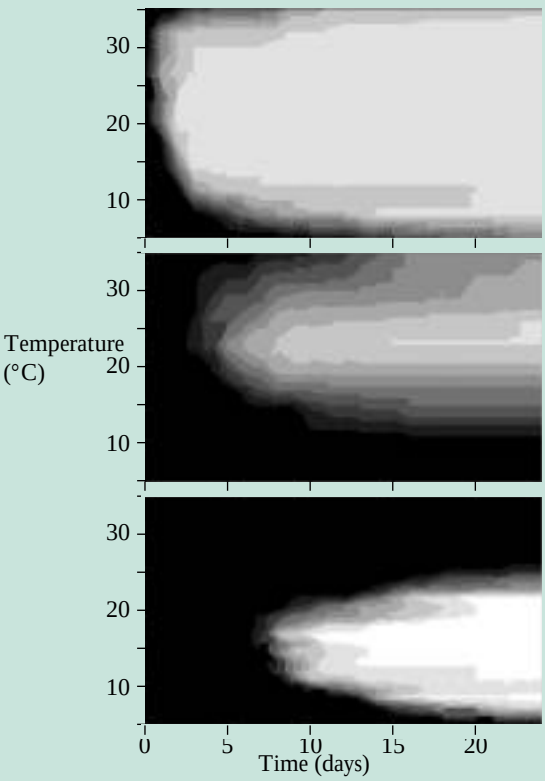

Figure 1 Time-temperature trait maps for germination of *Anisantha sterilis* (upper), *Brachypodium pinnatum* (middle) and *Saxifraga tridactilytes* (lower). From re-analysis of data supplied by the Unit of Comparative Plant Ecology, Sheffield.

The simple trait space of germination The concepts can be illustrated for the primary step of germination, which is commonly governed by conservative relations between time and temperature. Data collected and supplied by the Unit of Comparative Plant Ecology, Sheffield were used to construct a space of time, temperature and % germination for a range of species. In a preliminary survey[2], the weeds and other wild plants examined displayed rate-temperature curves similar to those found many times previously with crops. In principle therefore, a wide range of cultivated and wild species could be examined by a similar analysis. A deeper re-analysis of the data revealed time-temperature trait maps (Fig. 1) that are highly characteristic of a species or population. The contrasting *Saxifraga tridactylites* and *Brachypodium pinnatum* represent extremes in the flora, each differing in the time to first germination, the amount of non-germination indicated by the grey shelves in the maps, the temperature at which seeds do and do not germinate, and many other features, especially the spread of trait values within the population at any temperature or time. In contrast, the grass weed *Anisantha sterilis* (= *Bromus sterilis*) displays a very wide map, germinating near to 100% over much of the ranges of temperature and time.

The grey shelves are particularly important for population dynamics over long time scales, since they generally indicate regions of trait space where seeds do not germinate, but are generally alive in a condition of induced dormancy. This 'drop-out' from the actively developing population, as conditions move away from the optimum, is an indicative feature of populations. The drop-out phenomenon occurs in later developmental stages also, and has important implications for how we examine a net of trajectories.

We chose to examine drop-out in the common weed, feral oilseed rape, since it gives rise to overwintering

[1]University of Abertay Dundee (J. W. Crawford from February 2000)

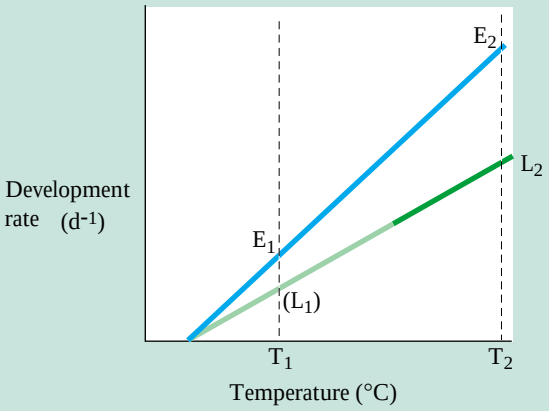

Figure 2 Representation of rate-temperature analysis of developmental stage, to illustrate the drop-out conundrum. Four phenotypes are indicated for early (E) and later (L) developers at two temperatures (1 and 2) - E1, E2, L2 and (L1); (L1) is in parenthesis because it would only have existed at that location if it had developed, but it had dropped out at low temperature. The question is - do non-developers, in this instance (L1) at low temperature, occupy a specific ranking in the population (e.g. L2) at optimal temperature?

and persistence in the soil if the seeds experience sub-optimal temperature or reduced water potential during imbibition. The trait maps in oilseed rape were analysed and quantified by converting time to a rate (1/t) and fitting curves of 1/t on T for a range of percentiles[3]. We then posed the question as to whether seeds that dropped out of the germination map at sub-optimal temperature arise from any part of the map at optimal temperature. For instance, did slow seeds at optimal temperature drop out at low temperature (Fig. 2). This seemingly mundane question has great implications for population biology and for tracing trajectories in particular. Usually, ecophysiologists compare a mean trait value at one developmental stage with a mean trait value at a later stage, and assume that the collection of individuals nearest the mean or mode is the same at both stages. Clearly, if they are not, because some have dropped out, then we are not relating like to like. In this instance, both circumstantial and molecular evidence confirmed that slow individuals at optimal temperature were indeed more likely to drop out at sub-optimal temperature. Variation between varieties and other seed lots was also shown in the physiological and molecular parameters.

Structure independent of composition in plant communities? Such germination and dormancy traits have great importance for the arable seedbank community - the populations of buried seed that provide

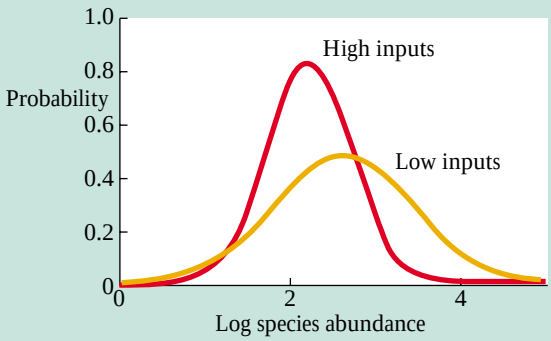

Figure 3 Shifts in species abundance curves in the arable seedbank in response to decreasing chemical inputs. Note abundance is on a log scale.

both a reservoir of diversity and future weed problems - which is one of the Unit's main foci. As already admitted, the balance of species is several steps removed from the detailed behaviour of individuals. Nevertheless, a frame is sought that enables us to move between and compare communities that consist of different species. Recent opportunities for synthesis in this area arose following completion of two major experiments on ADAS farms. The experiments examined the effect on a range of economic and biological indicators of less intense rotations that included more fallow and less use of herbicide. SCRI's role was to examine the seedbank. The dominants, and in fact most of the species, were different at the sites, but measures quantifying the communities displayed a consistent response to reduced inputs at the three sites. As inputs decreased, the mean and standard deviation of the species-abundance distribution both increased (Fig. 3). With abundance on a log scale, this meant that for each new species that increased above the detectable level, the common species produced many more individuals[4]. It was impossible in these circumstances to increase the number of rarer species without causing a potential major weed problem. The study generated ideas about the way production and arable diversity are to be balanced, i.e. there is an optimum point in the system that management should aim for, and is a constructive example of how SCRI's work on population biology can contribute to more applied, farm-scale experiments in the UK. The primary need for policy and management now is to put a price on rarity and on habitat for wildlife that can be directly compared with yield and profit.

Modelling trait space in physical space The trait map of germination provides the starting grid for the net of trajectories that describe later development and

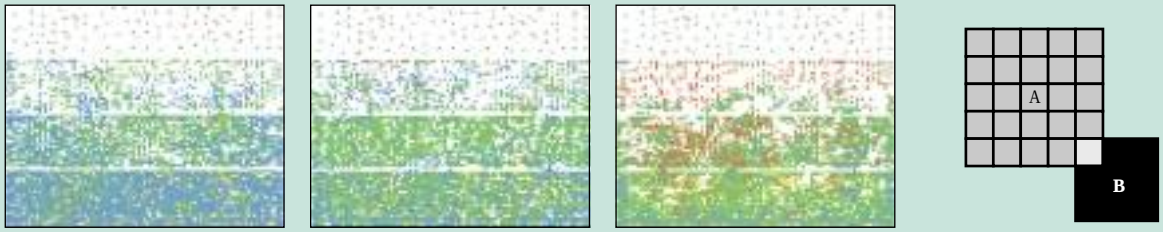

Figure 4 Spatial output of modelled plant types, showing patterns that give a clue to the arrangement of individuals and species in the field[5]. The resource is arranged in four bands, increasing in amount from top to bottom of each map. The three maps show the temporal change in proportion of four interacting plant types. Blue (rapid growth, early reproduction) gives way to Green then Red (slower growth, greater resource storage). Inset shows two individuals interacting through their resource capture area. The spatial patterns can be analysed to reveal 'species-abundance'and 'species-area' curves typical of much larger ecosystems.

that must ultimately, over several generations, give rise to the community-scale features of the seedbank and other vegetation. It is relatively easy to measure early development in a population and again the number and abundance of species. It is also relatively easy to measure the trajectories of individuals taken from systems and grown *ex situ*. The difficulty is that the trajectories of only a finite and quite small set of individuals can be measured so as to trace the links between the physiological trait and the community of species *in situ*. Some of the experimental designs in which these links are traced in annual populations have been described in previous reports. However, it is very difficult, if not impossible, to trace the links in perennial systems. Yet questions are repeatedly asked of these links: for instance, how does the introduction of a new trait, in say a new crop cultivar or weed, propagate through the populations to alter the productivity and diversity of the vegetation as a whole; and how does a restriction applied 'top-down' to the community, perhaps a change in micro-environment, agronomy or grazing intensity for instance, determine the types and numbers of individuals that can co-exist in that community? The challenge, then, is to show how a general collection of individuals defined by a set of traits form a community described by a set of species.

To probe this complexity, we have developed unique mathematical models of communities in which individual plant types are defined by physiological traits that govern the way they interact with their environment (Fig. 4). The traits have been carefully chosen, based on a wealth of wider research in plant ecophysiology and growth analysis in many environments. They describe the basic processes of uptake of resource, allocation of resource to plant parts, reproduction and dispersal. The environment is defined by a resource-base that itself can be assigned various characteristics such as holding capacity and replenishment rate. The 'plants' grow and interact with each other through the resource base. The basic model[5] was designed so that its traits could be parameterised by data from experiments on plant development and growth. This further, major step forward was achieved using data from physiological work carried out at MLURI on nitrogen cycling and carbon accumulation in the grassland species *Rumex acetosa.* This co-operation is an example of the melding of physiology and modelling through the Co-ordinated Programme in Vegetation Dynamics (a SERAD-funded initiative). Comparable work has defined the seedbank traits of common arable weeds such as *Poa annua*, *Stellaria media,* and *Chenopodium album,* and aims to reconstruct the seedbank community.

Running the model with plants types selected from the *Rumex acetosa* trait space, revealed that most types were quickly exterminated, after which a set of types then coexisted for long periods. Analysis of the abundance of these types revealed systematic patterns highly reminiscent of those in large ecosystems. It was as if the individual variation in one species was arranging itself over two-dimensional space in much the same way as the different species in a grassland, an arable field or an archipelago. This finding gives the stimulus to much further work on real populations. It means that quantitative hypotheses can be set on, for instance, the trait distributions – the diversity among individuals - that should be measurable at a small scale in the real environment.

Searching trait space with a genetic algorithm This modelling approach can also be the basis of a 'search' for the most appropriate plant community in a given

situation. A genetic algorithm search technique was therefore examined for its usefulness in this respect[5]. A plant type is identified by a letter (A, etc., a number of traits (1, 2, etc., where 1 might be maximum resource uptake, 2 might be distanced proportioned uptake, and so on) and a trait value (a, b, c, etc.). The total trait space that plants can inhabit in the search is defined, as in physiological studies, by all the traits and their values. A group of plant types, in effect a community, is represented by a string of trait values, which in search terminology is known as a 'chromosome' (Fig. 5). The community can be pre-defined to represent known functional types or selected randomly from trait space.

The spatial model is then parameterised for the community and whatever resource base is required. The aim of the search is to maximise some aspect of 'fitness' of the community, which could be some measure (for instance) of resource in the plants, and abundance distribution or period of coexistence. The model is run separately for a number of 'chromosomes' (communities), each of which has a fitness parameter assigned to it on the basis of its performance. When the communities have run their course, they can be subject to genetic operators that bring about change through 'recombination' (where the strings of trait values cross over) or 'mutation' where the value of one trait might change independently of the rest, or even the introduction of new trait values. Recombination, followed by re-running the model, can be made between communities that have been successful in previous generations as a means of searching for a community of maximum fitness (Fig. 5).

Experience with the search so far suggests that optima in trait space – combinations of individuals that give high values of fitness of the community - are rare, but extended searches might allow the identification of areas of trait space that generally contain optimal solutions. Approaches such as this enable us to get a feel for the potential importance of different plant traits,

In the genetic algorithm search technique, populations or communities are represented by 'chromosomes' or strings of trait values (see text). The following simplified example uses 13 original plant responses collapsed into six traits (1 to 6) to reduce the dimensionality of the search space. For the purpose of demonstrating the technique, each of the traits is given 16 possible values (a to p), again limited for simplicity. Therefore the symbols in the trait space are:

Plant types: A to J (10). Traits: 1 to 6 (6). Trait values: a to p (16)

The chromosomes are of the form

$C_{A1}\ C_{A2}\ C_{A3}\ C_{A4}\ C_{A5}\ C_{A6}\quad C_{B1}\ C_{B2}\ C_{B3}\ C_{B4}\ C_{B5}\ C_{B6}\ \ldots\ldots\ldots$

Where C_{A1} represents the first trait (resource uptake), for plant A, C_{A2} the second trait (distance proportioned uptake) for plant A, through the four other traits for plant A, and all traits for plant B, and so on up to plant J. An example chromosome for a set of ten plant types is

pijfpc hnfjbk ngkhnp jilhog ijhepd ehechm miedhk acmhjj gkpdic adfgck

The plants are separated by spaces, so the first plant is defined by the sixteenth (p) value of trait 1, the 9th (i) of trait 2, the 10th (j) of trait 3, etc. Each 'chromosome' represents a community of 10 plant types. The fittest community in this example is defined as the one that maintains all plant functional types for the longest period in coexistence. The upper limit of time was defined as a 1000 cycles, so that fitness was an integer between 1 and 1000. The model is run for a number of chromosomes (communities), 20 in this instance, and the ones that perform best according to the criteria then 'recombined' by cross over of the strings of characters at assigned points. The runs shown below identified one very fit community that evolved rapidly. The mean fitness of all 20 communities lagged behind because of the diversity of solutions. The error bars indicate the standard deviation in fitness values at each iteration.

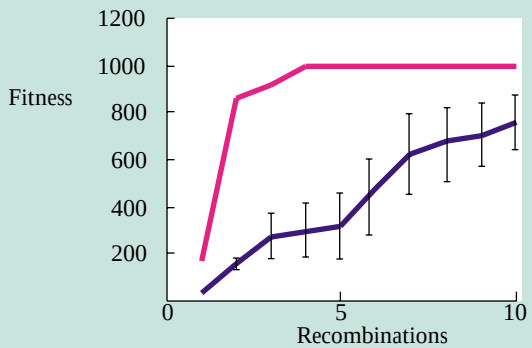

Figure 5 Searching trait space with a genetic algorithm (adapted from Bown[5])

and might enable us to assess the consequences of introducing new traits in plant varieties or invading species.

The model is now being parameterised for seedbank annuals, where a second 'layer' is needed for the dynamics of the buried seed. More detailed and more targeted experimentation is being planned to test the specific hypothesis that the approach is indicating. The concepts and associated analysis are now well developed for plants, but could in principle be applied to invertebrates. The ultimate aim is to treat the system as a collection of individuals of whatever taxonomic group.

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Production and diversity in multi-trophic systems

J.G. Hillier, C. Hawes, G.R. Squire & A.N.E. Birch

A scheme is presented for studying the interactions between three trophic layers in arable ecosystems. Every living member of an ecosystem seeks to acquire resource from the trophic layer below and convert this resource to self, or to offspring. Trophic interactions between organisms have a major impact on the structure of a given community, and specifically on its contribution to food production and to biodiversity. The trajectory of any individual in trait space is, as was considered in the previous article, a function of its genetic traits, and the external influences on it. Just as plants occupy, at any given time, a position in trait space, so do other components of an ecosystem such as pests, natural enemies, and members of other trophic levels.

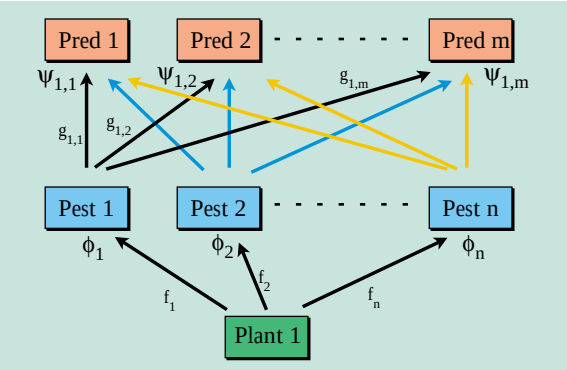
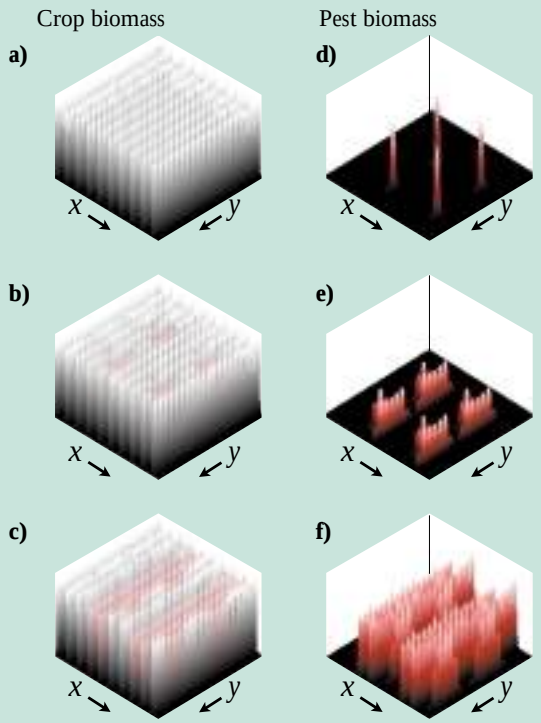


Figure 1 Representation of a tri-trophic system. Text describes the symbols.

Due to the complexities of the trophic, competitive and spatial interactions between organisms in a given habitat, any change in management is likely to propagate through the system with effects that are difficult to predict. This uncertainty is particularly important when considering the impact of, say, the introduction of a new insecticide or a new crop variety which has been bred or genetically modified for insect resistance or herbicide tolerance. It also limits Integrated Pest Management strategies in which, for example, natural enemies are used as a biotic factor to suppress a pest population. The work described is the first step towards defining and quantifying the components of the arable tri-trophic systems, including aspects of spatial grouping and dispersal.

The basic scheme The tri-trophic system is examined through a linked mathematical and experimental approach. The first aim has been to develop a modelling framework that both draws in existing experimental knowledge and enables precise hypotheses to be set and tested in the laboratory, glasshouse and field. The scheme in Figure 1 shows a single plant type interacting with several herbivore types, and these in turn with several predators of the herbivore. Following earlier workers, the many potential interactions between these layers are simplified to two functions, linking each layer. That between crop and pest depends on the function, *f*, defining the rate at which crop is eaten, and ϕ,defining the amount of pest biomass produced per unit crop eaten. The values of *f* and ϕ are specific to the crop-pest combination. The symbols, *g* and ψ, define the corresponding functions linking predator to pest. The scheme allows for generalist herbivores and predators, for which the two functions take values greater than zero for all interactions, and for specialists, for which the functions may take values greater than zero for only one or a few specific combinations. Adding other plants types (not shown), such as weeds or different crop varieties in a mixture, increases the potential number of pests and predators brought into the scheme, but does not alter its basic structure. The inclusion of the dimension of time allows for feedback among the three layers, and will allow for *f*, ϕ, etc. changing with the phenology of the plant, though still within the scheme shown. Expressed in time, each plant type in the absence of a pest can be represented by a seasonal growth curve (e.g. logistic or expo-linear), which itself can be split into radiation interception (surface) and conversion (photosynthetic efficiency) to allow discrimination between herbivores that, say, bite and chew leaves, e.g. the Lepidopterans, and those that suck plant sap, e.g. Homopterans. However, the salient point is that this simplification directs our attention to measurable variates that discriminate between plants.

Extending the model spatially The ability of an individual to capture resource is strongly influenced by spatial factors. The structure of the vegetation level can have a large impact on the searching and dispersal behaviour of a pest and its natural enemies. Spatial



The mathematical model is the 2-dimensional analog of the following.

$$\frac{\partial C}{\partial t} = \theta(x)C\left(1-\frac{C}{K}\right)-fCH$$

$$\frac{\partial H}{\partial t} = \phi f CH-\mu H+\frac{\partial}{\partial x}\left(D(x)\frac{\partial}{\partial x}H\right)$$

Where:
C is crop density
H is herbivore density
θ is the crop growth rate
K is the crop carrying capacity
f is the *per unit herbivore per unit crop* consumption rate
ϕ is the conversion efficiency
μ is the *per unit herbivore* mortality rate
D is the diffusion coefficient.
In a 2-D grid, θ(x) is set to 0 in regions where there is no crop (i.e. nothing grows). In this way, spatial arrangements of crops are simulated. D is also allowed to vary spatially to simulate variations in the surface on which the pest moves.
References: Shigesada & Kawasaki (1997); Murray (1989, Chapter 12).

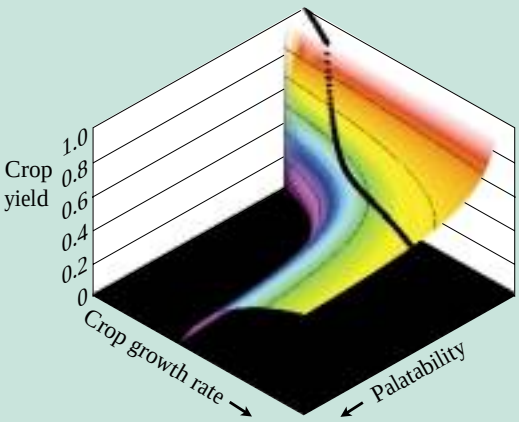
Figure 2 A spatially explicit 2-D crop-pest model showing crop biomass (a, b, c) at increasing time after invasion and pest biomass (d, e, f) at corresponding times. Height of bars represents biomass at individual plants.

grouping, dispersal and migration can have great effects on the behaviour of the systems in Figure 1. In a simple example, a population of a herbivore type feeding on a plant type increases over time, but also spreads out as result of density-dependent or random movement. If flightless, the herbivore's spread depends on the way its own characteristics interact with factors of the plant, notably its architectural features – the angling and connectivity of its surfaces, the distribution of food sources over its structure and wider aspects of stand density and spatial arrangement. It is necessary, therefore, to determine how pests and predators will move in response to their genetic programming and to environmental stimuli. Again, simulation has been used first to aid synthesis of existing data and to set measurable goals in experimentation.

A simple example of a small, plot-scale simulation model with two trophic levels is shown in Figure 2. Parts a, b, and c represent the biomass of a crop at three different times during the growing season, and parts d, e, and f represent the biomass of the herbivore at corresponding times. The crop is planted in rows, such that the herbivore can move more easily from plant to plant along than across rows. Each vertical rod shows the biomass of the crop and pest within one unit of the crop, for example, a single plant. Observe the non-uniform spread of the pest, which arises from the greater ease of movement along rows than across them.

The spatial spread model in Figure 2 has a small set of parameters, which can be quantified from existing information where available, or measured experimentally. The crucial botanical parameters affecting spread of flightless herbivores exist in three dimensions (not two as in Figure 2) and include features of the vertical architecture and physiology of plant stands. Work is in progress to define the salient aspects of vertical and horizontal connectivity in crop-weed stands by image analysis of photographic slices of the stand at different times during the season. Experimental systems are being tested that will enable the links to be made between architecture and rate of insect spread within and between crop fields.

The complication of plant energy trade-offs One of the practical aims of this work is to offer optimal designs for both plant breeding of new genotypes and the construction of multi-purpose stands. The work here links to the individual-based approach to diversity described in the preceding article. Within the plant level in Figure 1, individuals can partition the



The mathematical model is a system of 2 Ordinary Differential Equations.

$$\frac{\partial C}{\partial t} = \theta C\left(1-\frac{C}{K}\right)\frac{-fCH}{H+fhC}$$

$$\frac{\partial H}{\partial t} = \frac{\phi fCH}{H+fhC} - \mu H$$

Reference: Jost *et al.* 1999.

We impose a constraint on the crop C so that the following holds

$$\frac{\theta h}{\phi f} = q$$

where q is constant. This imposes an energy trade-off. For instance, additional investment in growth (increase in θ) leads to one of the following:
1) increase in ϕ (the food quality or palatability).
2) decrease in h (the "handling time". This can be thought of as the time it takes a unit of pest to process a unit of crop).
3) increase in f (the maximum crop consumption rate).

In the example presented in the dialogue the first of these is assumed, giving us that $\theta = Q\phi$ with $Q=qf/h$ representing the "strength" of the trade-off. This is the equation of the line cutting across the yield surface .

Figure 3 Modelled variation in yield with change in plant growth rate and palatability to herbivores. Contours show lines of equal yield. The black line shows the yield of a single plant type having a defined trade-off between growth rate and palatability (see text).

available resource in a number of areas, important among them being (i) plant growth, (ii), toxins for defence against pest attack (lowering the food quality to the pest), and (iii), structural defence which inhibits attack or hinders spread. Since a plant has only a finite rate of energy acquisition, being limited in the amount of sunlight it can intercept and convert, there must be large regions of the trait space which are unavailable to it. If a plant invests a larger part of its energy into growth, then it has to put correspondingly less into defence, i.e. it produces less toxin or invests less in structural defence. A plant can't be a lettuce and a cactus. This complicates the issue of plant improvement, since breeding for increased growth, with which we would anticipate increased yield, may be offset by a decrease in plant defence which makes the crop more susceptible to attack by pests or more desirable as a food source for a pest, thereby negating or producing the opposite of the desired agronomic trait.

As illustration, the example in Figure 3 shows a 'yield surface', which defines how yield might be expected to vary with changes in the palatability and growth rate of the crop. The surface is derived by using simple functions of the type described previously. Maximum yield in the presence of a pest would be reached at maximum growth rate and minimum palatability to herbivores; this is the area of trait space that plant breeding aims for, but in reality is impossible to inhabit. The contours in the figure indicate lines of equal yield, the different points on which are necessarily achieved by different combinations of growth rate and palatability.

The wider challenge is to place the individual in this scheme. As a first step, we accept the existence of a 'trade-off' between plant growth and defence, and so quantify individuals by the strength of the trade-off (a growth/palatability function), which is the rate of loss of growth per unit decrease in palatability (or how much slower plants grow if they use a unit of their resource to produce toxin). Plants that lose little intrinsic

growth while making themselves highly unpalatable to pests might be desirable in crop breeding lines. The black line cutting across the contours in Figure 3 shows one such growth/palatability type, and might represent a crop genotype in a breeding programme or a weed ecotype in an arable field. Any number of other types could be added, but are excluded for clarity. The line (= plant genotype) traverses the yield surface in an interesting way. As we follow the line from the back of the graph (low growth, low palatability), the yield first declines: the surface decreases too steeply since the gain from increased growth rate is overpowered by the loss from increased palatablity. Nearer the front of the graph, the effects are reversed, such that the gain from increased plant growth rate is greater than the loss from increased palatability. Whether the individual gains or loses depends as much on the context - the system parameters - as its physiological traits.

Biodiversity and integrated management The argument can now return to the matter of increasing invertebrate diversity. Many intensive improvement programmes are concerned with the interactions between one plant type (the crop variety) and one herbivore (the main target pest). Intentionally or not, one or both of f and ϕ are altered by plant breeding to reduce the effect of the herbivore on the crop. Even if chemical formulations are not used to augment the genetic change, the alteration has ramifications for other herbivores that might eat the plant and possibly predators that consume herbivores made 'toxic' by eating the plant. Additionally, the decline of one herbivore might be matched by the rise in others through change in competitive advantage. Such tri-trophic effects have received particular attention in risk assessment studies on GM crops that have enhanced anti-pest properties. However, our scheme can be used to examine the impact of GM crops in the same way as corresponding effects of conventional crops and of weeds that produce toxic, anti-insect chemicals.

If the intention is to increase biodiversity, however, tri-trophic effects have to be managed rather than avoided. Means have to be found of increasing the number of different values of f and ϕ and then g and ψ in a system, and also of the traits that alter the survival and movement of the herbivores and predators. Increasing the number of plant types potentially increases the complexity of the interactions in Figure 1, but a systematic approach to balancing the traits among individual plants should be preferable to simply increasing the number of plant species. With reference to the arguments around Figure 3, plant types should be mixed that cover a range of growth/palatability lines for the environment in question. The result would be to optimise the trade-off at a broader scale than the individual plant or genotype.

The inclusion of other species in the model results in continual feedback between the different components of the system. For instance, each herbivore type has the potential to affect others through its influence on the amount and quality of plant mass. In principle, the complexity can be examined by techniques similar to those used to probe vegetation communities. Provided the tri-trophic system can be parameterised - and there is no reason why it should not - then searches of the type outlined in Figure 5 of the preceding article should be able to point to the fittest community of plants, herbivores and predators.

Conclusions The aim of this report was to introduce a new area of work at SCRI, which has taken the approach of synthesising the existing mathematical theory and empirical knowledge as a means of defining the salient questions, before embarking on experimentation. Additional subjects tackled in this way, not mentioned above, include the evolution of resistance in the pest population to the genetic change introduced to the plant. As in the comparable research on trait space and interactions in plant communities, a particular function of the models is to condense a complex set of potential traits and variates to a manageable number that are common across organisms and ecosystems. The emphasis will now be on measuring the appropriate traits of crops, pests and natural enemies in field experiments, the prototypes of which were tested during 2000.

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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 principal remit is to support the SERAD 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. In 1999, new research funding allowed further expansion of work in veterinary epidemiology, particularly in understanding the dynamics of zoonotic diseases including E. coli *0157. Links were further strengthened with Scottish universities through establishment of a formal link with the Department of Statistics and Modelling Science, University of Strathclyde, focused principally on our new West of Scotland Unit, and the appointment of two research students with the University of Aberdeen. The SCRI group continues to support the rapidly expanding SERAD-funded programme in genomics. Recent activities of the group in this and other areas are described below.*

Work in statistical genetics progressed with the development of methodology for estimating a linkage map from dominant and codominant markers in tetraploid species such as potato. This has been implemented in a computer program and will form the theoretical basis for QTL mapping in tetraploid species. Methodology for multi-trait QTL mapping in diploid species has also been developed, and applied to map QTLs affecting six yield characters in barley. The importance of this work was recognised by an invitation to review statistical methods for linkage analysis at a meeting organised by BBSRC and the British Council on 'New horizons for marker technologies and their application for cereal genomics.'

The release of new molecular sequence analysis software (EMBOSS) and new, Web-based, bioinformatics services has considerably affected the way that SCRI scientists analyse their molecular sequence data and the consequent support required from BioSS. Updated training courses are planned for 2000/01 when the EMBOSS graphical user interface becomes available. Considerable progress has been made in developing improved hidden Markov models to detect evidence of mosaic sequences (due to recombination) in DNA multiple alignments, as part of a BBSRC-funded project under their Bioinformatics initiative. The method has been programmed in MATLAB. Collaboration continues with the Applied Statistics Department, University of Reading, on distance-based approaches to detecting recombination in large datasets. Among several collaborative projects with SCRI scientists involving phylogenetic methods, an analysis of protein sequence data was used to illustrate enzyme activity and down-regulation in transgenic plants.

Sampling designs have been devised for soil surveys in the Micronet project and other Soil-Plant Dynamics programmes. These involve establishing random transects from a set of systematically arranged origins and provide a compromise between providing an overall assessment of the site and quantifying spatial dependence by the variogram.

Modelling stochastic structures in soil

G.W. Horgan[1] & I.M.Young[2]

It is well appreciated by environmental scientists and ecologists that the apparently uniform, appearance of an area of ground hides a rich complex structure of organic and inorganic material, and porosity, that is variable in its properties at almost every scale at which it can be examined. Statistics, which is the science of variability, can play an important role in modelling and understanding this variation. There are several ways in which a model can be applied. We may use it to *describe* the variability, and to compare aspects of it in different situations. We can use a model to *predict* what will happen when parameters of the model are varied. Comparing predictions with observations provides a *test* of the validity of the model. Perhaps the most satisfying use of a model is when it gives insight that helps to *understand* aspects of a system that cannot be revealed readily by observation alone.

The analysis of pores and cracks in soil provides good examples of the way in which stochastic models can complement experimental work. As the medium through which water, gases and micro-organisms move through soil, their importance cannot be overstated. The structure of the pore space is complex and, being three-dimensional, is difficult to observe. Descriptions in terms of fairly simple concepts such as pore size distributions and indices of tortuosity will be inadequate. Although all models of pore space will fall short of reality, more complex models will be more realistic and useful, but more demanding to work with. Fortunately, continuing improvements in the power of affordable computing allows us to be ever more ambitious in this regard.

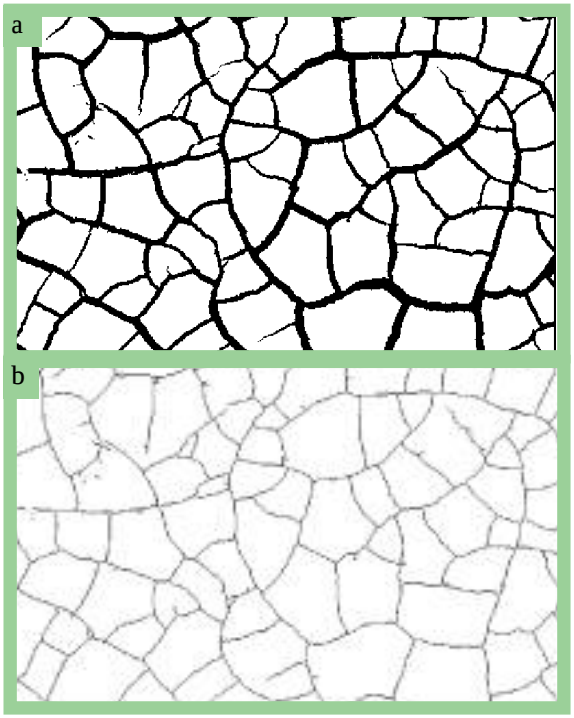

Figure 1 (a) Image of cracks in soil collected from Cruden Bay, sieved, and dried in a 10cm dish at 20°C. (b) Skeletonisation operation to reduce the cracks to single pixel thickness before estimating length density.

Image Analysis

Image Analysis is the extraction of information from digital pictures. It sits somewhere between Image Processing, which is the modification of a picture to produce another picture, and Image Understanding, which tries to build a complete representation of all the relevant content of an image - a type of Artificial Intelligence. In practice, these three disciplines overlap and interact.

We can illustrate image analysis by considering the image in Figure 1(a). It shows cracks in soil collected from Cruden bay, sieved and dried at 20°C. We are interested in quantifying aspects of the crack pattern. For example, the length density is relevant. Measuring the length requires an initial image processing step: we must reduce the cracks to single pixel thickness, a process termed skeletonisation. It is done by iteratively removing pixels, providing crack connectivity is unaffected, until no more can be removed. The result is shown in Figure 1(b). Some further tidying will be needed, either by removing unwanted artefacts from the skeleton, or by some initial smoothing of the image. Crack length can be measured by simply counting pixels, with allowance made for the number of pixels per unit length for lines at different angles. Assuming a lack of any directional preferences in crack growth, which seems reasonable, allows adjustment by a simple correcting factor.

The curvature of the cracks is thought possibly to be related to soil composition. This is trickier to measure. It requires more than looking at patterns of pixels and their neighbourhood. We need to trace each crack across the image as it passes many crack junctions.

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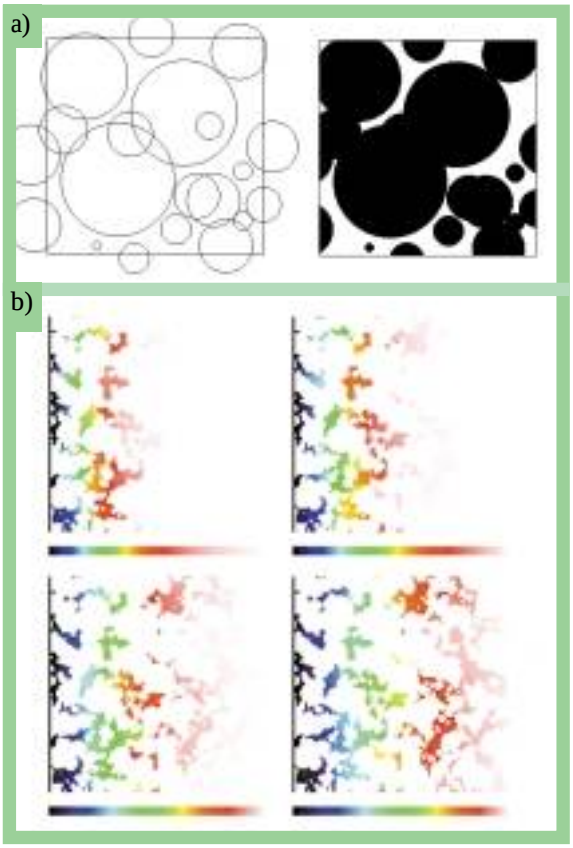

Figure 2 (a) Illustration of a two-dimensional slice of the Boolean model of soil pores: Spheres with a random radius are placed at random positions in space and allowed to overlap. The remaining spaces, shown here in white, are the pores. (b) Gas diffusion at four time points through a Boolean pore space. Gas diffuses from a source on the left. Intensity is shown by colours from white (low) through to black (high). Diffusion through an empty cylinder is shown underneath each image for comparison.

This was done by identifying segments of cracks (the lengths of crack between junctions) in the skeletonised image and deciding which segments of cracks were continuations of which other cracks. Segment length and angle were the main criteria used for this. Once whole cracks had been listed, some measure of curvature could be readily calculated.

Pore space diffusion

Although observation of the three-dimensional nature of soil pores is difficult, it is possible to construct models of the pore space that are simple to define, yet give rise to complex realisations. We have used the Boolean model for this purpose. It is defined by placing random sets at random positions in space and allowing them to overlap. Because of their simplicity, spheres with some distribution of radius are a common choice for the random sets. This is illustrated in Figure 2(a). The pore space is the complement of the spheres - the space that remains. We have found that an exponential distribution of sphere radius gives realisations which, in two-dimensional sections, resemble those of real pores.

We have used this model to explore the effect of model parameters (mean sphere radius, percent of pore space) on the properties of gas diffusion. Figure 2(b) shows the density of a tracer gas diffusing from left to right through a piece of soil, at four time points. Diffusion through an open cylinder is shown underneath each image for comparison. Properties of the diffusion process, such as steady-state flux, and the time to establishment of this steady-state, can be estimated. These properties can be compared with those obtained from experiment. We have found good agreement, which helps confirm the validity of the Boolean model. For example, steady-state flux appears approximately proportional to the square of the porosity. At very low porosities, no diffusion occurs. This can be understood in terms of percolation theory, whereby long-range connectivity of pores only occurs above some critical porosity threshold.

What is important for diffusion?

The modelling just described enables us to summarise and predict the effect of pore characteristics on diffusion. However, it does not tell us what properties of the pore space are important for diffusion, or what their influence is. Why does the diffusivity vary for

Geometric measure	Effect on flux	Effect on delay
1. p: the porosity		
2. p_l: left boundary porosity		
3. p_r: right boundary porosity		+
4. L_l: mean path length from left		
5. L_A: mean of all path lengths		+
6. p_p: proportion of pixels on paths		–
7. p_5: area within 5 pixels of paths	+	+
8: p_{10}: area within 10 pixels of paths		
9. W_G: Geometric mean path width.	+	
10. W_N: mean path bottleneck width		
11. D_1: Area of deadend pixels		–
12. D_2: Square root of deadend area		+
13. D_M: Maximum deadend length		
14. C_1: Porosity near sample centre	+	+
15. C_2: Harmonic of measure 14.		

Table 1 Effect on diffusion of pore geometry.

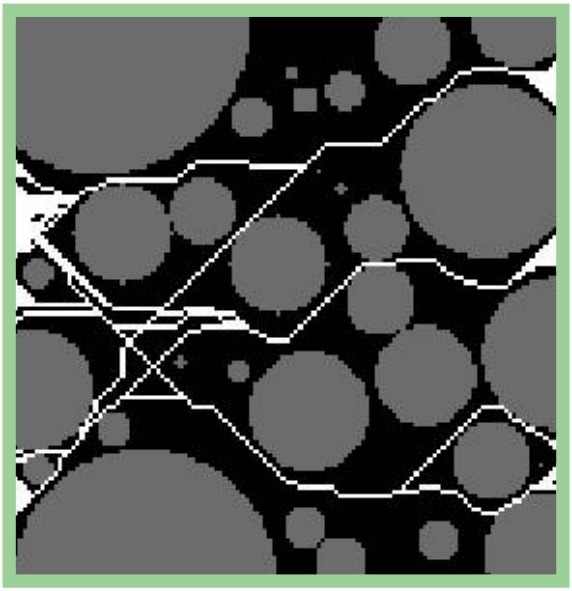

Figure 3 Paths through a simulated random pore space. A path is the shortest route from a point on the left to a point on the right of the pore space. It is the proportion of pore space near these paths, rather than their length, which has the greatest influence on the amount of steady state diffusion.

several samples with the same porosity? What geometric properties of the pore space can explain this?

We have investigated this issue by simulating diffusion in a number of random pore spaces, calculating a range of geometric characteristics, and looking at the associations between them. Two-dimensional models were used for simplicity, while permitting a substantial range of geometric structures. With gas diffusing from a source on one side of a sample, as described above, we were not surprised when principal component analysis of the diffusion curves revealed most variability to be accounted for by the amount of steady state flux, and a delay effect in the establishment of the steady state.

There are many ways in which the pore space geometry may be summarised. We calculated 15 summaries (see Table 1). Those based on path lengths are illustrated in Figure 3: a path is the shortest route from a point on the left to a point on the right of the pore space.

Multiple regression of the principal components of the diffusion curves on pore geometry measures showed that the main influence on steady state flux was the area within 5 pixels of a path. Diffusion will be highest when much of the pore space is concentrated near these paths. The main influence on delay is the proportion of pore space near the centre of the sample. The measures which contributed significantly to variability in diffusion, and the sign of their marginal effect, are shown in Table 3.

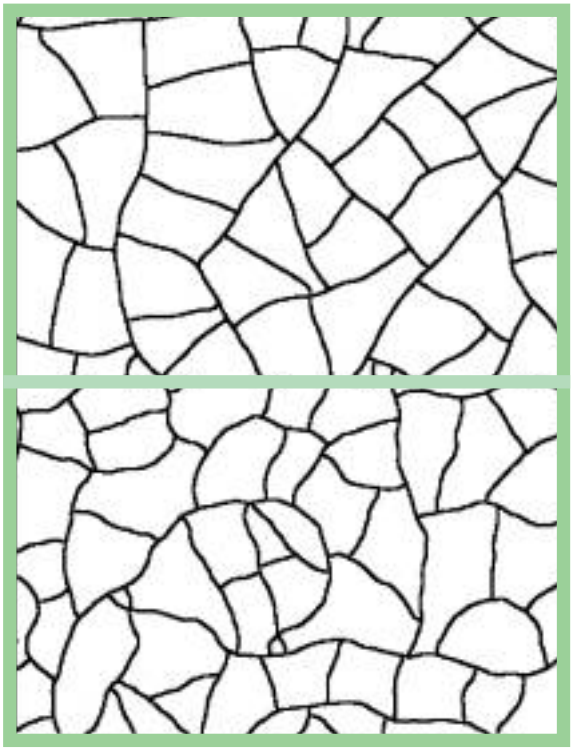

Figure 4 Simulated soil cracks. Crack direction changes more rapidly in the lower image.

The geometry of soil cracks

We saw earlier how image analysis allows us to measure features of soil crack geometry. A challenging question these images pose is to say what processes lead to these observed patterns. Modelling may be able to help understanding of processes that cannot easily be observed.

We found no models in the literature of stochastic geometry which in any way resembled real cracks. Those which had been proposed had sacrificed realism for mathematical tractability. After much scrutiny of images like Fig 1(a), and many attempts to devise algorithms which generated something resembling them, we found that five processes were necessary:

1. Random walk crack growth which continually makes small changes in direction.
2. New cracks forming on existing cracks and initially growing perpendicularly.
3. Crack attraction at small distances.

4. A tendency for large aggregates to split.
5. Stability below a certain aggregate size.

Each of these processes requires a parameter to describe it (process 3 needed two), leading to six parameters in total. Elegance is lost, but the result is realistic. Some realisations are shown in Figure 4. An important conclusion is that all five processes are necessary. For example, if process 4 is weak, the resulting simulations look nothing like cracks. The modelling has demonstrated the need for this process in understanding how cracks form.

It can be seen that the real cracks have even richer structure: varying thickness and a possibility of stopping before reaching other cracks. The model could be extended to incorporate this. Heterogeneity of crack patterns can also be seen in some crack images. This too could be handled by processes a step higher in a hierarchy. As we said at the start, soil structure is rich and complex at many scales.

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. During 1999, the facility was moved to new laboratories in the refurbished Building S.

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

The re-located Stable Isotope Facility in Building 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. During the year the data acquisition and processing software was updated to the Y2K compliant Mass Lab 1.3 package.

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.

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. During 1999, the data processing capability and injector hardware of the older instruments were upgraded.

Media Kitchen

W. Ridley

The Media Kitchen was established in 1996 and provides a wide range of sterile microbiological, mycological, plant tissue culture, media preparation and disposable plasticware for the Institute's laboratory staff.

Operating as a research facility under the central administration overhead, to minimise bureaucracy, each user site is 'shadow-tolled' for its throughput of consumables, etc. The outputs of the Media Kitchen and the increase year by year can be seen in Figure 1. Overall, through bulk purchasing agreements, prices have held since 1996 and huge savings have been made for the Institute.

The Media Kitchen is staffed by two full-time and one part-time worker, and the facility is also supported by the efforts of Walter Burry (full-time) and James McMillan (part-time), who were recruited from the Helm Project in Dundee.

Orders are delivered on a daily basis to 12 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 or by e-mail for delivery, usually 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 waste microbiological materials. This frees the innovative scientists, visiting workers, trainee students and support technicians from the repetitive

and time-consuming tasks associated with media preparation, and secures a standardised quality of service throughout the Institute. At the same time, this saves 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. The success of the Media Kitchen indicates that a larger working area will be necessary in order to meet the ever-increasing demand for this essential core facility

	1997	1998	1999	Increase 1999 on 1997	Increase 1999 on 1998
Tips	1,393,350	1,430,000	1,973,800	+ 42%	+ 38%
Eppendorf tubs	520,095	524,000	842,200	+ 62%	+ 61%
Agar plates (poured)	37,011	43,600	56,084	+ 52%	+ 29%
Other items	24,654	45,080	47,928	+ 95%	+ 7%

Figure 1 Media kitchen output.

Scientific Liaison and Information Services

W.H. Macfarlane Smith

We live in a world today where the pace of scientific advance has never been faster. The public awareness of science is greater than ever before but, equally, the concerns about scientific developments exceed anything known in the past. Certainly, the demand for information seems to be almost insatiable, much of this fuelled by the concerns following the BSE problems in cattle and the resulting CJD outbreak in Man. The issue of the genetic modification (GM) of crops ties up ever more of the Unit's time in an endeavour to explain the real and potential advantages of the new technology and to counter the insidious and frequently erroneous propaganda of the anti–GM pressure groups. Through the Institute's participation in events at the local Botanic Gardens, at 'Sensation' (the new Dundee Science Centre), at the Haddington Science Festival, the National Garden Show, the Edinburgh International Science Festival, and our own Open Days, large numbers of people have been able to see for themselves, the true nature of SCRI's research. Of equal importance, they have been able to ask questions of well-informed staff involved with the research and to have a proper debate on the pros and cons of biotechnology and other new scientific and technological developments. Participation in radio and television programmes, and one-on-one press interviews have increased markedly and have helped again to raise public awareness of the Institute's work. However, there is still a section of the media which is more concerned with confrontation between those of different views than with making the effort to inform better the large numbers of non-scientists in the UK. A considerable number of talks have been given to agricultural discussion groups, some numbering well in excess of 100 members, and to other interested organisations, including the W.R.I., Women's Graduate groups in Universities, Rotary and Probus – it would seem that all ages and sectors of society are interested. It is also clear that everyone attending these presentations is concerned to understand the biological revolution which is taking place and to be assured that the benefits will be as much available to the UK as to other countries in the World. It is particularly important that young people should understand the issues. To this end, parties from schools, colleges and universities have been welcomed at SCRI. Staff have also travelled to give one-off talks to such institutions and have delivered papers on new developments in biotechnology and the genetic modification of crops, at a range of conferences. Knowledge of the wide range of research in the Institute has become increasingly important in undergraduate teaching. As well as receiving our regular visitors from the Universities of Aberdeen, Abertay, Dundee, Edinburgh, Glasgow, Heriot Watt, Newcastle, Paisley, St Andrews and Strathclyde, staff now participate in teaching parts of the courses at some of the above.

The collaborative effort with MRS to bring to SCRI those individuals and organisations who might be interested in commercial links, has continued and been expanded. It has also been a pleasure to see a further increase in visitors from overseas including Australia, China, Japan, New Zealand, Russia, Ukraine and the USA.

Political interest in the work of SCRI has increased and all the major political parties have visited in recent times. It was a particular pleasure to welcome on two occasions, Mr John Home Robertson, Deputy Minister of the Scottish Executive Rural Affairs Department. His first visit was part of a familiarisation exercise on taking responsibility for the SABRI's.

The second was to undertake the official opening of the new £1.6 million Research Glasshouse (see page 48) and other refurbished offices and laboratories.

The various groups within Information Services have again had a busy and productive year. The demands

on Visual Aids to provide a wide range of services in photography and graphics continue to increase and output has again been raised. A major constraint on further increases will be the quality and capacity of the specialised equipment. The high quality of production for publications, brochures, conferences and public events, such as the Dundee Millennium Exhibition, has attracted much favourable comment. It was a particular pleasure to see these skills recognised by the award of a Bronze Medal at the National Garden Show.

The Library has continued the process of reviewing its accessions and holdings. The re-location of little used stock to a Library Annex has now been completed. Pressures on all public-sector bodies to ensure total compliance with the Copyright laws continue to increase. Material improvements to the Library are being made with the replacement of old shelving. Additional computer terminals are a part of the forward planning, as is an expansion of the office space and the provision of a ramp for disabled access. The advent of electronic journals continues to cause problems as every publisher, for the short- to medium-term, has a different method of delivery and different licence conditions. It is clear that there are to be no cost savings from moving to the electronic format, and the security and continued access to the archive are also questions that have not been answered satisfactorily. However, the advantages of the electronic delivery of content to the desktop are being exploited as far as the budget will allow. Links with the University of Edinburgh are likely to be discontinued and new arrangements are currently being finalised with the BBSRC for access to online databases and electronic journals.

As part of a major management re-organisation in the Institute part-way through the year, a new Unit of Information Technology was created. This will be operated by staff previously in the Unit of Bioinformatics and Information Technology and will have many of the same responsibilities. In future years, its activities will be the subject of a separate report. A major review of electronic communications, both within and outwith the Institute, showed a number of problems with the existing Local Area Network, parts of which are more than ten years old and use only Thinnet cabling. A project for the re-cabling of the Institute to Twisted Pair cable standard, has now been drawn up and costed. It is hoped to proceed with this in the coming year.

The importance of Health and Safety in all places of work continues to grow and, as always, the Institute accords such matters a very high priority. Accordingly, a separate report is presented this year. The Institute's policy on Health and Safety is printed at the front of this year's Annual Report.

The Institute's Internet pages have been revised and expanded while increasing use of the Intranet is being made both for routine administrative purposes and to keep staff fully informed of management processes and changes.

Finance and Human Resources Unit

I.F. Harrington & I. Paxton

The Finance and Human Resources Unit (FHR) is responsible for not only the day-to-day smooth running of the Institute but also for longer-term assessments and planning. Its work includes two major areas, Personnel and Finance. Staff costs represent the major component of expenditure, accounting for 61% of the Institute's recurrent budget.

Not surprisingly, therefore, the work of the Personnel Section within the Unit is vital to the success of the Institute. The Personnel Section carries out the administrative induction for new members of staff and supervises probation procedures during their first year of employment. It is responsible for monitoring absences with particular emphasis on those attributed to ill-health. Personnel handle requests for job evaluation at the request of both individuals and Institute management. In addition, it co-ordinates the recruitment of Institute staff and, during the year, 31 posts were advertised, attracting 678 applications from external and internal sources. The training budget is administered by Personnel on behalf of the Institute Training Committee. Personnel staff collate training requests from staff and also arrange training with the appropriate training providers. Personnel are involved in monitoring the progress of the research students based at the Institute. During the year, approximately 35 students were working at the Institute. Assistance is provided to new staff, visitors and students with accommodation and other welfare related matters. Throughout the year, Personnel staff were involved with the Institute's Investors in People Initiative.

The Institute continues to operate under considerable financial pressure as the Grant-in-Aid which it receives from Government has declined and will continue to decline 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 SERAD is still an important source of finance. However, funds are gained increasingly from other sources to provide the necessary infrastructure to maintain and expand the quality and quantity of science within the Institute.

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. The team also maintains over 1700 items on its fixed asset register, ranging from PC Computers to laboratory buildings.

Estate, Glasshouse & Field Research Unit

G. Wood

1999 was an exceptionally demanding yet productive year for the Estate, Glasshouse & Field Research Unit. Almost 50% of the total usable land area was required for field trials. There is a trend towards increasing size of individual trials: two trials (one brassica, one cereal) alone accounted for more than 10 ha. The SCRI/SAC/BPC Potato Trials Open Day on 12 August 1999 was a tremendous success. This event is now an established feature in the technology transfer programme pertaining to potato research and advisory matters. In conjunction with the scientists involved with the trials, monitoring of the sites of previous GM experiments on SCRI land continued throughout the year. These trials included potato and strawberry releases in 1996, 1997, and 1998. An HSE Specialist Inspector also made an unannounced spot-check of all these sites and found no residual or groundkeeper potato or strawberry material. Such trials and the associated monitoring procedures, reports, and inspections, provide valuable data with regard to the risk assessments associated with GM crop field releases.

The large area occupied by tunnel structures was increased again, with several new polytunnels being erected for specialist use, and a number of existing tunnels were re-covered. A new irrigation system with automatic on/off control and pressure regulation was designed and installed throughout this area. Plant production in glasshouses, tunnels, and growth cabinets placed great demands on the limited space available. The situation was not helped by the ageing nature of some facilities and the difficulties in ensuring regular maintenance in others. Several cabinets were out of action for lengthy periods and others had inadequate light levels for the proposed research. Such problems placed extra burdens on the staff looking after plants within these facilities.

These were exacerbated by staff shortages over a large part of the year. The exceptional demands, however, were met due to the great commitment, ability, and co-operation of Unit staff, coupled with a more proactive involvement with clients. All the staff are to be applauded for their efforts. It was a considerable logistical achievement to meet all the clients' requirements.

The line managers in the Unit were very conscious of limitations in budget allocations and shortage of manpower. However, their careful management of resources was effective in ensuring that the priority quality standards were not compromised. Several line managers were responsible for the astute management of funds and contracts with clients, and income generated by the EGFR Unit direct to the Institute exceeded expectations.

Major components of the Countryside Premium Scheme, comprising the planting of nearly 1000 metres of new hedging and the installation of over 1500 metres of new rabbit fencing, were completed at the start of the year. Approximately 300 linear metres of dry-stone dyke restoration were completed at the end of the year, to improve several field boundaries.

A significant contribution was made to the specification, design, and planning of a new state-of-the-art research glasshouse complex. The application to establish this facility at SCRI was successful, and construction work commenced late in 1999. The complex was opened in June 2000 by Mr John Home Robertson, Deputy Minister for SERAD (see also page 48), and will come into full operation in the latter part of this year. During the year, Unit staff also made a significant contribution (factual and advisory) to the Institute's review of its Science Strategy. Consequentially, in the year 2000, representatives from this Unit will be major contributors to the groups looking at (a) plant growth facilities (field and protected/controlled environments), (b) seed potato production options, and (c) efficient and effective utilisation and operation of the new research glasshouse complex, in line with the requirements of the new science aims.

John McAllion MP, MSP, presents SVQ certificates to members of EGFR staff, November 1999.

The staff training and development programme continued successfully. The number of core staff pursuing SVQs increased from three to five. Two other staff undertook training in line management/supervisory/ team-leader skills. Another member of staff successfully completed her HND (in Horticulture) during the year. Four new staff appointments were made towards the end of the year. We welcomed Derek Matthew to the field trials staff from August, together with Marion Grassie, Alasdair Munro, and Graham Pitkin to the glasshouse staff from October.

Barry Robertson left on 28 May 1999 after 24 years service to the Institute. He joined SHRI on 7 July 1975 when he started an Apprenticeship in conjunction with the Agricultural Training Board (now LANTRA). From apprentice to senior line manager in the Glasshouse Unit, he had been involved in and responsible for the production of countless millions of a vast array of different types of plant material spanning a multitude of research programmes. His contribution has had a highly significant impact on the success of the plant production systems in place at SCRI. Latterly, he had been involved increasingly in operational team and client interface management. He was also a major player in the introduction of sorely needed modern technologies with regard to auto-environment and auto-irrigation control systems in our glasshouses and with regard to auto-seed sowing equipment and seed germination processes. The skill, experience, knowledge and commitment Barry takes with him will be a great loss to SCRI. We wish him and his family good luck for their future in Norwich where Barry took up his new position as Glasshouse Manager at the John Innes Centre.

Engineering and Maintenance Unit

S. Petrie

The Engineering and Maintenance Unit offers a technical design and maintenance service throughout the Institute. Preservation of Institute assets is of paramount importance and careful, skilled inspections are frequently carried out. 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. 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 Unit provides a general stores facility and a cleaning and security service. The workshops are generally well equipped to deal with the maintenance tasks assigned to them.

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, 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. Scientists can now confidently bring new and diverse projects to the Institute knowing that a team is on hand to ensure the facilities will meet whatever requirement they may have.

During 1999, several areas of the Institute were refurbished to either enable new and expanded areas of work to be carried out, or simply to improve the existing facilities. The main project undertaken this year was the construction of the New Research Glasshouse. This has resulted in a facility which provides approximately 1000 m^2 of high quality glass along with a 500 m^2 headerhouse. The glasshouse area is divided into 24 individually controlled cubicles, while the headerhouse contains laboratories, tissue culture rooms, plant growth rooms and controlled environment chambers.

To offset the increased running costs associated with the new glasshouse, the Virology Glasshouse is being decommissioned during the coming year.

The Unit is also responsible for negotiating utility contracts with electricity, gas, water and telephone companies, although successful negotiations in previous years have now made further savings in these areas difficult. The Unit costs for electricity and gas were held around the same level as last year's. Telephone call charges were again reduced while water charges continue to rise.

The Unit monitors usage and efficiency of all the four utilities and although there will always be room for improvement, levels of use are now unlikely to fall significantly without major capital investment.

In recent years, ever increasing and more demanding legislation has had an effect on the work and the working practices of the Unit. The Institute must and does provide a safe working environment for its employees and visitors, but the cost of doing so is increasing annually. Much of the work to ensure their safety goes unseen by the majority of staff and often there are no tangible benefits to be gained from it. With the severe financial difficulties being faced by the Institute, it would be easy to become complacent in this area, and it is to the Institute's credit that it continues to find the necessary resources to fund this properly.

We are also educating staff into understanding that legislation now clearly defines areas of work, such as those associated with electrical and gas systems and equipment, that they cannot enter into without undermining the Institute's legal position. It is sometimes difficult for non-technical staff to understand that simple tasks such as changing a 13 amp plug or fuse must always be carried out by the relevant trained person within the organisation.

More and more time, effort and resources are being spent to cope with the 'what if' scenario all of which add to the ever increasing workload of the Engineering and Maintenance Unit.

Mylnefield Research Services Ltd

N.W. Kerby & J.B. Snape

Mylnefield Research Services (MRS) Ltd was established in 1989 as the commercial arm of the Scottish Crop Research Institute (SCRI), to diversify the funding base and to realise the potential of intellectual property (IP) generated at SCRI. The reasons for establishing MRS as a separate legal entity from SCRI are i) to protect the charitable status of the Institute and to take advantage of the gift aid mechanism to maximise financial returns, and ii) to protect the Institute from commercial liability. MRS is a fully-owned subsidiary of SCRI which appoints the Board of Directors of the company. Mr Alastair MacCallum resigned as Chairman of MRS on 29 April 1999 and was replaced by Mr Jim Godfrey.

The Mission Statement of MRS is:
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.

MRS provides a range of services to SCRI, including providing information on funding opportunities, costing projects, assisting in writing project proposals (especially issues connected to intellectual property and commercialisation plans), project management and managing the Institute's intellectual property portfolio. MRS also has video conferencing facilities and meeting rooms that are available for use by SCRI.

MRS is also responsible for marketing the expertise and resources of the Institute, and in 1999 was Highly Commended for its marketing activities by the Perth and Kinross Chamber of Commerce. In 1999, a company website was launched (www.mrsltd.com) which has attracted interest from all over the world, especially North America and Scandinavia. MRS has produced a broad range of publicity material, which has been widely disseminated. During 1999/2000, the number of commercial companies visiting MRS increased significantly.

Since its incorporation, MRS has been self-sufficient in providing its own accommodation and staffing. This was achieved without start-up funding, Government subsidy or venture capital.

In the financial year 1998/1999, turnover exceeded £1.77 million, of which £1.05 million was transferred to SCRI for services supplied. Income is generated in four areas, namely contract research, collaborative research, royalties and services.

Contract research income accounts for more than 50% of MRS' income and includes contracts with SmithKline Beecham (blackcurrant breeding), McCains (potato breeding), Biosource Genetics Corporation (viral vector technology) and other major agchem and food processing companies. New contracts were signed in 1999/2000 worth in excess of £1.5 million pounds.

A significant amount of potential business has been lost as a result of the media hostility towards genetically modified food, with a number of companies changing their policy on the application of this technology. It is clear that, in the short- to medium-term, the commercial opportunities for the development of genetically modified crops will be limited, at least in Europe.

MRS is involved in several collaborative research projects, including five Link Schemes and three multi-partner EU projects, usually undertaking project management duties. MRS' contribution to these projects is often not fully-funded and is never profitable. However, MRS' participation is often crucial in ensur-

J.B. Snape (rear) with I. Toth and Beth Hyman, winners of the Altran Foundation Award for Innovation 2000, for their test kit for *E. coli* O157.

ing that valuable contracts are placed at SCRI and that these projects are run professionally and effectively.

MRS is responsible for licensing and protecting plant variety rights throughout the world for cultivars bred at SCRI. Royalty income from these varieties exceeded £154,000 in the financial year 1998/1999, with the strawberry variety Symphony being particularly successful. The brassicas, especially Caledonian Kale and a number of swedes, also performed exceptionally well, although the portfolio of potato varieties again proved very disappointing. During 1999, significant changes to the way in which SCRI potato varieties will be marketed in the future will hopefully lead to a significant increase in royalty income in the next few years. MRS bought back the rights to the blackcurrant variety Ben Alder from PBI Cambridge. Ben Alder is grown in Europe and Canada, and the royalties generated should pay off the investment in less than 2 years.

The lipid analysis unit had another successful year and consolidated its routine analysis business by establishing a serum lipid analysis service. A specialist website (www.lipid.co.uk) for lipid chemists was launched in 1999 and has received highly favourable reviews from both the academic and commercial sectors. Dr Bill Christie, formerly head of the Chemistry Department at SCRI, was appointed as a consultant to the Lipid Analysis Unit in August 1999, and was awarded an MBE in the New Years Honours List in 2000.

Dr Charles Weller, formerly of St Andrews University, was placed at MRS for 3 months in 1999 to work as a technology scout on a pilot project funded by Scottish Enterprise. Other staff joining MRS in this period included Duncan Adam (Lipid Analyis), and 11 scientists who were employed on the Biosource Genetics Corporation project between April 1999 and July 1999. Five members of staff commenced employment on another externally-funded project during November 1999. The following scientists left the employment of MRS in 1999/2000:- Sharon Canavan, Wendy Craig, Bleddyn Hughes, Andrew Mudie, Jonathan Tonberg and Joanne Russell.

MRS supports the training needs of its staff so that it can effectively compete in today's challenging technology transfer market. It hopes to be assessed for Investors in People (IIP) in calendar year 2000.

In August 1999, the Baker Report on technology transfer from UK Government Research Institutes ('Creating Knowledge Creating Wealth' – Realising the Economic Potential of Public Sector Research Establishments) was published. MRS welcomes the findings of the report and looks forward to the proposals being adopted by Government.

Acknowledgements
MRS gratefully acknowledges its sponsors and the cooperation 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
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	Dr S Wale
Secretary and Treasurer:	Mr D L Hood
Registered Office:	c/o Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA
Membership Numbers:	*295*

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.

D.A.S. Cranstoun and Ann Foster.

The Society has continued to support projects for a second year such as 'Malting Quality Analysis in Winter Barley Mixtures', and new projects such as 'Indicated Nitrogen Investigation in a Potato Crop.' 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.

Potatoes in Practice, 1999.

The Society Annual General Meeting has, in the past, provided an opportunity for distinguished academic, media, and governmental representatives to express their views on topics of interest to the agricultural and horticultural membership of the Society.

The balanced approach of the Society to Genetic modification and transgenic crops was continued at the AGM in May when Ann Foster, the Director, Public and Government Affairs, UK of Monsanto PLC Agricultural Sector, took as her topic, 'Where do we go from here?'

The Society Soft Fruit Walk continues to bring forward new products in research, and their presentation to growers, processors and journalists challenges staff

and participants alike. 'Potatoes in Practice', the Society Potato event, held in conjunction with the British Potato Council, the Scottish Agricultural College, and the Institute, was hard work for those involved in the organisation but their efforts were favourably received and this event is rapidly becoming a focus for the industry.

The Crop Sub-Committees held occasional meetings, which are considered a useful opportunity for discussions between the scientific staff, growers, processors, and retailers. 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 Director, Professor Hillman, is restored to health and thanked the Society for its best wishes for his recovery.

Society membership regrettably continues to decline along with the decline in farming numbers. This will become more apparent in 2000. Overseas membership has almost disappeared.

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.

The Newsletter is distributed to Members and media, and contributed articles, together with photographs of interest, should be forwarded for the attention of the Secretary and Treasurer.

Health & Safety

M.J. De,Maine

The Institute's Health & Safety management was audited by SCRI's internal and external auditors. The auditors examined procedures and record keeping and drew attention to some minor points which were either rectified immediately or set in motion for completion in the near future.

Over the course of the year, 49 members of staff undertook a half-day course on manual handling run by JHW Training. Thirty-two candidates sat a half-day internal course on Health & Safety awareness and risk assessment for laboratory managers and work group supervisors. The First Aid Officer and deputy attended a one-week course for first aid instructors held by Liberty Occupational Health. First aid refresher training and training of new volunteer first-aiders to HSE-approved standard was carried out in four courses throughout the year. One course was audited, as part of HSE's continuous assessment of first aid training standards, and the institute received formal approval and confirmation of its certificate authorising the use of our own qualified trainers. A half-day course on health and safety awareness for senior managers was presented to heads of Divisions and Units and their deputies, by management training consultants AMT International. Fire safety training was carried out for all available SCRI staff over a one week period. The training consisted of two video presentations and a practical demonstration of fire extinguisher use given by Fire Fighting Equipment Limited. A video presentation and discussion on tractor safety was held with 21 members of the Institute's Experimental Field and Glasshouse Research and Engineering and Maintenance units.

The Safety Co-ordinator attended a meeting of the BBSRC safety officers at BBSRC headquarters, Swindon to discuss health and safety matters related to the BBSRC and SABRI institutes. There were two meetings of the statutory SCRI Health, Safety and Welfare Committee which were attended by management delegates, union and other staff representatives. Meetings of work area safety advisers with the Safety Co-ordinator took place every two months throughout the year where health and safety issues were discussed. These meetings are a medium for passing comments and recommendations to management on a frequent basis. In addition, there were four meetings of the Genetic Modification Safety Committee and four meetings of Institute fire officers and work area fire wardens, which were all held according to a planned timetable.

The SCRI/MRS Manual of Health & Safety Management was revised and updated. A virtual copy was made in 'html' language and placed on the Institute's intra-net. Any member of staff can now access the manual at any time from any networked personal computer on-site, and virtual links enable the user to navigate to subjects of interest using the contents list.

Staff Association

Jane Fairlie

The Scottish Crop Research Institute continues to have a very active Staff Association with approximately 240 members. The Association is now in its 7th year, and its primary aim remains that of raising money for charity through raffles, prize draws, functions and donations from companies.

The charity is chosen each year by members at an Annual General Meeting and, in 1999, the chosen charity was Cystic Fibrosis Trust. The disease can be carried by 1 in 20 people in Scotland, which has the highest incidence in the UK, and about £300K is raised in Scotland annually. The Trust provides support for families through physiotherapy and nursing care, and is linked to the British Heart foundation and the main Cancer charities. Mrs Sandra Stevenson, the Regional Fund Raising manager for the Trust, was presented with a cheque for £1147 in March 2000.

The Staff Association organises a number of outings including: golf competitions, angling competitions, surfing, white-water rafting, paint-balling and shopping trips. There is a weekly aerobics class, as well as netball, volleyball, softball, badminton and football teams, who are provided with equipment and strips from Association funds. In June, the Association hosts a Summer BBQ for the families of staff, and a Christmas Ceilidh, Disco, and children's Christmas Party are held annually.

Each month, a draw takes place to win cinema tickets and a meal for two, and there are periodic Prize Draws to win a pair of theatre tickets with dinner, which are generously donated by local restaurants and theatres. 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. Staff also benefit from the Association's Corporate Membership of the National Trust for Scotland which provides five membership cards for their use. 'Which' magazine is also provided for the SCRI library. Membership of the Staff Association is £1.50 per month, and the office bearers and committee are elected annually at the AGM. The charity chosen for the year 2000 is Roxburghe House, in Dundee.

Jane Fairlie (l) presents a cheque to Mrs Stevenson.

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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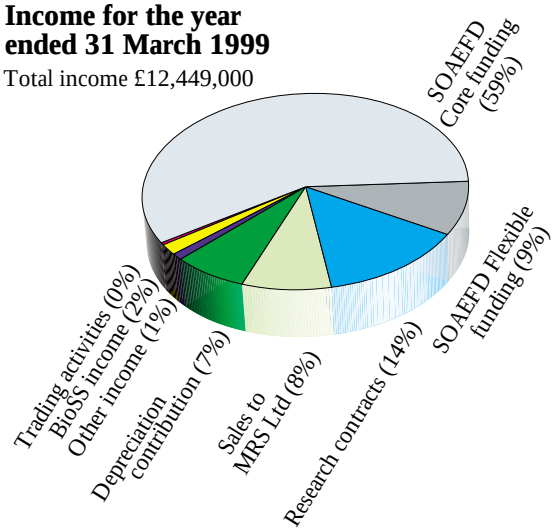
Summary of the Accounts

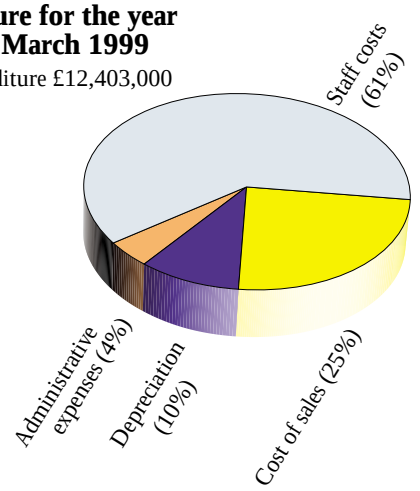
Income for the year ended 31 March 1999

Total income £12,449,000



Expenditure for the year ended 31 March 1999

Total expenditure £12,403,000



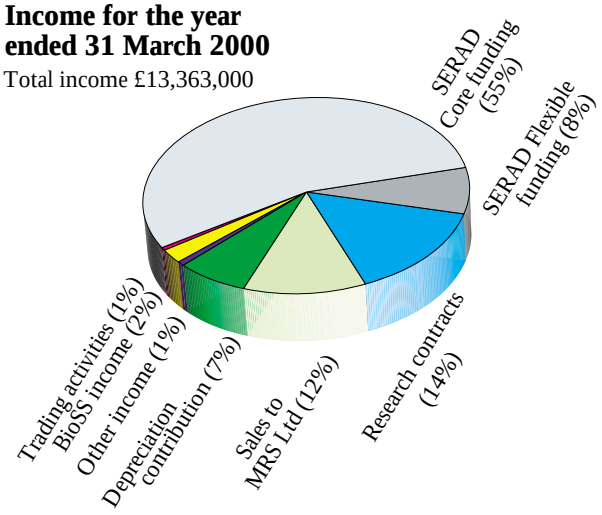
Balance sheet at 31 March 1999 **Total value £19,247,000***

Assets		Liabilities	
Fixed assets	94 %	Capital reserve	93 %
Stocks	0 %	Income & expenditure account	3 %
Debtors	6 %	Current liabilities	4 %

* following revaluation of the SCRI land and buildings

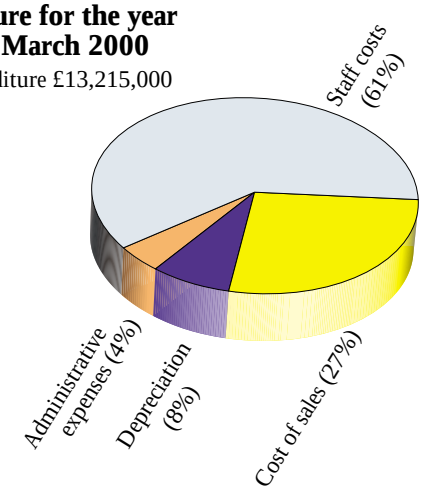
Income for the year ended 31 March 2000

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		Liabilities	
Fixed assets	90 %	Capital reserve	89 %
Stocks	0 %	Income & expenditure account	3 %
Debtors	10 %	Current liabilities	8 %

The Governing Body

Left to right, Back row : W. Powell, E. Angus, K. Dawson, I. McLaren, M.J.Emes
Front row : C.W. Goldstraw, Sir John S. Marsh, J.R. Hillman, J.E. Godfrey, P. Whitworth, M. Eddie, J.J.F. Belch
Absent : R.J. Cogdell, J. Evans, K. Hopkins, B. King, A.R. Slabas.

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 Centre for Agricultural Strategy, University of Reading; the Royal Agricultural Society of England; and Humberside Training and Enterprise Council. 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 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, O.B.E., 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 Commision from 1989 to 1997. He is Vice-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 7 technical books, including 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 Queens 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 Director of Research, 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. He has extensive collaboration with Industry, including Biogemma, Zeneca, Linnaeus and Unilever. He is 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 as Technical Director, Snacks in March 1996. 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 member of the Board of ECSA Research Ltd (ERL) (the research company formed by ESA to progress the industry's ECLAIR project to improve the tolerance of potatoes to low temperature sweetening using genetic manipulation. Part of this ECLAIR project has been carried out at SCRI.). He has now retired from the board of ERL. He was appointed to the Governing Body of SCRI in 1997.

Staff list

as at 31 March 2000 (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.[1,2,3,4]	Band 1
Deputy Director	Professor W. Powell, B.Sc., M.Sc., Ph.D., D.Sc. [5,6,7] (w.e.f. 1-7-00)	Band 2
Company Secretary	D. Watt, L.L.B., C.A. (w.e.f. 12-6-00)	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 : H.V. Davies, B.Sc., Ph.D., C.Biol., F.I.Biol[6,8]	Band 3
Deputy Head : D.W. Griffiths, M.A., Ph.D, C. Chem., M.R.S.C.	Band 4
I.M. Morrison, B.Sc., Ph.D.[9]	Band 4
M.A. Taylor, B.Sc., Ph.D.[10]	Band 4
R. Viola, B.Sc., Ph.D.[11]	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) (Prom. Jul 99)
G.J. McDougall, B.Sc., Ph.D.	Band 5 (SPD)
D. Stewart, B.Sc., Ph.D.	Band 5 (SPD)
G.W. Robertson, B.Sc., C.Chem., M.R.S.C.	Band 5
Heather A. Ross, H.N.C., Ph.D., C.Biol., M.I. Biol.	Band 6 (PD)
H. Bain, H.N.C., L.R.S.C.	Band 6
Fiona Falconer, H.N.C.	Band 8
Diane McRae, O.N.C.	Band 8
Patricia Dobson	Band 10 (P/T) (Appt. Apr 99)
Julie A. Duncan	Band 10 (P/T)
Jean Wilkie	Band 10
C. Torrie	Band 11 (P/T) (HELM)
Quality Assurance Officer : T. Shepherd, B.Sc., Ph.D.	Band 6 (PD)
Unit Administrators :	
Elizabeth L. Stewart	Band 8
Terrie Ritchie	-
SERAD FF	
P.P.M. Iannetta, B.Sc., Ph.D.	Band 6 (PD)
EU	
I. Muckenschnabel, B.Sc., Ph.D.	Band 6 (PD)
Louise V.T. Shepherd, B.Sc., M.Sc., Ph.D.	Band 6 (PD) (Appt. Feb 00)
Fiona C.M. Brown, H.N.D., B.Sc., M.Phil.	Band 7
SmithKline Beecham	
R.D. Hancock, B.Sc., Ph.D.	Band 6 (PD)
M.R. MacLeod, B.Sc., Ph.D.	Band 6 (PD)
Ruth Razzo, B.Sc.	Band 8 (Appt. Feb 00)
Nicola McCallum, B.Sc.	Band 10
MAFF	
Sarah Tiller, B.Sc., M.Sc.	Band 7

Unit of Cell Biology (CB)

Head : K.J. Oparka, B.Sc., Ph.D.[6]	Band 3 (IMP)
Deputy Head : S. Santa Cruz, B.Sc., Ph.D.	Band 4 (Prom. Jul 99)
I.M. Roberts, H.N.C., Dip.R.M.S.	Band 4
G.H. Duncan, H.N.C.	Band 5
Sheila Glidewell, M.A., M.Sc., Ph.D.	Band 6 (PD)
Kathryn M. Wright, M.A., Ph.D.	Band 6 (PD)
Fiona Carr	Band 8 (P/T)
Director's Group	
B.A. Goodman, B.Sc., Ph.D., C.Chem., F.R.S.C.	Band 4
Unit Administrator :	
Fern Watt	-
SERAD FF	
Petra Boevink, B.Sc., Ph.D.	Band 6 (PD)
C. Lacomme, B.Sc., Ph.D.	Band 6 (PD)
Alison Roberts, B.Sc., Ph.D.	Band 6 (PD)
Katja Allen, B.Sc.	Band 7 (Appt. Sep 99)
EU	
L. Simon-Buela, M.Sc., Ph.D.	Band 6 (PD) (Appt. Jan 99)

Division of Genetics

Head : J.W.S. Brown, B.Sc., Ph.D.[9] Band 3 (IMP) (Prom. Jul 99) — Deputy Head : G.C. Machray, B.Sc., Ph.D.[1] 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)
Gillian Clark, H.N.C., B.Sc.	Band 7
B. Harrower, H.N.D., B.Sc., M.Sc.	Band 7
Jill Middlefell-Williams, H.N.C.	Band 7
A. Booth, H.N.C.	Band 8
Diane Davidson	Band 8 (P/T)
J.D. Fuller	Band 9
Jennifer Watters, H.N.D., B.Sc.	Band 9 (P/T)
DNA Sequencing / Genotyping Facility	
Clare McQuade, B.Sc.	Band 7
Media Kitchen	
Wendy Ridley	Band 7
Evelyn Warden	Band 9
W. Burry	Band 11 (HELM)
Maureen Burton	Band 11 (P/T)
J. McMillan	Band 11 (P/T) (HELM)
Unit Administrator :	
Elizabeth L. Stewart	Band 8
SERAD FF	
Jennifer Watters, H.N.D., B.Sc.	Band 7 (P/T) (Appt. Dec 99)
BBSRC GAIT	
Julie 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

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
G. Bryan, B.Sc., M.Sc., Ph.D.	Band 5 (SPD)
Julie Graham, B.Sc., Ph.D.	Band 5 (SPD)
P. Davie, O.N.C.	Band 8
Jane McNicoll, H.N.C., B.Sc.	Band 7
Nicky Bonar, H.N.C.	Band 8
Sharon Mudie	Band 8 (Appt. Feb 00)
M. Macaulay, H.N.C., B.Sc.	Band 8
Kay Smith, Dip. H.E.	Band 8
Gail Simpson	Band 9
Unit Administrator:	
Sheena Forsyth	Band 8
SERAD FF	
Karen McLean, B.Sc.	Band 7
BBSRC	
Linda 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)
HORTLINK	
Heather McCafferty, B.Sc., Ph.D.	Band 6 (PD)
A. Stevenson	Band 10 (P/T)
Leverhulme	
A. James, B.Sc.	Band 6 (PD) (Appt. Nov 99)

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.[5,6]	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. 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)
I. Chapman, B.Sc.	Band 5
M.J. De,Maine, B.Sc., M.Phil.	Band 5
Helen E. Stewart, C.Biol., M.I.Biol.	Band 6 (PD)
Ruth M. Solomon-Blackburn, B.A., M.Sc.	Band 6
Jackie Lyon	Band 7
G.E.L. Swan	Band 7
D. Todd, B.Sc.	Band 7
R.N. Wilson, N.C.H.	Band 7
G.R. Young, H.N.C.	Band 7
M.P.L. Campbell	Band 8
Sandra L. Gordon, H.N.C.	Band 8
R. Keith	Band 8
A. Wilson	Band 8
Patricia E. Lawrence	Band 9
Moira Myles, O.N.C.	Band 9
Sharon Neilson	Band 9
Alice Bertie	Band 10
A. Margaret McInroy	Band 10
Unit Administrators :	
Sheena Forsyth	Band 8
EU	
Jan Moir, B.Sc.	Band 7 (P/T) (Appt. Mar 99)
Hayley Baldie	Band 8
D. Jean Harkins	Band 9 (Appt. May 99)
MAFF	
Caroline Thompson, B.Sc., Ph.D.	Band 6 (PD)
Trudi Gillespie, B.Sc.	Band 7
DTI Link	
Rhonda Meyer, B.Sc., Ph.D.	Band 6 (PD)
Jennifer Ritchie, O.N.C.	Band 10
Miscellaneous funding	
P. Lanham, B.Sc., Ph.D.	Band 6 (PD)
H-GCA	
P. Rajasekaran, B.Sc., Ph.D.	Band 6 (PD) (Appt. Sep 99)

Division of Pathology

Head : P.F. Palukaitis, B.Sc., Ph.D.[6,12,13] 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]	Band 3
J.M.S. Forrest, B.Sc., Ph.D.	Band 4
D.J. Robinson, M.A., Ph.D.[9,14]	Band 4
M. Taliansky, Ph.D., D.Sc.	Band 4
Lesley 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)
S.A. MacFarlane, B.Sc., D.Phil.	Band 5 (SPD)
B. Reavy, B.Sc., D.Phil.	Band 5 (SPD)
T. Canto, B.Sc., Ph.D.	Band 6 (PD)
A. Ziegler, B.Sc., Ph.D.	Band 6 (PD)
Maud M. Swanson, B.Sc., Ph.D.	Band 6
G.H. Cowan, H.N.D., M.Sc.	Band 7
Sheila M.S. Dawson, H.C.	Band 7
Alison Dolan, H.N.C.	Band 7 (P/T)
Wendy J. McGavin, B.Sc.	Band 7
Kara D. McGeachy, H.N.C.	Band 7
Gillian L. Fraser	Band 8 (P/T)
Unit Administrator :	
Fern Watt	-
SERAD FF	
Nicola Wood, B.Sc., Ph.D.	Band 6 (PD)
Linzi Jorgensen, H.N.D.	Band10 (Appt. Nov 99)
DFID	
Jane S. Miller, B.Sc., Ph.D.	Band 6 (PD)
DTI/LINK/BBSRC	
Karen Harper, B.Sc., Ph.D.	Band 6 (PD)
BBSRC	
Wendy Smith, B.Sc.	Band 7
EU	
Rosemary Clarke, B.Sc., Ph.D.	Band 6 (PD)
Michele Liney	Band 7 (Appt. Feb 99)

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. Birch, B.Sc., Ph.D.	Band 5 (SPD) (Prom: 1 Jul 99)
Vivian Blok, B.Sc., M.Sc., Ph.D.	Band 5 (SPD)
D. Cooke, B. Sc., Ph.D.	Band 5 (SPD) (Prom: 1 Jul 99)
J.T. Jones, B.Sc., Ph.D.	Band 5 (SPD) (Prom: 1 Jul 99)
I. Toth, B.Sc., Ph.D.[15]	Band 5 (SPD) (Prom: 1 Jul 99)
Alison K. Lees, B.Sc., Ph.D.	Band 6 (PD)
Lizbeth J. Hyman, B.A., M.Sc.	Band 6
R. Lowe	Band 6
R. Neilson, H.N.C., M.Sc., Ph.D.	Band 6
Jacqueline Heilbronn, H.N.C., B.Sc.	Band 7
Ailsa Smith, B.Sc.	Band 7 (P/T)
Naomi A. Williams, H.N.C.	Band 7
Anne M. Holt	Band 8 (P/T)
Sheena S. Lamond	Band 8
Mairi J. Nicolson, B.Sc.	Band 8
Alison J. Paterson, H.N.D.	Band 10 (P/T)
Unit Administrators :	
Maureen Murray	Band 8
Fern Watt	-

SERAD FF

K. Bell, B.Sc., Ph.D.	Band 6 (PD) (Appt. Feb 99)
Lindsey Gray, B.Sc.	Band 6 (PD)
S. Whisson, B.Sc., Ph.D.	Band 6 (PD) (Appt. Feb 99)
Lydia Castelli, B.Sc., M.Sc.	Band 7

SERAD/BPC

P. van de Graaf, B.Sc., M.Sc., Ph.D.	Band 6 (PD) (Appt. Nov 99)

EU

M. Armstrong, B.Sc., Ph.D.	Band 6 (PD) (Appt. Apr 99)
D.C. Guy, H.N.D.	Band 7
W. Morris, B.Sc.	Band 7 (Appt. Mar 99)
Alison Prior, B.Sc.	Band 7
Jane Wishart, B.Sc.	Band 7

BPC

Anna Avrova, B.Sc., Ph.D.	Band 6 (PD)
A.J. Hilton, B.Sc., M.Sc.	Band 6 (PD)
M. Elliot, B.Sc.	Band 7
Sandie L. Linton, B.Sc.	Band 10 (Appt. Mar 99)
Louise Sullivan, B.Sc.	Band 10

MAFF/BPC

D. Cullen, B.Sc., Ph.D.	Band 6 (PD) (Appt. Oct 99)

Division of Plants, Soils and Environment

Acting Head : G.R. Squire, B.A., Ph.D. — Band 4

Unit of Soil-Plant Dynamics

Head : K. Ritz, B.Sc., Ph.D.[16,17]	Band 4
Deputy Head : A.G. Bengough, B.Sc., Ph.D.	Band 5
B. Boag, B.Sc., Ph.D.[6]	Band 4
B.S. Griffiths, B.Sc., Ph.D.	Band 4
Linda L. Handley, B.A., B.Ed., M.Sc., Ph.D.[18]	Band 4
R.E. Wheatley, B.Sc., Ph.D.	Band 4
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
D.C. Gordon, H.N.C.	Band 6
Winifred M. Stein, H.N.C., B.Sc.	Band 6
Sandra Caul, H.N.C.	Band 7
Susan Verrall, H.N.C.	Band 7 (P/T)
D. Crabb	Band 8
Unit Administrator :	
Sharon Inglis	Band 8

SERAD FF

A.M. Johnston, B.Sc., Ph.D.	Band 5 (SPD)
Lynda Deeks, B.Sc., Ph.D.	Band 6 (PD)
X. Zhang, B.Sc., Ph.D.	Band 6 (PD)
Joanna Chessell, B.Sc.	Band 7
Susan Verrall, H.N.C.	Band 7 (P/T)
M.O. Henry, B.Sc.	Band 8
Evelyn Good	Band 9 (P/T)

DTI LINK

N. Nunan, B.Sc., Ph.D.	Band 6 (PD)
K. Wu, B.Sc., Ph.D.	Band 6 (PD) (Appt. Mar 99)

DETR

Kirsty Harris, B.Sc.	Band 7 (Appt. May 99)

Unit of Vegetation Systems

Head : G.R. Squire, B.A., Ph.D.	Band 4
Deputy Head : D.K.L. MacKerron, B.Sc., 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
B. Fenton, B.Sc., Ph.D., C.Biol., M.I.Biol.	Band 5
S.C. Gordon, H.N.C.	Band 5
Gaynor Malloch, D.C.R., B.Sc.	Band 7
Gladys Wright, H.N.C.	Band 7
G. Dunlop, O.N.C.	Band 8
Unit Administrators :	
Sharon Inglis	Band 8
Maureen Murray	Band 8

SERAD FF

M. Young, H.N.D., M.Sc.	Band 6 (PD)
Irene E. Geoghegan, M.Sc.	Band 7

MAFF

J. Hillier, B.Sc., Ph.D.	Band 6 (PD) (Appt. Jul 99)
Sian Hockaday, B.Sc., M.Sc., Ph.D.	Band 6 (PD) (Appt. Jul 99)

DETR

Cathy Hawes, B.Sc., Ph.D.	Band 6 (PD) (Appt. Aug 99)
Jane Reay, B.Sc.	Band 7 (Appt. Sep 99)

Division of Finance & Administration

Head (w.e.f. 12-6-00) : 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: Anne Pack Band 7
Catherine Skelly Band 7
Dianne L. Beharrie, Dip. Ed. Band 8
Joyce Davidson Band 8
Rhona G. Davidson Band 8
Pam Duncan Band 8
Kristy L. Grant, B.A. Band 8
Barbara V. Gunn Band 8
Theresa Ower, B.A., M.Sc. Band 8
Sandra Phillip, B.A. Band 8 (Appt. Nov 99)
Stella Bell Band 9 (Appt. Sep 99)
Louise Fiddes 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
Sarah E. Stephens, B.Sc., M.A., A.L.A. Band 6
S.F. Malecki, A.B.I.P.P. Band 7
Ursula M. McKean, M.A., Dip. Lib. Band 7
G. Menzies Band 7
Janette Keith Band 10
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 (Prom. Jan 00)
P. Smith, B.Sc. Band 6
I. Black, H.N.C. Band 7
Theresa Ower, B.A., M.Sc. Band 8 (Tr. from Admin Jan 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. 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
R. Pugh Band 9
W. Scott Band 9
C. Conejo Band 10
J. Flight Band 10
N. McInroy Band 10
D.L.K. Robertson Band 10
J. Rowe Band 10
M.J. Soutar Band 10
J. Oldershaw Band 11
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 (Appt. Oct 99)
J.R.K. Bennett Band 7
W.D.J. Jack, B.Sc. Band 7
D.S. Petrie Band 7
D.G. Pugh Band 7 (Prom. Dec 99)
A.W. Mills Band 8
R. Ogg Band 8
Angela M. Thain, H.N.C. Band 8
J.T. Bennett Band 9
E.R. Caldwell, H.N.C. Band 9
B. Fleming Band 9
Alison Dobson, H.N.C., H.N.D. Band 10
I. Fleming Band 10
A.C. Fuller Band 10
Marion Grassie, H.N.C., B.E.D. Band 10 (Appt. Oct 99)
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 (Appt. Aug 99)
A.D. Munro, H.N.D. Band 10 (Appt. Oct 99)
M.D. Neill Band 10
J.K. Wilde Band 10
J. Abernethy Band 11 (P/T) (HELM)
M. Orchiston Band 11
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)
Janet M. Dickson, B.Sc.	Band 5
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
Muriel A.M. Kirkwood, D.A.	Band 8
Karyn Linton	Band 9 (P/T)
Diane Glancy	Band 10 (P/T)
Amy G. Stewart	Band 10 (P/T)
Administration Officer : Elizabeth M. Heyburn, M.A.	Band 7
Jacqueline Clabby	Band 8 (Appt. Nov 99)

Ayr Unit

Head : Sarah 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)

Aberdeen Unit, MLURI

Head : D.A. Elston, B.A., M.Sc.	Band 4
Jacqueline M. Potts, B.Sc., M.Sc., Ph.D.	Band 6 (PD)
Grietje Zuur, M.Sc., Ph.D.	Band 6 (PD)
Elizabeth 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
Christine 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. Begg, B.Sc., Ph.D.	Band 6 (PD) (Appt. Mar 00)

SERAD FF

D. Allcroft, B.Sc., M.Sc.	Band 6 (P/T)
Maria 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)
Lynn Broadfoot, B.Sc.	Band 6 (Appt. Jan 00)

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 : Anne Ross, H.N.C., C.P.P.
Administrative Assistant : Lesley Beaton, H.N.C., D.M.S.
Personal Secretary/Administrative Assistant : Margaret Barton, H.N.C.
D. Adam, H.N.D. (Appt. Jun 1999)
Emma Brown, B.Sc., M.Sc. (Appt. Apr 1999)
E. Campbell, M.Sc. (Appt. Nov 1999)
Sharon Canavan
S. Chapman, B.Sc., Ph.D. (Appt. Apr1999)
Wendy Craig, B.Sc., Ph.D.
M. Dorward (Appt. Apr 1999)
Jane E. Fairlie, O.N.C.
Sophie Haupt, Dip.Biol. (Appt. Apr 1999)
P. Hedley, B.Sc., Ph.D. (Appt. Nov1999)
I. Hein, M.Sc. (Appt. Nov 1999)
Bleddyn Hughes, B.Sc., Ph.D. (Appt. Nov 1999)
Angela Ingram, B.Sc.
S. Nikki Jennings, B.Sc.
M. Jones, B.Sc., M.Sc., Ph.D. (Appt. Apr 1999)
Hui Lui, M.Sc. (Appt. May 1999)
Susan Mitchell, B.Sc.
A. Mudie, B.Sc.
Claire M. Reid, B.Sc.
Sheena Rowbottom, O.N.C., H.N.C.
Joanne Russell, B.Sc., Ph.D.
E. Ryabov, M.Sc., Ph.D. (Appt. Jun 1999)
Lisa Smolenska, B.Sc. (Appt. Jul 1999)
Julie Squires, B.Sc., Ph.D.
Jenny Stewart, H.N.D., B.Sc. (Appt. Jun 1999)
J. Tonberg, B.Sc., Ph.D.
Rachel Toth, B.Sc., Ph.D. (Appt. May 1999)
Tracy Valentine, B.Sc., Ph.D. (Appt. Apr 1999)
Charlie Weller, B.Sc., Ph.D. (Appt. Nov 1999)
Mary Woodhead, B.Sc., Ph.D.
Vanessa Young, B.Sc. (Appt. Nov 1999)

Resignations

Name	Unit	Band	Month
Nicole H. Augustin	BioSS	7	August 99
C. Clegg	SPD	6	January 00
J. Crawford	SPD	4	January 00
W. De Jong	Genom	6	January 00
Cathryn Hau	BioSS	6	July 99
Karyn Linton	BioSS	9	June 99
Z. Luo	BioSS	6	March 99
S. Pearce	MBN	-	November 99
Carol Reid	BioSS	6	July 99
B.D. Robertson	EGFR	7	May 99
D. Robinson	SPD	4	January 00
Sarah Simms	Admin	8	September 99
A. Smith	PB	8	November 99
I.M. Young	SPD	4	January 00
T.M.A. Wilson	Admin	2	March 99

Staff Retirements

Name	Unit	Band	Month
Joyce Davidson	Admin	8	July 99
W.W. Christie	Chem	3	August 99

Voluntary and Flexible Retirements

Name	Unit	Band	Month
H. Bain	PB	6	March 00
G. Dunlop	VS	8	March 00
Lesley George	PB	8	December 99
R.J. Killick	Admin	3	January 00
G.W. Robertson	PB	5	March 00

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 Janet 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

Alison 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.
Nia 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.
Konstantina Boutsika	MBN	Development of molecular diagnostic protocols for detecting "spraing" tobravirus disease of potato and its vector trichodorid nematodes.
Emma C. Brown	MRS	*Tobacco rattle virus* as a viral vector for gene silencing.
A. Campbell	BioSS	Inferential tools for stochastic epidemic modelling.
Joanna Chessel	SPD	Reactive transport in soil.
Qing Chen	MBN	*Xiphinema americanum*-group virus-vector nematodes: development of a diagnostic protocol.
Elaine 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.
Sofia Fonseca	BioSS	Stochastic geometry of muscle fibre development.
Jacqueline 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.
Maria Holeva	MBN	A molecular study of the type III secretion system and its surrounding DNA in the potato pathogen *Erwinia carotovora* subsp. *atroseptica*.
Sonia 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.*
Edwige Isidore	Genom	Construction of an ultra high density linkage map of potatoes.
V. Ivandic	AG	Simple sequence repeats in relation to adaptation in barley.
Erine 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.
Andrea Le Fevre	BioSS	Statistical aspects of lameness in dairy cattle.
Fevronia Lioliopoulou	Vir	Studies on molecular interactions between PMTV and its vector, *Spongospora subterranea* f.sp. *subterranea*.
Lucy Mackinnon	GE	Transformation of hemp – a multi-purpose fibre crop.
Gaynor Malloch*	VS	Genetic variation in the family Byturidae.
Milena Maule	BioSS	Stochastic modelling in plant epidemiology and ecology.
Hazel McGovern	SPD	The influence of soil biota on soil structural conditions.
Rebecca Nsubuga	BioSS	Statistical study of the epidemiology of *E. coli* O157 infection in cattle.
Elizaveta Pachepsky	VS	Modelling phenotypic and genotypic interaction in species-rich grassland.
Barnaly Pande	Genom	Linkage mapping in 4x potatoes.
Ederlinda Pascual	CB	Oxidation processes in coffee.
Alexandra Popovich	Vir	Development of a rapid screening system for gene function.
Alison 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.
Caroline D. Robinson	BioSS	Bayesian methods for segmenting X-ray CT images of sheep.
K. Rutherford	BioSS	Fractal analysis of animal behaviour.
Geetha Shilvanth	MBN	Enhancement of resistance to *Botrytis* grey mould of chickpea using PGIP genes.
Lisa Smolenska	Vir	The use of potato virus X for high level production of foreign proteins in plants.
Edwige Souleyre	PB	Carbohydrate metabolism during ripening in the fruit of strawberry.
Kiri 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.
D. Todd*	AG	The genetic effects and consequences of selection for processing potential in the early generations of a potato breeding programme.
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.
Jane Wishart	MBN	Characterisation of *Meloidogyne* species using molecular and immunological techniques.
C-P. Witte	PB	Modification of urea metabolism in transgenic potato.
Joanna 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

Short-Term Workers and Visitors

Name	Country of origin	Unit	Month/yr of arrival	Length of stay
Jelena Andrejeva	Estonia	Vir	Aug 98	1 year
L. Bidel	France	SPD	Aug 99	2 weeks
N. Bishimbaeva	Kazakhstan	GE	Nov 99	1 month
G. Bishop	Australia	BioSS	Apr 99	1 month
Pilar Blanco	Spain	GE	Nov 98	2 years
Karen Bollan	UK	AG	Feb 99	3 months
S. Carmichael	UK	GE	Feb 00	3 months
H. Chenia	S. Africa	MBN	Nov 99	1 month
Catherine Chesney	France	MBN	Feb 99	6 months
F. Cillo	Italy	Vir	Apr 99	1 year
C. Ckerblom	Sweden	BioSS	Sep 99	32 months
S. Czarnes	France	SPD	Sep 98	1 year
M. Daconceicao	France	MBN	Jul 99	6 weeks
Catherine Dolan	Ireland	MBN	Jul 99	2 weeks
Emma Easton	UK	GE	Feb 00	3 months
H. El-Menaie	Kuwait	AG	May 99	3 months
Debbie Feeney	UK	SPD	Mar 99	2 months
F. Fleischmann	Germany	MBN	Oct 99	2 weeks
B. Gillard	UK	SPD	Mar 99	1 month
C. Gillespie	UK	BioSS	Jul 99	2 months
S. Godfrey	UK	GE	July 99	6 weeks
S. Gordon	UK	MBN	Jan 99	5 months
S. Grazzani	Italy	PB	Feb 99	4 months
E. Grenier	France	MBN	Nov 99	3 months
M. Helmy	Egypt	AG	Apr 99	1 month
L. Hopp	Germany	SPD	Dec 99	2 weeks
A. Howden	UK	AG	Jun 99	3 months
K. Hrubikova	Slovakia	AG	Jan 99	1 month
J.S. Hsich	Taiwan	AG	Sep 99	1 year
M. Hughes	UK	GE	Oct 99	2 months
K. Hyun Soon	Korea	PB	Jun 99	1 month
Päivi Immonen	Finland	Vir	May 99	3 months
Helen Jacobs	UK	SPD	Feb 00	3 years
T. Jung	Germany	MBN	Jun 99	2 weeks
Natalia Kalinina	Russia	Vir	Mar 00	3 months
Thirumala Kanneganti	India	Vir	Jan 00	6 weeks
Petra Lepka	Germany	MBN	Jun 99	2 weeks
S. Lopez	Spain	PB	Sep 99	4 months
Lynne Meikle	UK	CG	Jul 99	10 weeks
J. Nechwatal	Germany	MBN	Jun 99	2 weeks
Kulpash M. Nurkiyanova	Kazakhstan	Vir	Oct 99	6 months
Klara Nyerges	Hungary	Vir	Jul 99	1 month
C. Olsson	Sweden	MBN	Jun 99	2 weeks
W. Oßwald	Germany	MBN	Oct 99	2 weeks
R. Palmer	UK	MBN	Jun 99	3 months
Yang Peilong	China	Vir	Apr 99	2 months
Vlada Peneva	Bulgaria	MBN	Apr 99	6 weeks
Taina Pennanen	Finland	SPD	Feb 00	10 months
G. Pillay	S. Africa	MBN	Jul 99	2 weeks
Maria Plekhanova	Russia	Vir	Sep 99	2 weeks
Delphine Pointel	France	Vir	Jun 99	3 months
F. Romaneix	France	PB	Jun 99	4 months
J. Rouault	France	BioSS	Sep 99	1 year
Gosia Ryback	Germany	MBN	Nov 99	2 weeks
P. Saldarelli	Italy	Vir	Feb 99	3 weeks
T. Sasaya	Japan	Vir	Oct 98	1 year
S. Sattar	UK	SPD	Apr 99	4 months
Alexandra Schlenzig	Germany	MBN	Jun 99	2 months
R. Shaik	S. Africa	MBN	Nov 99	1 month
P. Shaw	UK	MBN	Jun 99	4 months
Jacqui Sheridan	UK	MBN	Feb 99	2 months
A. Sletten	Norway	MBN	Sep 99	3 months
Darja Stanic	Slovenia	Vir	Mar 99	2 weeks
J. Staub	USA	AG	Apr 99	6 months
L. Terradot	France	Vir	Apr 99	2 months
H. de Turckheim	France	GE	July 99	6 weeks
K.S. Varaprasad	India	MBN	Aug 98	6 months
P. Veronico	Italy	MBN	Aug 99	3 months
Zhou Xueping	China	Vir	Oct 99	1 month
J. Zheng	China	MBN	May 99	5 month
X. Zhou	China	MBN	Oct 99	1 month

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.G. Bengough	Member	Scottish Soils Discussion Group
Vivian C. Blok	Committee Member	AAB Nematology Group
B. Boag	Member	UK National Committee for Biodiversity
J.E. Bradshaw	Chairman	Potato Section of EUCARPIA
R. Brennan	Secretary	Organising Committee for 8th International *Rubus* & *Ribes* Symposium
D.J.F. Brown	Co-Chairman	Russian Society of Nematology International Symposium
	Member	American Society of Nematology Ad Hoc Committee, International Federation of Nematology
	Member	European & Mediterranean Plant Protection Organization Ad Hoc Committee, *Xiphinema americannum* group nematodes
H.V. Davies	Member	EU Scientific Committee on Plants
	Member	CHABOS Scientific Sub-Committee
	Member	MLURI Promotion Board
R.P. Ellis	Member	BSPB Cereal Crop Group
	BSPB Representative	Scottish Agricultural College Recommended List Advisory Committee
D.A. Elston	Chairman	Royal Statistical Society, Highlands Local Group
B.P. Forster	Co-ordinator (4H)	International barley chromosome committee
	Committee member	EU COST, Gametic embryogenesis
	External Examiner	M.Phil. Biotechnology, University of Reading
G.J. Gibson	Member	BBSRC, Mathematics & Modelling of Agriculture & Food Systems Panel
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
	Member	RSS 2001 Conference Committee
C.A. Hackett	Council Member	British Region, Biometrics Society
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
J.R. Hillman	Chairman	SCRI/SASA/SAC Liaison Group
	Chairman	Tayside Biocentre Group
	Deputy Chairman	Board of Directors, Mylnefield Research Services Ltd
	Member	Committee of Heads of Agricultural and Biological Organisations in Scotland
	Member	ECRR Board of Management
	Member	SNSA Adviser to Committee
	Member	Court of University of Abertay Dundee
	Member	Senate, University of Dundee
	Member	University of Strathclyde Sub-Board for the Degree of B.Sc. in Horticulture
	Member	Tayside Economic Forum
	Member	PSRE Network Steering Committee
	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	Royal Statistical Society, Highlands Local Group Committee
E.A. Hunter	Member	Scientific Committee of Agro-Industries Conference, Pau, January 2000
	Member	RSS local (Edinburgh) committee
A.T. Jones	Member	Organising Committee for 8th International *Rubus* & *Ribes* Symposium
S.A. MacFarlane	Member	AAB Virology Group
W.H. Macfarlane Smith	Member	BBSRC Joint Committee on Health & Safety
	Member	BSPB Oilseed & Industrial Crops Group
	Member	ECRR PR Officers' Group
	Member	SABRI Safety Officers' Group
	Member	NPTC Plant Variety Development Panel
	Member	Organising Committee EUCARPIA 2001
G.R. Mackay	Member	Steering Committee of Global Initiative on Late Blight
	President	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	Member	BBSRC GAIT Initiatve Panel
	Member	BBSRC Agri-Food Committee Network Group
	Member	SIMBIOS Management Group
M.A. Mayo	Secretary	International Committee on Taxonomy of Viruses (ICTV)
	Member	Advisory Committee on Releases to the Environment (ACRE)
	Chair	Plant Virus Sub-committee of ICTV
	Member	Virus database Sub-committee of ICTV
	Member	IUBS/IUMS International Commission on Bionomenclature
U.M. McKean	Joint Chair	Scottish Agricultural Librarians' Group
R.J. McNicol	Convenor	Organising Committee for 8th International *Rubus* & *Ribes* Symposium

Name	Position	Committee or Organisation
I.M. Morrison	Member	SCI Agriculture & Environment Committee
	Member	COST 814-II Alternative Fibre Crops Group
	Member	NFUS, Energy & Industrial Cropping Group
A.C. Newton	Member	Crop Protection in Northern Britain Organising Committee
	Member	United Kingdom Cereal Pathogen Virulence Survey Committee
	Web Server Manager	British Society for Plant Pathology
	National Representative	COST 817 Airborne Pathogens on Management Committee on Cereals
G. Ramsay	Member	UK Potato Quarantine Unit Review Committee
K. Ritz	Member	BBSRC Plant & Microbial Sciences Committee Network Group
D.J. Robinson	Member	DETR Advisory Committee on Releases to the Environment
	Member	ICTV Tobamovirus & Tobravirus Study Group
	Member	ICTV Umbravirus Study Group
G.R.Squire	Chairman	CHABOS Working Group on Vegetation Dynamics
	Project Coordinator	SERAD Coordinated Programme in Dynamics of Species – Rich Vegetation
S.E. Stephens	Joint Chair	Scottish Agricultural Librarians' Group
	Member	Information Services Group - Scottish Library Association
	Member	Tayside and Fife Library and Information Network
	Working Group Member	British Research Institutes Serials Consortium (BRISC)
	Forum Group Member	Research Councils Library and Information Consortium (RESCOLINC)
M. Talbot	Chairman	Inter-departmental Statisticians Group, UK Plant Varieties & Seeds Committee
	Member	Statistics Committee of International Seed Testing Association
	Member	Technical Working Party on Automation and Computer Programs, International Union for the Protection of Plant Varieties
	Member	Royal Statistical Society Statistical Computing Committee
	Secretary/Treasurer	Edinburgh Centre for Rural Research
M. Taliansky	Member	Plant Virus Subcommittee, International Committee on Taxonomy of Viruses (ICTV)
	Chairman	Umbravirus Study Group, International Committee on Taxonomy of Viruses (ICTV)
Lesley Torrance	UK Representative	COST Action 823 'New Technologies to improve phytodiagnosis in Europe'
	Member	ICTV Plant Virus Sub Committee
	Chair	ICTV Furovirus and Allies Study Group
	Member	Cooperation in Science and Technology Management Committee
I. Toth	Member	Board of Directors, British Society of Plant Pathology
R. Viola	Organiser	COST 49 Workshop on Algal Polysaccharides, Dundee
R.E. Wheatley	Member	Plant Microbe Interaction Group Committee, Society for Applied Microbiology
B. Williamson	Treasurer	Association for Crop Protection in Northern Britain
	Treasurer	Organising Committee for 8th International *Rubus* & *Ribes* Symposium
	Member	Committee for 12th International *Botrytis* Symposium
F.G. Wright	Member	BBSRC/EPSRC Bioinformatics Panel
	Member	BBSRC Collaborative Computational Project CCP11 in Biosequence and Structure Analysis

Editorial Duties

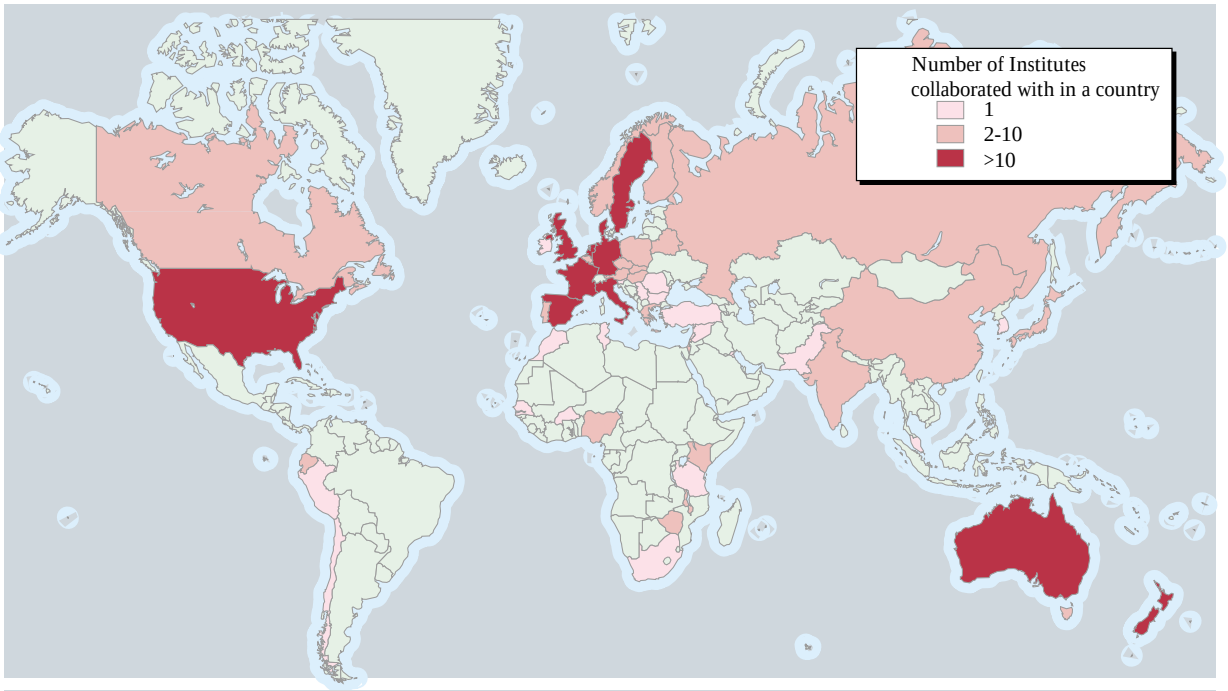
Name	Position	Journal Title
H. Barker	Editor	*Descriptions of Plant Viruses*
	Editorial Board	*Annals of Applied Biology*
A.G.Bengough	Editor	*Annals of Botany*
B. Boag	Editorial Board	*Annals of Applied Biology*
	Editorial Board	*Nematologia Mediterranea*
	Editorial Board	*Journal of Nematology*
D.J.F. 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 Board	*Plant Journal*
M.F.B. Dale	Editor	*Annals of Applied Biology*
H.V. Davies	Board Member	*Phytochemistry*
G.J. Gibson	Associate Editor	*INA Journal of Mathematics Applied in Medicine & Biology*
C.A. Glasbey	Associate Editor	*Biometrics*
	Associate Editor	*Journal of Royal Statistical Society, Series B*
B.S. Griffiths	Editorial Board	*Pedobiolgia*
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	Editorial Board	*British Journal of Nutrition*
E.A. Hunter	Editorial Board	*Food Quality & Preference*
A.T. Jones	Editor	*Descriptions of Plant Viruses*
D.K.L. MacKerron	Associate Editor	*Journal of Horticultural Science*
	Editorial Board	*Euphytica*
B. Marshall	Editor	*European Journal of Agronomy*
M.A. Mayo	Editorial Board	*Virology*
	Editorial Advisory Board	*Encyclopedia of Virology*
	Editor	*Archives of Virology*
	Editorial Advisory Board	*Encyclopedia of Life*
I.M. Morrison	Executive Editor	*Journal of the Science of Food and Agriculture*
R. Neilson	Deputy Chief Editor	*Russian Journal of Nematology*
P. Palukaitis	Senior Editor	*Molecular Plant-Microbe Interactions*
	Associate Editor	*Virology*
	Editorial Board	*Journal of General Virology*
M.S. Phillips	Associate Editor	*Journal of Nematology*
K. Ritz	Subject Editor	*Soil Biology Biochemistry*
	Editorial Board	*FEMS Microbiology Ecology*
D.J. Robinson	Editorial Board	*Journal of Virological Methods*
	Editor	*Descriptions of Plant Viruses*
G.R. Squire	Editorial Board	*Experimental Agriculture*
	Advisory Board	*Crop Physiology Abstracts*
D.L. Trudgill	Advisory Board	*European Journal of Plant Pathology*
	Editorial Board	*Nematologica*
	Editorial Board	*Fundamental and Applied Nematology*
	Associate Editor	*Journal of Nematology*
B. Williamson	Deputy Chairman	*Annals of Applied Biology*
F.G. Wright	Editorial Board	*Heredity*

Awards and Distinctions

Name	Unit	Degree/Award/Distinction/Appointment
W.W. Christie	PB	MBE for Services to SCRI
W.W. Christie	PB	Fellow of the Royal Society of Edinburgh
P. Palukaitis	Vir	Adjunct Professor, Cornell University, USA
P. Palukaitis	Vir	Visiting Professor, Agricultural University of Athens, Greece
R.Viola	PB	External Associate Professor, University of Parma, Italy
D.J.F. Brown	MBN	D.Sc., Open University
M.R. Armstrong	MBN	Ph.D., University of Dundee
Nicole Augustin	BioSS	Ph.D., University of St. Andrews
O. Brendel	SPD	Ph.D., University of Newcastle
S.J. Ferris	BioSS	Ph.D., University of Edinburgh
Rachel Fewster	BioSS	Ph.D., University of St. Andrews
C.S. Jones	PB	Ph.D., University of Dundee
S. Hameed	Vir	Ph.D., University of Dundee
P. Lava Kumar	VS	Ph.D., University of Sri Venkateswara, India
D. Milbourne	Genom	Ph.D., University of Dundee
R. Neilson	MBN	Ph.D., University of Dundee
A. Perera	Genom	Ph.D., University of Dundee
Alison Roberts	CB	Ph.D., University of Dundee
Louise V.T. Shepherd	PB	Ph.D., University of Dundee
Nicole Soranzo	Genom	Ph.D., University of Dundee
G. Walker	BioSS	Ph.D., University of Leeds
Fiona C.M. Brown	PB	M.Phil., University of Abertay Dundee
Theresa Ower	IT	M.Sc., University of Abertay
Jennifer Watters	GE	B.Sc. (Hons), Open University
Alison G. Dobson	EGFR	H.N.D. Horticulture
I. Toth/L.J. Hyman	MBN	Sir Ian Wood Award for Scottish Innovation
J.S. Swanston	AG	NEBOSH General Certificate
T.A. Mason	EGFR	S.V.Q. 2 Intensive Crop Production
A.C. Fuller	EGFR	S.V.Q. 2 Intensive Crop Production
Gillian G. Pugh	EGFR	S.V.Q. 2 Intensive Crop Production

Dr Bill Christie receives the MBE for Services to SCRI

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 1999 alone, SCRI collaborated with 104 organisations in 42 countries and, within the UK, collaborated with over 100 organisations.

SCRI Research Programme
1999-2000

SERAD funded research programme showing: SERAD 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/444/95	ROA Low temperature stress in *Ribes, Rubus* and other woody genera	McNicol R J
SCR/445/95	ROA Collection and evaluation and genetic resources of *Rubus, Ribes* and *Fragaria*	McNicol R J
SCR/449/95	ROA Advanced information techniques for the study and management of vegetation systems	MacKerron D K L
SCR/452/95	ROA Genetic architecture of tetraploid potatoes and production of enhanced germplasm	Bradshaw J E
SCR/454/95	ROA Structure of soil microbial and faunal communities, their interaction with vegetation and the relationship to soil processes and health	Griffiths B S
SCR/456/95	ROA Genetics and ecophysiology of abiotic stress tolerance in *Hordeum vulgare* (barley) and *Arabidopsis thaliana*	Forster B P
SCR/462/96	ROA Molecular mechanisms of plant virus replication and movement and the effects of resistance genes on these processes, using cucumoviruses and tobraviruses as contrasting model systems	Palukaitis P F
SCR/464/96	ROA Biochemical and molecular control of carbohydrate metabolism and the modification of starch structure in potato	Davies H V
SCR/471/96	ROA Mathematical analysis of dynamics and scaling in heterogeneous and hierarchially coupled systems I: the soil/microbe complex	Crawford J W
SCR/479/96	ROA Maintenance, improvement, evaluation and exploitation of biodiversity in germplasm collections of potato	Mackay G R
SCR/481/96	ROA Evaluation, improvement, maintenance and exploitation of biodiversity in germplasm collections of brassicas for improved pest resistance (particularly cabbage and turnip root flies) and nutritional value	Birch A N E
SCR/482/96	ROA Detection, identification, genetic variation and ecology of virus and insect, mite and nematode pests and virus vectors, especially of soft fruit crops, and strategies for their effective control	Jones A T
SCR/483/96	ROA Soft rot erwinias and blackleg disease: aetiology, epidemiology and pathogenicity, selection of resistant potato cultivars and their mechanisms of resistance	Lyon G D
SCR/487/96	ROA Mathematical analysis of dynamics and scaling in heterogeneous and hierarchially coupled systems II: complex biochemical networks	Crawford J W
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/495/97	ROA Transcriptional and post-transcriptional regulation of plant gene expression	Brown J W S
SCR/496/97	ROA Production of novel diagnostic reagents, in particular genetically engineered antibody-like proteins and investigation of their potential for use in research, biotechnology and diagnosis	Torrance L
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/499/97	ROA Free radical processes in plants and plant-derived foods	Davies H V
SCR/501/97	ROA Develop and operate methods for the detection and quantification of genetic resistance to a wide range of economically important fungal and bacterial pathogens of potato	Bradshaw J E
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.	Trudgill D L
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	Young I M
SCR/526/99	ROA Integrative mapping of the long arm of barley chromosome 5H	Machray G C
SCR/527/99	ROA Development of a graphical database for the visualisation of genotypic and phenotypic data in barley	Thomas W T B
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/529/99	ROA Phenotypic optima in the maintenance of physiological and genetic diversity in plant communities	Squire G R
SCR/530/99	ROA Measurement of the natural abundances of the stable isotopes of nitrogen ($\delta^{15}N$) in ammonium of soils and natural waters	Handley L L
SCR/531/99	ROA Chemical strategies for the study of leaf surface waxes and toxic glucosides of potato, brassica and soft fruit in plant-insect interactions and the inter-relationships of toxic glucosides with product quality	Griffiths D W

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
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	Trudgill D L
SCR/488/96	FF Modelling soil-water/structure functions to assess the efficiency of pesticides in agricultural soils against pathogenic nematodes	Young I M
SCR/505/97	FF Molecular approaches to manipulate the development and composition of strawberry fruit	Davies H V
SCR/506/97	FF Use of stable isotope techniques to determine the origin, movement and effects of nitrate in the catchment area of the river Ythan	Handley L
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
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/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	Robertson W M
SCR/821/96	FF Exploitation of novel and known lectins in agricultural and biological research - an interdisciplinary approach to improve crop protection and productivity, animal (including human) welfare and health	Stewart D
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
SCR/823/97	FF Significance of physical heterogeneity for scaling of solute chemistry in soils from fine scale to subcatchment	Crawford J W
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	Santa Cruz S

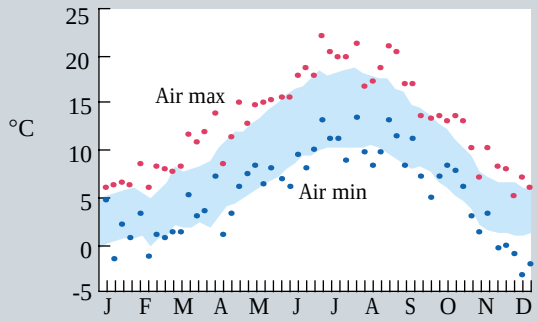
SCR/831/98	FF Insect-resistant transgenic plants in tri-trophic interactions: ecological, biochemical and molecular impact on aphiophagus ladybird predators	A N E Birch
SCR/832/99	FF Identification and assessment of nutritional relevance of antioxidant compounds from soft fruit species	Davies H V

Meteorological Records

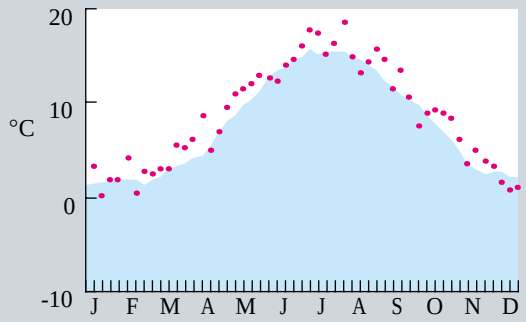
D.K.L. MacKerron

Detailed meteorological records are kept regularly at SCRI. The graphs shown are for weekly values for 1999 and the long term average for 1961-1990( ).

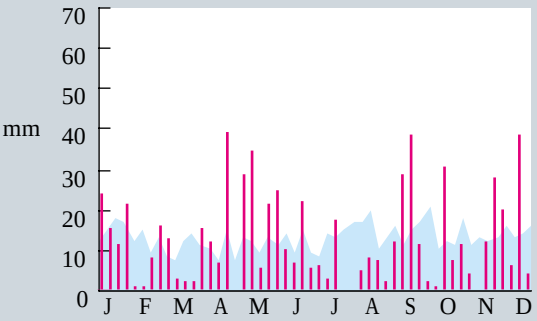
Air temperature



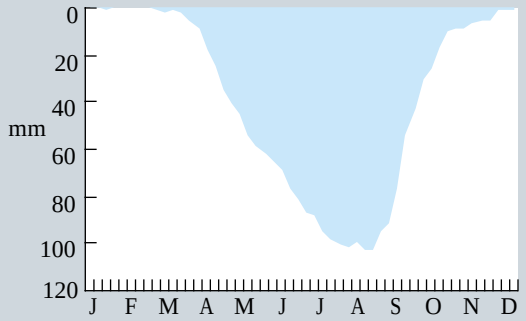
10cm soil temperature



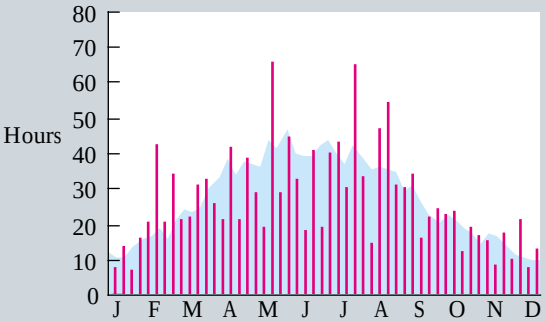
Rainfall



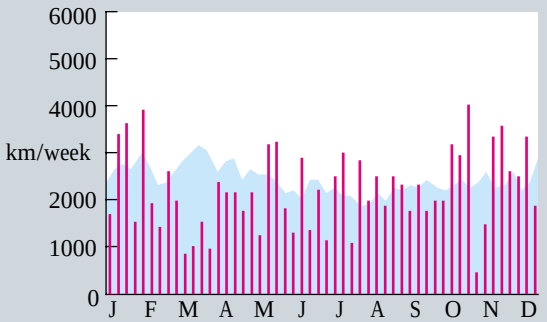
Soil moisture deficit *



Sunshine



Run of wind



* 1999 values for SMD not available

Cumulative Index 1990 - 1999/00

In addition to the list below, in every SCRI Annual Report during this period, there are reports of Mylnefield Research Services Ltd; the Research Services; a General Report including accounts, staff lists, publications, research project lists; Overviews by each Head of Department; and a Report by the Director.

Plant genetics

Breeding and genetics (1997/8 onwards)

Soft fruit and perennial crops

Molecular biology

Plant molecular and cell biology (1997/8 onwards)

Cellular and environmental physiology

Chemistry

Plant biochemistry and phytochemistry (1997/8 onwards)

Fungal and bacterial diseases

Plant viruses

Zoology

Biomathematics and Statistics Scotland

Host pathogen interactions and crop protection

(1997/8 onwards)

Plants, soils and environment (1997/8 onwards)

Other Articles

Institutes supported by the Biotechnology and Biological Sciences Research Council

BBSRC Office	Polaris House, North Star Avenue, Swindon, Wilts SN2 1UH	01793-413200
BBSRC Bioscience IT Services	West Common, Harpenden, Herts AL5 2JE	01582-714900
Babraham Institute	Babraham Hall, Babraham, Cambridge CB2 4AT	01223-496000
Laboratory of Molecular Signalling	Babraham Institute, P.O. Box 158, Cambridge CB2 3ES	01223-336600
Institute for Animal Health	Compton, Near Newbury, Berkshire RG20 7NN	01635-578411
Compton Laboratory	Compton, Near Newbury, Berkshire RG20 7NN	01635-578411
Pirbright Laboratory	Ash Road, Pirbright, Woking, Surrey GU24 0NF	01483-232441
BBSRC & MRC Neuropathogenesis Unit	Ogston Building, West Mains Road, Edinburgh EH9 3JF	0131-667-5204
Institute of Arable Crops Research	Harpenden, Herts AL5 2JQ	01582-763133
IACR - Long Ashton Research Station	Long Ashton, Bristol BS18 9AF	01275-392181
IACR - Rothamsted	Harpenden, Herts AL5 2JQ	01582-763133
IACR - Broom's Barn	Highham, Bury St. Edmunds, Suffolk IP28 6NP	01284-812200
Institute of Food Research	Norwich Research Park, Colney, Norwich NR4 7UA	01603-255000
Reading Laboratory	Earley Gate, Whiteknights Rd, Reading RG6 6BZ	01189-357000
Institute of Grassland and Environmental Research	Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB	01970-828255
Aberystwyth Research Centre	Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB	01970-828255
North Wyke Research Station	Okehampton, Devon EX20 2SB	01837-82558
Bronydd Mawr Research Station	Trecastle, Brecon, Powys LD3 8RD	01874-636480
Trawsgoed Research Farm	Trawsgoed, Aberystwyth, Dyfed SY23 4LL	01974-261615
John Innes Centre	Norwich Research Park, Colney, Norwich NR4 7UH	01603-452571
Roslin Institute	Roslin, Midlothian EH25 9PS	0131-527-4200
Silsoe Research Institute	Wrest Park, Silsoe, Bedford MK45 4HS	01525-860000
Horticultural Research International	Wellesbourne, Warwick CV35 9EF	01789-470382
HRI, East Malling	West Malling, Maidstone, Kent ME19 6BJ	01732-843833
HRI, Wellesbourne	Wellesbourne, Warwick CV35 9EF	01789-470382

Scottish Agricultural and Biological Research Institutes

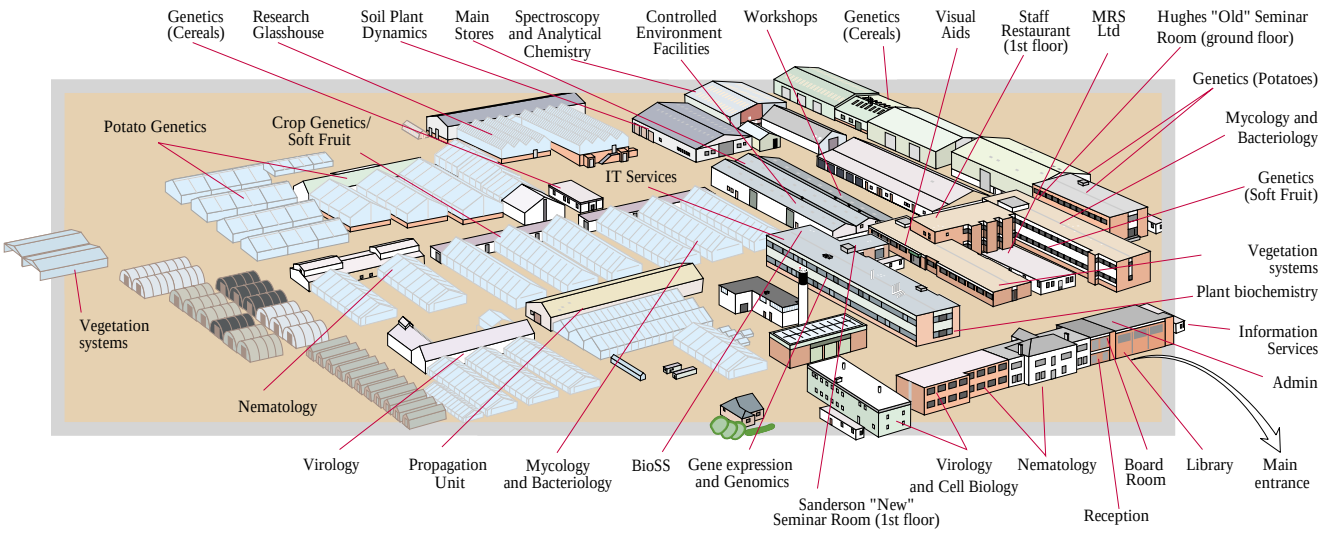
Hannah Research Institute	Ayr, Scotland KA6 5HL	01292-476013
Macaulay Land Use Research Institute	Craigiebuckler, Aberdeen AB9 2QJ	01224-318611
Moredun Research Institute	Pentlands Science Park, Bush Loan, Penicuik, Midlothian EH26 0PZ	0131-445-5111
Rowett Research Institute	Greenburn Road, Bucksburn, Aberdeen AB21 9SB	01224-712751
Scottish Crop Research Institute	Invergowrie, Dundee DD2 5DA	01382-562731
Biomathematics and Statistics Scotland (Administered by SCRI)	University of Edinburgh, James Clerk Maxwell Building, King's Buildings, Mayfield Road, Edinburgh EH9 3JZ	0131-650-4900

List of Abbreviations

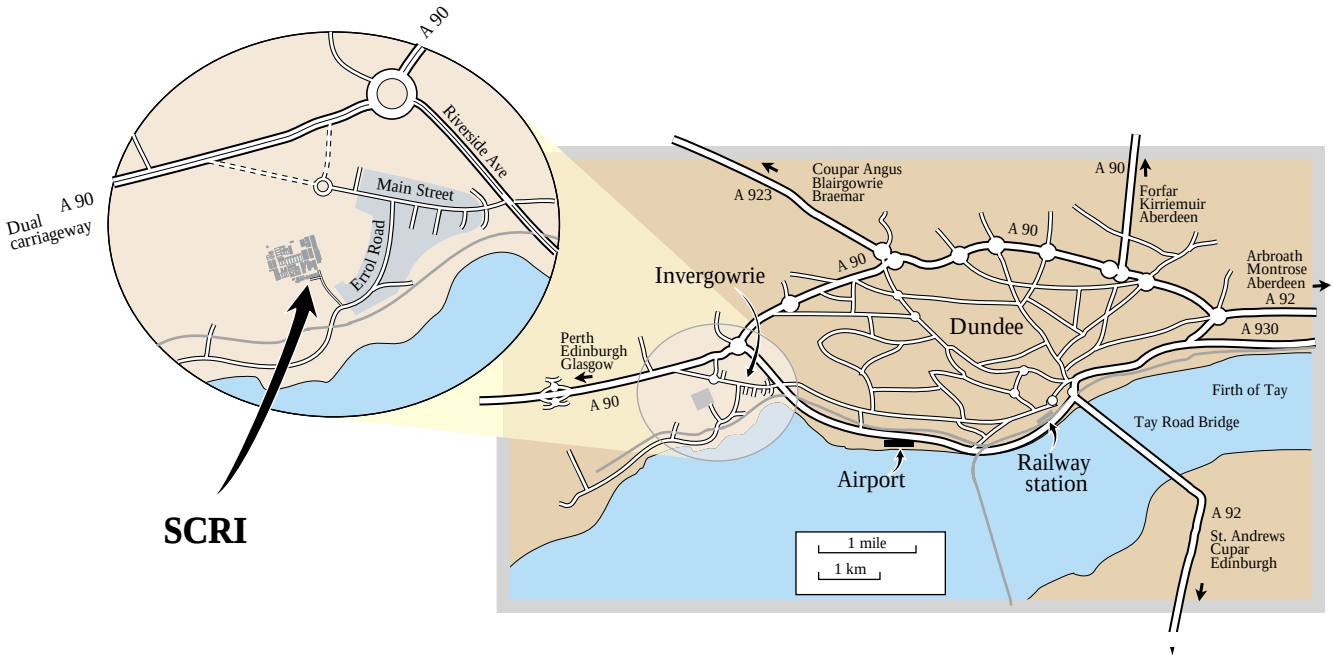
AAB	Association of Applied Biologists
ACRE	Advisory Committee on Releases to the Environment
ADAS	Agricultural Development and Advisory Service
BBSRC	Biotechnology & Biological Sciences Research Council
BCPC	British Crop Protection Council
BioSS	Biomathematics and Statistics Scotland
BPC	British Potato 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
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 (SEARAD)
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
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
SERAD	Scottish Executive Rural Affairs Department
SET	Scottish Enterprise Tayside
SNSA	Scottish Nuclear Stocks Association
SPD	Senior Post-doctorate
SSCR	Scottish Society for Crop Research
SSFG	Scottish Soft Fruit Growers Ltd
STS	Sequence Tagged Sites
UNDP	United Nations Development Programme
WHO	World Health Organisation

The Scottish Crop Research Institute

Site plan



Access to Scottish Crop Research Institute



SCRI is on the east coast of Scotland, midway between Edinburgh and Aberdeen.

It is located at Invergowrie 6km west of the centre of Dundee. Access is via Riverside Avenue, Main Street and Errol Road.

British Rail has direct InterCity services between Dundee and London, Edinburgh and Glasgow and other UK cities.

Flights are available to Dundee Airport from Edinburgh, Manchester and Aberdeen, and scheduled services operate from many domestic and international destinations to Edinburgh and Glasgow.