# Scottish Crop Research Institute

# Annual Report 1997/98



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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 knowl-

edge 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

die Grundkenntnisse in gesetzt. 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 obietti-

vo 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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# Introduction by the Director

#### John R. Hillman

The Scottish Crop Research Institute (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, SCRI assumed managerial responsibility for the Scottish

Agricultural Statistics Service (SASS), recently renamed Biomathematics & Statistics Scotland (BioSS), and received a transfer of posts from the former Macaulay Institute for Soil Research as recommended in the 1985 Department of Agriculture and Fisheries for Scotland (DAFS) Agricultural Research and Development strategy document.

# Plants - promoting the creation and protection of wealth and the quality of life.

#### The Mission of SCRI is:

to sustain excellence and our international reputation for strategic research in crop, plant and related sciences, and to facilitate the application of new knowledge to end-user industries.

#### The Aims of SCRI are:

- \* to provide a major international centre for research of the highest quality on agricultural, horticultural and industrial crops important to northern Britain and the rest of the World, by sustaining a broad, yet fully integrated programme of fundamental, strategic and applicable research designed to contribute to, and complement other sectors of the UK science base;
- \* to increase fundamental knowledge in the biological sciences while improving crop quality, utility and value through the application of conventional and novel molecular genetic breeding techniques and improved agronomic practices, and by developing more sustainable, environmentally sensitive methods to protect crops from depredations by pests, pathogens and weeds;
- \* to create wealth and protect investment in our essential plant-based industries by exploiting the advantages and solving the problems of crop production in northern Britain while seeking to improve the quality of life and safeguard the global environment;
- \* to promote public awareness and understanding of relevant environmental and bioscience issues through technical and lay publications and targeted presentations;
- \* to encourage, train and reward staff with relevant skills in crop genetics, plant biotechnology and physiology, chemistry, plant pathology, biomathematics and environmental studies, agronomy and the field trialling of new crop varieties.

SCRI is a non-profit-making limited company established under the Companies Act, has charitable status and is a Non-Departmental Public Body because over 50% of the total funding is received as grant-in-aid from Scottish Office Agriculture, Environment and Fisheries Department (SOAEFD, formerly DAFS) and all members of the Governing Body are appointed by the Secretary of State for Scotland. Staff are not formally civil servants, but are members of the SOAEFD Superannuation Scheme, and SOAEFD 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 SOAEFD. The Pay and Grading System, and Staff and Management Codes are administered by the Biotechnology and Biological Sciences Research Council (BBSRC). The mission and aims of SCRI are presented in Table 1.

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 the range of complementary skills assembled at the Institute, from fundamental molecular genetics to glasshouse- and field-trialling of potential and finished varieties of crops, is not to be found elsewhere within any civil or private sector agri-business centre in the UK or Europe.

mental and strategic research, and technology transfer are unique 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. See Figure 1 for management structure.

Genetics and enhanced breeding of selected crops, in an area of high phytosanitary standards, 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. Close 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 the commercial arm of the Institute, Mylnefield Research Services (MRS) Ltd (Managing Director, N W Kerby), 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.

SCRI also 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. SSCR achieves this by:

- organising interactive field walks and enduser/researcher discussion sessions:
- financing science-based advisory publications for the benefit of its members;
- stimulating crop-based sub-committees to support targeted research projects [eg breeding, selection and trialling, of spring malting barleys adapted to the Scottish climate];
- reinforcing SCRI representation with trade associations, Levy Boards, and other user-groups;
- administering 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

#### Introduction

Commission Research Agency, comprise the Committee of Heads of Agricultural and Biological Organisations in Scotland (CHABOS).

BioSS (Director, R A Kempton) 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.

Annual Reports, this Report details only a small selection of the 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, the associated Friendly Society. Significant advances continue to be made in both fundamental and strategic science, with

contributions to the protection and understanding of the environment. Discoveries are reported of direct and indirect benefit to agriculture, horticulture, forestry, land management and biotechnology. Dedicated and talented scientific and support staff in every department and section of the Institute, and BioSS, and MRS Ltd., account for our stature, successes and delivery of achievements. One important change introduced last year and applied to this and future SCRI Annual Reports is, with the exception of the publications listing, the transfer of reporting from the calendar to the financial year.

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 SOAEFD for their continu-

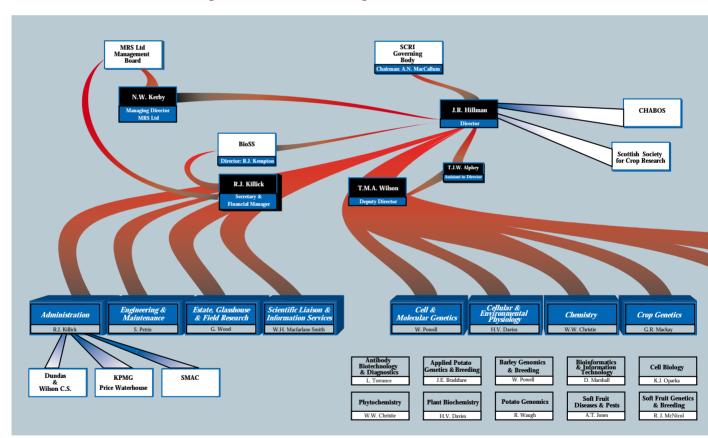
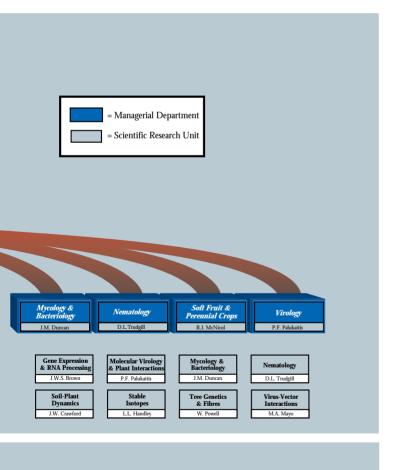


Figure 1 SCRI Departmental and Research Unit structure.

ing 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 CHA-BOS 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 remains buoyant in generally very difficult times for science in the UK, justifying its existence in every respect. We have every confidence in meeting future challenges. Scientifically 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\*

#### **Preamble**

Irrespective of the fact that 1997-1998 was a period of dynamic scientific advances, some of which had direct economic benefits, scientific research itself was under considerable financial pressure. The public sector in Europe seemed unable to show long-term commitment to research programmes and to the scientists themselves. In some countries, the status of scientists was adversely affected both by regulatory failures and by profound ignorance of the rôle of science in modern society. Private-sector and charitable support of research and development (R&D), however, increased substantially, particularly in the life sciences. Global economic output, agricultural output, and the world's population continued to grow, but the slump in Asia was starting to take effect on western economies.

Molecular biologists at the University of Washington reported that by the end of 1997, partial genetic sequences from 40,000-50,000 human genes -approximately half the total - had been recorded in databases world-wide. The detailed sequencing of the three billion base-pairs of the human genome was just beginning, with only about 2% of the total analysed by the end of the year. Contrasting with the human genome, however, the relatively small genomes of *Escherichia coli*, a yeast, and 11 other micro-organisms were completely sequenced.

Analysis of a 379-base-pair sequence of maternally inherited mitochondrial DNA extracted from the humerus of a Neanderthal skeleton, thought to be between 30,000 to 100,000 years old, indicated that the Neanderthal DNA was unlikely to have contributed to the mitochondrial DNA pool of modern humans. This research at the University of Munich, confirmed at Pennsylvania State University, validated the separate-species status for Neanderthals proposed by William King in 1864. To assess properly the extent to which Neanderthal genes exist in the gene pool of modern humans, however, it will be necessary to analyse Neanderthal biparental nuclear DNA.

On 16 December 1997, the microelectronics industry commemorated its 50th birthday when William Shokley, John Bardeen, and Walter Brattain invented the transistor as an alternative to the vacuum tube. By

<sup>\*</sup>This review updates and enlarges on themes developed in my previous Reports of the Director in the SCRI Annual Report series.

1997, microprocessor technology was producing chips each containing as many as 7.5 million transistors, according to the Semiconductor Industry Association, and the projected world-wide sales of semiconductors reached \$138 billion.

The Internet and World Wide Web continued to change the face of the on-line access industry. Driven by new high-speed modems, the web was quickly becoming the interface to information retrieval, and has been a major tool in all research organisations. New telecommunications products and services were developed to provide the high-speed data networks needed to transfer information. A standard for 56 kilobits-per-second (kbps) modems was expected to be 1998 by agreed in the International Telecommunication Union. Meanwhile, digital sub-

scriber lines which would download at speeds of up to 245 kbps, and cable modems downloading at 40 Megabits per second, were becoming available. More than 10,000 magazines and journals became available on-line. 'Virtual shopping', the new Internet economy, is thriving. By the year 2000, according to the Gartner Group, global on-line computer sales will reach \$20 billion, an increase of 233% over 1998 levels of about \$6.1 billion. Jupiter Communications estimates that by 2000 there will be 33 million online shoppers compared with 16 million in 1998. Disintermediation has become redefined to include so-called 'frictionless capitalism' in which 'middlemen' and intermediaries such as travel agents,

car dealers, stockbrokers, insur-

ance salesmen, most shop assistants, and even solicitors dealing with routine transactions, will increasingly become redundant. Governments have yet to come to terms with regulatory and taxation aspects of on-line shopping which can by-pass oppressive spending taxation regimes.

In order to address the millennium computer 'bug' or 'timebomb', in which many computers and electronic devices may not read 2000 as a valid date, and cause

data corruption, programs to crash or even lead to instrument failure, various countries introduced radical measures to force programming checks and resolution of the problem. In the UK, the Government established Action 2000 (www.bug2000.co.uk) as the official campaign body, substantially upgrading its funding compared with its predecessor body, Taskforce 2000. Year 2000 compliance (Y2K) is now a key feature in audits of performance indicators of private and public-sector bodies. Research and instrumentation-based organisations face difficulties and potentially huge costs in judging Y2K compliance.

Mathematics and science achievement, learning opportunities for girls and adults, morals and ethics ('values'), international higher-education coalitions, comparative data on performance, the rôles and costs

of universities, and underemployment of graduateand postgraduate-level personnel, were key issues in education during 1997/1998.

The nature of randomness and its assessment in terms of approximate entropy or disorder was a particular feature of mathematical occupation in 1997. For chemists, the year was noted for the fact that official names were finally adopted for elements 101-109 on the periodic table after the International Union of Pure and Applied Chemistry modified its approach to priority of discovery. The resolution of the conflict over names of these elements, which were synthesised between the 1950s and 1980s, cleared

the way for naming the recently discovered elements 110, 111, and 112. Even so, confidence in the belief that the periodic table graphically depicts that many physical and chemical properties of elements recur in a systematic fashion with increasing atomic number, had been somewhat undermined over recent years with the realisation that elements 104 (rutherfordium) and 105 (dubnium) apparently departed from periodicity. In 1997, however, confidence in the so-called periodic law was restored when research on element



106 (seaborgium) at the Institute for Heavy Ion Research at Darmstadt concluded that seaborgium does indeed behave in the formation of compounds like lighter members of group 6 on the table, as periodic law predicts.

For physicists, 1997 was a particular year of achievement. At the Stanford Linear Accelerator Center, photons were arranged to interact with a pulsed beam of high-energy electrons, giving rise to pairs of electrons and positrons, thereby demonstrating for the first time the creation of matter from radiation. Several research groups were engaged in calculating the mass of the difficult-to-observe neutrino. The debate in quantum physics over the Einstein-Podolsky-Rosen paradox, relating to particle spin, was enlightened by research at the University of Geneva, where pairs of quantum mechanically entangled photons were separated and piped over optical fibres to two measurement sites several kilometres apart. Measurements at the two sites showed that each photon 'knew' its partner's state in less time than a signal travelling at the speed of light could have conveyed the information. This faster-than-light 'telepathy', as Einstein called it, is implicit in quantum theory, where the spin of a particle exists in all possible states simultaneously, and is not even defined until a measurement has been made on it. Therefore, if a measurement is made on one of two entangled particles, only at that very point in time would the state of the other be defined. The first atom laser was created at the Massachusetts Institute of Technology.

Terrestrial science, engineering and technology have derived wide-ranging benefits from space research, not least in miniaturisation, reliability, remote sensors and sophisticated data analyses. During the year, scientific instruments on the Earth-orbiting Hubble Space Telescope were upgraded; there were ten manned space launches and several unmanned space exploration probes and satellites; two additional moons were discovered in orbit around Uranus; and the Comet Hale-Bopp provided a spectacular celestial display. A close-up view of Mars was transmitted by the National Aeronautical and Space Agency's Pathfinder and its roving robot, Sojourner - the first moving vehicle ever deployed on another planet. The Galileo spacecraft came within 586 km of the Jovian moon Europa. Results of the survey by the Hipparcos satellite, launched in 1989 by the European Space Agency, included the determinations of positions of 100,000 stars with a precision two orders of magnitude better than those achieved on Earth; the positions of another one million stars were calculated with somewhat less precision. Another special outcome of the Hipparcos survey was a new determination of the distance to, and luminosity of, the Cepheid variable stars in the Milky Way. These stars are used to calibrate the distances to remote galaxies.

An extraordinary public outpouring of mass grief accompanied the death in a road accident of Diana, Princess of Wales. The press and television coverage was greater for this untimely death than for any other news event in the 20th century, including the major events of World War II. By contrast, scientific issues tended to be ignored or misrepresented.

The arts gained a prominent position politically and in the news media. Clinical analysis of artistic output in 1997, however, indicated worrying concerns. Stylistic plurality often incorporating technologically and interpretively puny skills; a stunning ignorance of science, engineering and technology; transgression of the boundaries of traditional artistic media; an insatiable quest for publicity, frequently involving images calculated to disturb; integration with performance art - especially film and video with rapidly changing images appropriate only for brief attention spans; collectively made the arts less relevant, more flippant and therefore more peripheral to mainstream life than hitherto. Such apparent dynamic evolution in the arts, no matter how superficial, contrasted with the retention by the arts profession of a conventional framework of exhibitions, shows and performances, and also with the attention they give to the reports of the critics. Many nations struggled to resolve the relationships between the overt and indirect uses of funds from the taxpayer for both the creative and evanescent not-so-creative arts, and the contribution of the subsidised arts towards wealth creation and the quality of life. The juxtaposition of the arts, design, science, technology, engineering, functionality, cultural and societal development, sustaining of cultural distinctions, literature, music, entertainment, and challenges to conventional thinking, are complex and were rarely analysed in the depth they deserve. Certainly, the brilliant and devastating hoax article published in 1996 by two physicists, Alan Sokal and Jean Bricmont, revealed the vacuity of much of postmodernist subjectivism. Nonetheless, it still seems that the arts are more likely than the sciences to provide the opportunity for the expression and recognition of unique genius as gauged by the responses of the public and politicians.

There are organisations trying to stimulate art/science collaborations, *e.g.* The Arts Catalyst, The Wellcome Trust, the Novartis Foundation, various universities *etc.*, but meetings often have faced translation problems both linguistically and philosophically. Few seem to appreciate the beauty of science compared with its actual and potential utility. Can artists address conceptually complex scientific models and hypotheses, and can there be a two-way dialogue between artists and scientists?

#### **Economics and Politics**

The financial year 1997-1998 registered the fastest growth in world output for the last 10 years edging up to 3%; the World Bank and the International Monetary Fund (IMF) forecast that the world economy per se in 1997 grew by slightly more than the 4% recorded in 1996. The inflation rate in most countries was relatively low or declining, and fiscal deficits were being brought under control as a result of tax remaining more buoyant than expected, and the effects of rigorous controls on public spending. Growth was particularly resilient in the USA, where an underlying trend of rapid increase in productivity enabled rapid growth of the gross domestic product (GDP) in tandem with low inflation. This so-called 'new economic paradigm' was referred to as the 'Goldilocks' economy - neither too hot nor too cold. In the European Union (EU), excluding the UK, the deflationary strictures imposed by the Treaty on European Union specifically in regard of a common currency (economic and monetary union - EMU) were coming to an end. Even so, the fiscal rigour meant that domestic demand was sluggish irrespective of low interest rates; the growth that took place was mainly in the export sector which derived competitive benefit from a strong US dollar and pound sterling. By March 1998, inflation in the euro-11 countries those joining EMU - was 1.2%, with an unemployment rate of 11.3%, and a positive trade balance of ecu 7.7 billion. The combined GDP of the euro-11 euro-zone countries was 80% of the GDP of the USA. Preparations were put in hand to establish the European Central Bank (ECB) by June 1998; its base would be in Frankfurt.

The UK economy gained momentum although the manufacturing sector showed signs of acute stress arising from the strength of sterling against the continental European currencies. The GDP of the Irish Republic grew at the fastest rate of any of the European economies. At 3.6% and 4.5% of GDP in 1996, according to the Organisation for Economic

Co-operation and Development (OECD), the state pension schemes in the Irish Republic and the UK were substantially below other EU countries which averaged 8.8%. Italy's pension outlays at 13.9% face drastic reform, not least in the era of EMU. The rôle of EU subsidies and payments started to come to the fore.

The Japanese economy faltered with GDP growth declining from 3.6% per year to 0.9% in 1997. The Bank of Japan was forced to bail out several bankrupt banks and inject liquidity into the Japanese economy. The growth rates in Australia and New Zealand moderated, but the economies of the former Soviet Union registered a growth rate of 1.2%, the first increase since they were constrained by central planning more than 8 years ago. China tried to combine strict political control with relatively relaxed economic freedom, leading to stresses in parts of the economy.

GDP is an inaccurate measure of a nation's prosperity. It does not, for example, distinguish between polluting and clean-technology industries. It also counts the cost of cleaning-up cumulative pollution as a positive addition to a nation's wealth. Indicators for sustainable development will need to include measurements of air, water and land pollution, production of 'greenhouse' gases that affect the climate, consumption of natural resources, imports and exports used to create economic wealth but which also produce waste and consume energy during transport. Future sustainable growth will need to produce more wealth with fewer resources. GDP measurements tend to overstate national income.

In the less-developed world, growth tended to be strong, with the exception of South-East Asia. Preliminary data indicate that the rate of growth in the less-developed countries (LDCs) remained at 6%, double that of the stronger more-developed countries (MDCs). There were encouraging signs of recoveries in the economies of Latin American countries and in some of the former communist countries that had pursued comprehensive stabilisation and reform policies. Russia became a full, permanent member of the Group of Seven (G7, now G8) of the major economic powers (the other members are Canada, France, Germany, Italy, Japan, UK and the USA).

Other groups in the world of international financial diplomacy in 1997-1998 included the G3, G10, G22 and G24. The G3 comprised Germany, Japan, and the USA - EMU may well give the G3 a formal rôle to discuss exchange rates. The G10 comprised the origi-

nal G7 plus Belgium, The Netherlands, Switzerland, and Sweden; these were contributors to the IMF credit line and their central bank governors met regularly in Basel. The G22, the Willard group, was a new USA-inspired group for one-off meetings, and comprised G8, plus Argentina, Australia, Brazil, China, Hong Kong, India, Indonesia, South Korea, Malaysia, Mexico, Poland, Singapore, South Africa, and Thailand. The G24 consisted of eight developing countries each from Africa, Asia, and Latin America the 24-member committee represented IMF and World Bank shareholders, and each committee member represented a single country or multi-country constituency. EMU and the soon-to-be-established ECB could create circumstances where individual European country representation would shrink on these international committees and be replaced by the ECB.

Inadequate reform policies impeded the development of Belarus and Slovakia, and high inflation was recorded in Belarus, Bulgaria, and Romania.

Recovery from the Mexican crises of 1995 was noted in South America, with Latin American economies achieving a 4% growth rate, but towards the end of the year, the Asian crisis was starting to affect the South American economies.

During the 1990s, the economies of the Pacific Rim recorded the fastest rates of growth, spreading from the first phase of 'tiger' economies (Hong Kong, Singapore, South Korea, and Taiwan) to other countries around the Pacific Rim and into South Asia. Structural changes took place in those economies, with movement away from agriculture and heavy industries to electronic consumer goods, clothing, fashion-trainer footwear, and automobiles. By the end of 1997, however, concern over current account deficits, trading ethics and processes, and restrained domestic growth, led to speculation-induced devaluation and signs of political unrest. This large-scale financial crisis started initially in Thailand, spread to Malaysia, the Philippines, Indonesia, and South Korea, indirectly affecting the large economies of China, India and Japan, For the western economies, there were portents of increased imports of even cheaper consumer goods from South Asia but severely reduced exports to that region, not least of agricultural exports.

Unemployment worsened in many EU countries and Japan, but improved to under 5% in the USA and to 5.2% in the UK, essentially expressing the differences

in the flexibility of labour market and political ideology. At a meeting of EU leaders in Luxembourg in November 1997, there was little agreement with the French direct interventionist solutions. The unemployment rate in Japan increased to more than 3.5% late in the year.

In 1997, the volume of world trade was projected to rise by 8% as import and export activities increased in the USA, and the export performance of most EU countries and Japan improved in tandem with their weakening currencies. There was, however, no significant improvement in the volume of trade in the LDCs. Nonetheless, with few exceptions, the LDCs did not experience severe problems in funding their current deficits and servicing existing loan portfolios, despite the fact that their current account deficit widened to a projected \$109 billion. Debt-servicing ratios, *i.e.* export earnings as a proportion of interest on total external debt, moved up to a projected 9.5%, reversing the decline that began in 1991.

Compared with the USA and UK where economic policy was framed to prevent the emergence of higher inflation rates, most countries in the EU adopted policies influenced by political rather than economic considerations. After the election of the new Labour government in May 1997, the Bank of England gained operational freedom through a Monetary Policy Committee to set interest rates to meet new inflation targets, a move welcomed by the IMF. Interest rates were raised five times to prevent the economy from overheating.

In the UK, ignoring the negative influences of the strong, possibly overvalued pound sterling, and tight public spending, the GDP rose by an estimated 3.5% compared with 2.4% in 1996. Consumer demand was aided by higher real incomes in which salaries and wages rose by 6.5% in money terms although publicsector salary increases were less than half this amount. Around £30 billion was received by members of mutual building societies that converted to banks. Contrasting with the 6% increase in private-sector investment spending, public-sector investments fell by 11%. Entry of the UK into EMU was ruled out by the new Government in view of the asynchrony of the UK economy with most of the economies of other EU members, and many of the features of the economic policy of the previous Conservative government were continued. Fiscal policy was subtly tightened in the July budget, which included a £3.5 billion 'windfall' tax on the privatised utilities to fund the new programme to reduce the number of young people unemployed. Abolition of tax credits on dividends was expected to raise £2.3 billion per year, and there were additional taxes on petrol and cigarettes, but there were no increases in headline-sensitive direct taxation. The appreciation of sterling led to an increase in the UK current account deficit.

Keynesian economic control regulates demand either by fiscal policy (tax and public spending), or by monetary policy (interest rates). Monetarist economic policy involves setting targets for the money supply, and setting interest rates to ensure these targets are achieved, as a consequence it is expected that inflation will be controlled. Modern growth theory ('post-neo-classical endogenous growth theory') relates to raising the growth rate of the economy by increasing the inputs of investment and education. The UK Government appeared to adopt components of all three forms of economic control.

From the UK's perspective, it has been argued that to be successful, a single currency demands a single monetary policy which is part of a single economic policy. The latter would be best achieved by a single government which in a democracy should be answerable to a single electorate. For EMU, there should be criteria, conditions and agreed processes not only for entry but also for withdrawal. Yet the introduction of the euro was decided primarily on political grounds viz. to provide further impetus for European integration. The entry criteria were fudged to permit a broad entry base. At the macroeconomic level, EMU might lead to six outcomes. (i) There will be a significant redistribution of wealth in the EU. Larger, internationally trading companies will benefit whereas smaller, localtrading companies will face costs and few overt benefits; prices across the EU countries will become transparent and comparable, leading to a greater degree of price arbitrage. A legal framework will be needed to remove barriers to free trade. (ii) With margins, profits and prices under pressure, EMU will be highly anti-inflationary and possibly even deflationary in the short-to-medium term. Predatory use of tax rates by EU members would have to be outlawed. (iii) EMU will probably have a positive effect on economic growth in the long term, and the need for tight fiscal policy will accelerate reform of public finances; nonetheless, a deep early recession would be disastrous politically. (iv) Unemployment will remain for years well above levels considered by most economists to be necessary for price stability; malfunctioning labour markets and defective social security systems have already placed a significant tax on jobs. Radical labour market reforms are needed if monetary union is to survive. (v) With expanding transaction volumes in euro-denominated markets, the euro will become a large reserve currency to rival the dollar, making the international financial system and its institutions, such as the IMF and the World Bank, less lop-sided towards the dollar. There is no doubt that the arrival of EMU poses a greater challenge to US economic and political orthodoxy than any previous stage of European integration, and changes in previously positive US attitudes towards EMU were reported in 1997 and early 1998. (vi) There will be extreme but not insuperable difficulty in applying a uniform monetary policy, through the transmission mechanisms (e.g. interest rates, exchange rates), to such a variable group of economies as currently exists in the EU. All these six predictions will be tested directly by the euro-11 countries (Austria, Belgium, Finland, France, Germany, The Irish Republic, Italy, Luxembourg, The Netherlands, Portugal, and Spain). The remaining EU countries (Denmark, Greece, Sweden, and the UK) will be involved onlookers, and features in common with the euro-11, e.g. the operation of the Common Agricultural Policy (CAP), and exchange rates, could suffer EMU-induced stress in the four outsiders.

With regard to agri-money arrangements after the introduction of the euro on 1 January 1999, the European Commission proposed to scrap the 'green' rates of exchange; use the market rate of exchange to determine the value of CAP prices and amounts; set the value of CAP direct payments in national currency terms by using the exchange rate in force on a particular day of the year; provide fully funded EU compensation for one year to compensate producers for the elimination of 'frozen' green rates of exchange; and finally consider the possibility for compensation for an appreciable revaluation to be in force until 31 December 2001.

An unpublished document circulated inside the European Commission (EC) by the Employment Directorate was claimed by the *Financial Times* in March 1998 to indicate that the 'black economy' in the EU was equivalent to 7%-16% of the EU's GDP. The black economy was defined as economic activity that is intrinsically lawful but not notified to the public authorities. The biggest black economies were claimed to be in Greece (29%-35% of GDP), Italy (20%-26%), and Belgium (12%-21%). This compared with the UK at 7%-13% and Finland at 2%-

4%. A significant component of the black economy is thought to relate to agriculture and horticulture, and compounds the effects of CAP fraud.

There was a 6.2% increase to over \$411 billion world-wide in advertising on all media. Most airlines and aircraft manufacturers experienced gains in income. The clothing industry, from apparel-manufacturing to fashion and retail, noted an upturn in sales and profits after several years of lacklustre sales. Automobile sales reflected the economic performance of the host nation regardless of the fact that nearly all the manufacturers were multinational traders.

In the chemicals industry, several multinational companies moved into high-priced low-volume speciality areas, most notably the 'life sciences' or 'bioscience' sector, encompassing pharmaceuticals, agricultural and horticultural chemicals, and biotechnology involving molecular genetics and diagnostics.

Commercial gambling was aided by changing public acceptance and the strange willingness of humans to allocate time and money to frivolous gambling pursuits. Casino-style gaming, video-lottery terminals and electronic gaming systems introduced in the USA were making headway in several countries as prohibition and moral distaste have given way to variable levels of tolerance. The UK National Lottery was remarkably successful and efficient at raising indirect taxes for reallocation by government.

The business trend in 1997 of mergers grew beyond manufacturing and trading companies, and incorporated insurance companies, agencies and brokers and soon engaged much of the entire financial services sector, including accountancy companies, banking, legal firms and securities. Lloyd's of London returned as a competitive force in the global insurance market, introducing a new internal-monitoring system designed to prevent huge losses of the like suffered between 1988 and 1992.

Various trade-liberalisation talks in 1997 included discussions between South America's two largest trading blocs - the Andean Community (Bolivia, Colombia, Ecuador, Peru, and Venezuela) and the Southern Cone Common Market (Mercosur, comprising Argentina, Brazil, Paraguay, and Uruguay). Both blocs constitute a market of 310 million consumers with a combined GDP of \$1.2 trillion). A hitherto confidential report ordered by the EC from its Budget Directorate noted that there would have to be massive compensation payment to EU farmers if a free-trade

agreement were to be reached with Mercosur. The Commission's Agriculture Directorate has noted that the main constraint on Mercosur farm production is the lack of markets, not land. In Vancouver, the annual meeting of the Asia-Pacific Economic Cooperation Forum agreed to liberate trade in nine categories of goods and services.

International exchange rates experienced considerable volatility in 1997. The pound sterling and US dollar advanced strongly against most currencies, sterling's strength reflecting robust economic growth but the UK's exchange rate was less competitive in March 1998 than at any time since the early 1980s. During the summer months, speculative attacks against many Asian currencies led to a slump in the value of most Asian currencies, and a threat to nearly all the remaining Asian currencies. The currency crisis in Asia started with a speculative run on the Thai baht in mid-May. By October, the Malaysian ringgit was depressed by 25%, the Indonesian rupiah by 33%, and the Philippine peso by 23%. The Singapore dollar lost 9% of its value. In November, the South Korean economy faltered and the won fell by over 35% against the US dollar. Ripple effects were noted in stock exchanges around the world.

By October 1997, the bull run in world stock markets over the previous 2 to 3 years gave way to turbulence in share prices. Sharp declines occurred in Japan and the point of collapse was reached in many other Asian markets, but the *Financial Times/Standard & Poor's* World Index registered a 13.2% gain in dollar terms for the year, with European shares gaining 34% and the Dow Jones industrial average ending the year 22.6% higher. No doubt, large-scale assistance from the IMF to Thailand, Indonesia, and South Korea improved investors' confidence.

Labour-market flexibility was a contentious issue in 1997. The flexibility of the US and UK labour markets was regarded by many as a major factor in improving efficiency and achieving low unemployment, whereas the highly regulated old socialist practices common in most EU countries had led to high labour costs and rising unemployment, a point reinforced by the IMF in its *World Economic Outlook*. European employers were expected to bear high social costs and offer a high level of worker protection, strong disincentives to expanding staff numbers and entrepreneurial behaviour. The financial viability of social protection/security programmes caused concern world-wide.

Of course, it is possible that the relative weakness of labour growth in the EU may not reside in the supply-side labour market but is a manifestation of demand-side depression in product or capital markets. If there is a shortage of ready capital for entrepreneurs, then the demand for labour becomes much less sensitive to the level of wages, no matter how much low wages are emphasised. Similarly, regulations that constrain output and profits will also have the effect of lowering employment levels.

Within the UK, following the May 1997 General Election, the new Labour Government declared its intention to leave in place most of the basic elements of the labour laws enacted by the previous Conservative administration between 1980 and 1993. Nevertheless, trades union membership would be permitted at the intelligence-gathering Government Communications Headquarters; a low-pay commission would be established to recommend a national minimum wage; the opt-out from EU labour proposals at the December 1991 Maastricht conference would end; and trade union recognition by employers would become a legal requirement.

Kofi Annan of Ghana took office on 1 January 1997 as the new Secretary-General of The Secretariat of the United Nations (UN), and shortly thereafter sought to introduce a tranche of efficiency-improving reforms in tandem with a budget reduction. The UN was owed more than \$2 billion by its member nations, the largest debtor being the USA with \$1.2 billion in unpaid dues.

Set up in 1961, the Paris-based OECD had as its primary aim the support of the economic liberalisation of the western democracies. The fall of communism, the establishment of the EU, the growing influence of the WTO, the rôle of the IMF in macro-economic policymaking, and a focus on Europe and North America, (only two Asian members and one from Latin America), have made it a less appropriate forum for formal talks on global issues than many other bodies. Negotiations to agree the Multilateral Agreement on Investment, the OECD's top priority, came to an impasse and may be shelved. Nonetheless, it has the world's major economies in its membership, carries out valuable data collection and economic analyses on behalf of its members, and can stimulate and smooth negotiations at the global level. In early 1998, there were calls for OECD membership to be enlarged.

International law continued to evolve from sets of rules governing relations between specific sovereign nations into a framework for joint actions on matters that directly affect individual citizens. Nations took greater recourse to international legislation, involving both treaties and multilateral conventions, in order to develop their own laws in collaboration with other nations. In addition, there was increasing subordination of national action to international adjudication as evidenced by the growing number of international courts and tribunals, many of which have set up Web sites on the Internet. During 1997, the International Tribunal for the Law of the Sea was established, the UN General Assembly approved the establishment in 1998 of a permanent International Criminal Court, a body flawed at the outset by claiming jurisdiction over non-signatories, e.g. the USA, and diluting the authority of the UN Security Council. Also in late 1998, a new European Court of Human Rights will be formed as foreshadowed by the entry into force in October 1997 of the 11th Protocol to the European Convention on Human Rights.

The World Trade Organization (WTO, formerly the General Agreement on Tariffs and Trade-GATT) Appellate Body adopted, in December 1996, Rules of Conduct that supplemented the existing understanding on Dispute Settlement Procedure and the Working Procedures on Appellate Review. With a full set of working texts, during 1997 the WTO decided on several appeals from WTO panel reports. These included the controversial condemnation of the EU's inclusion of bananas in the CAP. The USA, Ecuador, Guatemala, Hondurus, and Mexico successfully complained about the EU's highly regulated 1993 regime which gives indirect price support to EU territorial producers and banana exporters in the Lomé Convention covering certain African, Caribbean, and Pacific (ACP) countries linked to France and the UK. The regime was said to discriminate against non-ACP producers in the second biggest market in the world of about 4 million tonnes per year. ACP producers tend to be small-scale and few could adapt to compete in liberalised markets by 2002 and their banana-dependent economies would find adjustment to new crops or industries stressful. The EU hoped that a modified tariff regime and altered importing licensing and support arrangements would satisfy WTO rules.

Another case before a WTO panel involved the disputed exercise by the USA of global jurisdiction against trade with Cuba, Iran, and Libya through the Helms-Burton Act and the Iran and Libya Sanctions Act; this dispute between the EU and USA was resolved by a memorandum of understanding.

By the way of background information, the WTO is essentially the legal and institutional foundation of the multilateral trading system that arose from GATT. Established in 1948 initially as an interim measure until a charter could be drafted by a committee of the UN Economic and Social Council and ratified by member states, GATT became the only regime for the regulation of world trade, developing its own rules and procedures over time. The original charter was never ratified. Dedicated to the expansion of non-discriminatory international trade, GATT operated by a series of 'rounds' of multinational negotiations (Geneva 1947, Annecy 1948, Torquay 1950, Geneva 1956, Dillon 1960-1961, Kennedy 1964-1967, Tokyo 1973-1979, and Uruguay 1986-1994). It is expected that the measures agreed in the Uruguay round will be fully implemented in 2002, and will lead to the average duties in manufactured goods to reduce from 40% in the 1940's to 3%. In Marrakesh in April 1994, the 128 GATT negotiating states and the EU established the WTO to supersede GATT and implement the Uruguay agreement.

Like GATT, WTOs principal aims are to liberalise world trade through an agreed set of trade rules and market access agreements, as well as through further trade liberalisation agreements. The WTO also administers and implements a further 29 multilateral agreements in areas such as agriculture, government procurement, rules of origin, and intellectual property. By April 1997, there were 131 WTO members, and a further 28 governments being considered for membership. The present membership of the UN is 185.

According to the 1997 World Drug Report, compiled by the UN International Drug Control Program (UNDCP), the annual turnover in recreational and addictive drugs was estimated to be \$400 billion, or about 8% of total international trade, exceeding trade in iron, steel and automobiles. World production of coca leaf, the source of cocaine, more than doubled between 1985 and 1996, and poppy-derived opium production more than tripled. A world-wide drop in the street price of narcotics indicated that supplies were readily forthcoming. In the USA, the nation with the highest drug-consumption rate, a report in February 1997 by the Congressional General Accounting Office stated that despite a \$20 billion prevention effort over a decade, supplies of cocaine and heroin continued to flood into the country at levels that were more than adequate to meet demand. This report also noted that in 1995 only about 230 of the 780 metric tonnes (mt) of cocaine produced around the world were seized by the enforcement authorities, and only about 32 of about 300 mt of heroin. Antidrug efforts by the US and certain other MDCs relied heavily on foreign governments reducing the cultivation of the source plants by eradication and crop-substitution projects, and by prosecuting the major drug traffickers.

Following the signing of the Founding Act on Mutual Relations, Co-operation and Security, three former Warsaw Pact members - the Czech Republic, Hungary, and Poland - were invited, against the objections of Russia, to join the 16-member North Atlantic Treaty Organization (NATO), a process expected to take 2 years. A Euro-Atlantic Partnership Council was also established. The 36,000-strong NATO-led Stabilization Force (SFOR) in Bosnia and Herzegovina involved contingents from 20 non-NATO countries, enabling peace to be maintained between the three ethnic communities, but it became clear that SFOR's mandate, which will end in mid-1998, should be extended.

In recent years, it has become evident that ethnic and religious antagonisms are likely to express themselves in guerrilla warfare and terrorism rather than in fullscale military operations which have become too costly and too risky even for the more powerful nations. At the end of 1997, there were 30 conflicts of varying size and intensity recorded throughout the world; all adversely affected agriculture and food suppliers. The former Yugoslavia and Albania witnessed conflict. The UN Security Council refused to lift the economic sanctions imposed on Iraq in 1990 because it had not received full details of Iraq's weapons of destruction, and violation by Iraqi and Iranian warplanes of the no-fly zone in Southern Iraq led to a building-up of forces by the USA and allies in the Persian Gulf. There was debate within Israel on the value of military operations inside Lebanon. Civil war continued to ravage Afghanistan as the Taliban Islamic militia tried to extend its influence northward from the capital, Kabul. For the 15th year, the government in Sri Lanka was unable to crush by military means the rebel Liberation Tigers of Tamil Eelam. In early October 1997, Indian and Pakistani forces exchanged artillery fire across their disputed border in Kashmir.

North Korea remained a serious threat to stability in the region, starvation of its population adding a new dimension to the urgency of solving the problems facing the Korean peninsula. Little progress was made in efforts to convene the four-power (South Korea, North Korea, China, and the USA) peace talks to bring about the official end of the Korean war. In July, North and South Korean troops exchanged heavy gunfire across the demilitarised zone. It was estimated that more than one million people have been displaced in Cambodia after the Second Prime Minister ousted his co-premier in a coup.

In Colombia, government forces continued their often unequal struggle against two left-wing insurgent forces, both of which were allied with drug traffickers.

In Africa south of the Sahara, a broad arc of violence stretched from the Red Sea to the South Atlantic Ocean. The demoralised army of Zaire's President Mobuto Sese Seko failed to prevent the advances of the rebel Alliance of Democratic Forces for the Liberation of Congo-Zaire led by Laurent Kabila, and aided by troops from Rwanda and Uganda. By May, the rebel forces captured Kinshasa, and Kabila named himself president of the country which was renamed the Democratic Republic of the Congo. Tutsi-Hutu animosities in the eastern Congo generated renewed attacks by various rebel groups into Rwanda, Uganda, and Burundi. Serious fighting rocked the neighbouring Republic of the Congo. Elsewhere, fighting continued for the 14th year throughout the year in southern Sudan. An army coup in May 1997 overthrew the Government of Sierra Leone; the government was reinstated in February 1998 after a counter-coup involving Sandline International, a British firm of military consultants.

The transfer of power in Hong Kong to China at the end of June 1997 proceeded in an orderly fashion. At the same time, the UK rejoined the United Nations Educational, Scientific and Cultural Organization, having withdrawn in 1985 and obtained observer status in 1986. Rwanda and Yemen applied to join the Commonwealth and their applications were kept under review; Fiji was readmitted.

#### **Populations**

According to estimates prepared by the Population Reference Bureau, the world population at mid-year 1997 stood at 5.840 billion, 86 million higher than in 1996 and more than 800 million higher than in 1987, indicating that by the new millennium the population would reach 6 billion. The annual rates of increase declined from 1.52% in 1996 to 1.47% in 1997, reflecting a decline in birthrates in the LDCs. At the 1997 growth rate, the world's population would double in 47 years.

World-wide, 32% of the population was below the age of 15 in 1997, but the figure was 38% in LDCs excluding China. In the MDCs, only 20% were below age 15 as a result of the persistently low birthrate throughout Europe and Japan. The continued younger age distribution of the LDCs would result in relatively large numbers of women entering the childbearing age in the near future. Only 4% of the population in LDCs excluding China, was over the age of 65 compared with 14% in the MDCs.

By 1997, 43% of the world population lived in urban areas; 36% of the population of LDCs was urban compared with 74% in MDCs.

Life expectancy at birth world-wide in 1997 was 64 years for males and 68 for females; in LDCs the figures were 62 and 65, and 71 and 78 in the MDCs, respectively. The 1997 world infant mortality rate was 59 infant deaths per 1,000 live births. The figures for Western and Northern Europe were at 5 and 6, respectively. In LDCs in general, the overall rate remained at 64.

More detailed demographic analysis of the LDCs showed that in 1997, the population of the LDCs grew at 1.81% per year, 2.09% for LDCs excluding China. The total population of the LDCs was 4.666 billion, some 80% of the world's total. Of the 86 million people added last year to the world's population, 82 million were in the LDCs. In LDCs excluding China, women still average four children each, unchanged from 1996, and far above the number needed to stabilise the world population size.

As in previous years, Africa remained the region with the highest fertility, averaging 5.6 children per woman, 6 in sub-Saharan Africa. Nevertheless, surveys in Senegal and Zambia indicated a slow decline in fertility. The population in Africa was estimated at 743 million, with the world's highest growth rate of 2.6%, sufficient to double the population size in only 26 years. At 52 years for males and 55 for females, the life expectancy in Africa was the world's lowest; infant mortality at 89 infant deaths per 1,000 live births was the world's highest.

With a population of about 3.6 billion in 1997, Asia was the most populous of the world's regions. The growth rate remained at about 1.6% resulting in a population increase of about 56 million. Life expectancy stood at 64 for males and 67 for females. Women averaged 2.9 children each, 3.5 excluding China. Data released during 1997 by India indicated

that in 1995 the country's birthrate did not decline as much as expected, coincident with the government dropping specific demographic targets for its population programme.

In the MDCs, the population expanded by only 4 million in 1997, to 1.175 billion. The growth rate of the MDCs was 0.1% annually. Europe once again registered a negative rate of natural increase (birthrate minus death rate) of -0.1%. This was due primarily to the sharp drop in birthrates in the European republics of the former Soviet Union, and to continued low fertility in Western Europe. Bulgaria, Czech Republic, Italy, Latvia and Spain had the lowest birthrates in the world, with an average number of children per woman of only 1.2. Life expectancy at birth in Europe, including the European republics of the former Soviet Union, was 69 for males and 77 for females. Japan maintained its leading position on life expectancy of 77 for males and 83 for females. Finland reported the lowest infant mortality rate world-wide.

The OECD estimated that by 2050, there would be almost 6 retirees for every 10 people in the labour force in the OECD area, placing a huge burden on public finances and living standards. The World Health Organisation (WHO) estimated that by 2025, about 800 million people - one in ten of the world's population - will be over 65, compared with 390 million today.

There was considerable abatement in 1997 of the massive humanitarian crises of the early 1990s. The world's overall refugee population declined to 13.2 million in 1997 compared with 15.5 million the year before. Similarly, the overall population classified as 'of concern' to the Office of the UN High Commissioner for Refugees (UNHCR) fell to 22.7 million, representing one out of every 255 people on Earth. Of this vulnerable population, in addition to the 13.2 million refugees stated above, 3.3 million were returnees, 4.9 million were internally displaced persons (persons in a refugee-like situation but who had not crossed an officially recognised international border), and 1.4 million were others of humanitarian concern, largely victims of conflict. UNHCR, in common with a few other international bodies that meet enormous challenges, was accused of dubious accounting and incompetent management of its \$1 billion annual budget.

More than 2 million refugees returned to their countries of origin in the latter half of 1996 and first six months of 1997, highlighting the fact that voluntary

repatriation is the preferred solution for many of the world's refugees. Often, however, they returned to unstable political and economic situations.

The Great Lakes region of South Africa where more than two million Rwandans and Burundians fled their countries in 1994 remained the major focus of humanitarian concern. In Rwanda, whilst trying to overcome the social aftermath of Hutu-Tutsi conflict, the country tried to absorb approximately 2.8 million returnees since 1994. At the end of 1997, about 74,000 Congolese (former Zairian refugees) remained in Tanzania along with large groups of Burundian refugees. Implementation of the 1994 peace accord that ended 20 years of civil war in Angola enabled about 96,000 refugees to return to Angola by mid-1997, leaving about 200,000 Angolan refugees outside the country.

Renewed violence in Sierra Leone prevented the planned repatriation of about 375,000 refugees who had sought asylum mainly in Guinea and Liberia. The unfortunate coup in May 1997 led to the outflow of a further 38,000 refugees. Following the progress of the peace process in Liberia, it was anticipated that more than 500,000 refugees would return from Côte d'Ivoire, Ghana, Guinea, and Nigeria. Similar hopes were raised for 165,000 refugees returning to Western Sahara. More than 7,000 Somali refugees were repatriated from Eastern Ethiopia to Northwestern Somalia, and 7,000 out of more than 320,000 Ethiopian refugees returned home from The Sudan.

In the former Yugoslavia, the return of refugees to Croatia was negligible. In Bosnia and Herzegovina, more than 250,000 people had resettled in areas where their particular ethnic group was in the majority. The return of ethnic minorities to their former homes proved extremely difficult despite multinational efforts. Elsewhere in Europe, negotiations had commenced on the conflict between Georgia and its breakaway regions of Abkhazia and South Ossetia, a conflict which had displaced 38,000 Georgians and raised the spectre of former Soviet nuclear weapons falling into the wrong hands. In the countries of the Commonwealth of Independent States, attempts were made to address refugee flows and migratory movements, most enforced by the former Soviet powers, which had affected about 9 million people. By mid-1997, some 31,000 Albanian refugees sought temporary asylum, mainly in Greece and Italy. As a result of stricter visa requirements, more rigorous border controls, and in some countries, restricted social payments,

the EU experienced a decline in the rate of recognition of those applying for refugee status. The USA and Canada tried to address the issues of requests for asylum by those that had suffered from sexual violence and from discrimination based on gender.

Since the 1979 Soviet invasion of Afghanistan and the ensuing 18 years of civil war, more than 6 million Afghans were uprooted. At one-third of the nation's population, Afghan refugees constituted the largest refugee caseload of concern to UNHCR. Around 20,000 of the 60,000 Tajik refugees who had fled the 1992-1993 civil war in Tajikistan remained in Afghanistan, despite the continuing civil war.

Renewed fighting in Cambodia during the summer led to 28,000 Cambodians fleeing into Thailand. More than 24,000 Vietnamese boat people returned to Vietnam in late 1996 and the first half of 1997, but about 1,700 Vietnamese remained in Hong Kong after transfer of sovereignty from the UK to China in July 1997. In Myanmar (Burma) more than 220,000 Muslim refugees who had fled the country in late 1991 and 1992 returned but 21,000 remain in Bangladesh. More than 90,000 displaced Bhutanese of Nepalese origin remained in Southeastern Nepal after troubles in 1991 and 1992. In Sri Lanka, fighting displaced more than 500,000 people, 8,000 of whom moved to India.

It was estimated that more than one million people have been internally displaced in Colombia as a result of internal conflict.

#### **Development and Food Aid**

Development assistance by the industrialised countries to developing countries can be calculated in terms of a percentage of gross national product (GNP). The UN recommends a figure of 0.7%. According to OECD, development assistance in 1997 fell to around \$47 billion from the \$55.4 billion recorded in 1996. Half this decline is explained by the strength of the dollar which meant that aid payments by other countries were worth less in terms of the dollar. In addition, there was an impact of nations 'graduating' from developing country status. Despite these factors, there was a 3% decline in development assistance. Provisional data appear to indicate that aid as a share of MDC GNP has fallen to an average level of 0.22%, down from 0.25% in 1996, and the lowest figures since records began. Development-aid payments from the USA fell to just over \$6 billion, 0.08% of GNP versus 0.12% in 1996, which was already by far the least generous of any of the leading industrialised countries and partly explained by the fact that Israel, a major and continuing recipient of aid, is no longer classified as a developing country. In 1996, Denmark provided 1.04% of GNP as aid, and The Netherlands, Norway, and Sweden all gave more than 0.8% of GNP. Even excluding a \$3 billion emergency loan to South Korea, the World Bank's lending commitments reached a new high of \$8.8 billion in the first half of fiscal 1998. In the current fiscal year as a whole, the World Bank expects to deliver over 265 projects with a lending volume of \$21 billion to \$24 billion. There were strong indications that there had been improvements in project quality, and the number of problem projects declined.

Political opposition from Germany and Japan and others who question the principle of debt relief, delayed international efforts to relieve the debt burdens of the world's poorest countries. The UK made efforts in September 1997 to ensure that every poor country eligible for debt relief under the Highly Indebted Poor Country (HIPC) Initiative had made a start by 2000. Around poor 19 countries appear likely to comply with HIPC conditions of economic good behaviour in the foreseeable future. The HIPC Initiative has yet to deliver debt relief to a single country. Most of the 41 countries classified as heavily indebted are in sub-Sahara Africa, including 32 countries rated as severely indebted. The most heavily indebted countries are Nigeria (\$35 billion), Côte d'Ivoire (\$19 billion) and Sudan (\$18 billion). Although the debt of Latin America (\$650 billion) is much larger than that in Africa, relief agreements and stronger economic growth have made the problem more manageable. For Africa, arrears on the debt are rising rapidly as terms of trade, commodity price turbulence and diminishing aid packages have conspired to create the current situation where external debt cannot be properly serviced. To this almost insuperable problem must be added the fact that the debt owed to multilateral lenders (e.g. the IMF, World Bank, African Development Bank etc.) which do not reschedule debts - has grown also.

Doubts have been raised by Graham Searjeant, the economist, as to whether the UK should surrender its aid budget to the EU or to international agencies over which the UK has little or no influence for transfer to people who cannot be readily identified. In other words, development aid becomes more akin to a tax. France and Germany, and many other countries, still operate with tied aid budgets, to the benefit of their own industries and services.

The Food and Agriculture Organization of the United Nations (FAO) identified food emergencies in 31 countries during 1997, compared with 25 in 1996. Civil strife, natural disasters and crop failures resulting from adverse weather conditions, pests and diseases accounted for most of these emergencies. Selfimposed political problems added to the problems faced by Iraq and North Korea, and the transition to a market economy compounded the difficulties caused by bad weather in Armenia, Azerbaijan, Georgia, and Tajikistan. Nonetheless, short-term food prospects for most low-income, food-deficient countries (LIFDCs) improved markedly in 1997 as a result of better harvests and increased commercial food purchases. For the poorest LIFDCs, however, the Economic Research Service of the US Department of Agriculture (USDA) estimated that about 10 million metric tonnes (mmt) of food aid in the form of cereals was needed in the 1996-1997 crop year just to raise food consumption to an average of consumption levels in the period 1990-1995, notwithstanding the fact that the targeted enhanced level did not even meet minimum nutritional needs.

Regardless of needs, food-aid shipments in 1996-1997 sharply declined to around 4.8 mmt probably because of relatively high prices and constrained supplies. There is some debate as to whether there has been a slight recovery in 1997-1998, but FAO estimates of 5.0 mmt are substantially below the average level of 12.5 mmt recorded in the period 1992-1993 to 1994-1995. Around 85% of the volume in food aid is attributable to cereals, primarily wheat, and with the exception of China, is derived from the developed countries, mainly the USA and EU. LIFDCs in Asia and Africa, several countries emerging from the direct influence of the former Soviet Union, Latin America, and countries in the Caribbean were the main recipients of food aid.

A 1997 report by the World Bank pointed to the enormous benefits of multinational companies to the economic development of LDCs. Lowering of barriers to trade and investment, lower transport and communication costs, and co-ordinated production and distribution across national boundaries, have generated sophisticated products and services and stimulated the development of specialised intangible knowledge-based assets. Global production contributes directly to incomes and employment in the countries in which it takes place. Multinationals tend to be more productive, pay higher wages and conduct more international trade than local companies. 'Spill-over' benefits

include the improvement of the quality of local labour forces and enhanced indigenous management skills enabling technological advancement. The foreign direct investment has helped replace declining publicsector or government-based development assistance. Added advantages include improving knowledge of export markets. Quantifying the spill-over benefits is not straightforward, but the report calculated that each percentage point share of foreign direct investment in national output was associated with an extra 0.3-0.4% annual economic growth. Even though multinationals account for a fifth of global production of goods and services, a third of world trade, and much of its R&D activity, they can be regarded as essential to development strategies. Often regarded as sinister and dangerous not only by pressure groups, and a threat to national security, they have assisted developing countries face up to the removal of trade and investment barriers, promotion of domestic competition and privatisation, and investment in infrastructure and education. Potential problems such as countries creating aggressively competitive company taxation breaks and the companies borrowing domestic currency against fixed assets only to switch into foreign currency, still did not alter the conclusion that LDCs need multinationals.

#### **Agriculture and Food**

According to the FAO (http://www.apps.fao.org) total world agricultural production in 1997 rose by 1% above the previous record level of 1996, although this was accounted for by a marked increase in production in the LDCs whereas there was a decline of 0.5% in the developed countries. A similar pattern was observed in total food production and *per capita* food production. It would appear that the increase in food production in 1997 was in line with world population growth. More detailed analysis of the data reveals that the increase in agricultural production was attributable to an increase of livestock production by 2%, crop production and non-food agricultural products remaining at the same levels as in 1996.

During the period 1996-1997, crop output in individual countries increased at twice the rate of livestock production. In contrast, over the same period nonfood agricultural production (e.g. fibres, industrial vegetable oils, etc.) declined somewhat. Agricultural production in the EU stagnated over the last seven years coinciding with the steady reduction in production-related subsidies within the stranglehold of the CAP. In the USA, however, there was a healthy 2% rate of growth. The greatest expansion in crop and live-

stock production occurred in China with *per capita* food production increasing by 50% between 1990-1997.

In less-developed African countries, growth in food production lagged well behind population growth such that *per capita* food production was 3% lower than in 1990. The decline in agricultural production since 1990 noted in most of the countries of Eastern Europe and the former Soviet republics showed signs of bottoming out. Of all the former Soviet-influenced states, Azerbaijan, Bulgaria, Estonia, and Latvia experienced over several years the most pronounced declines in total agricultural production, total food production, and *per capita* production.

A study by the Economic Commission for Latin America and the Caribbean noted that agriculture, forestry, and fishing grew at a faster rate in that region than other sectors of the economy. New production methods led to a decline in the composition of agricultural workers in the economically active workforce. For the future, however, trade liberalisation from the Uruguay round would affect the highly protected agriculture sector. Moreover, almost one-third of all cultivatable land was moderately or severely degraded through poor agronomic practices.

In 1995, a report on world horticultural trade entitled The Game of the Rose estimated that 60% of all cut flowers that crossed international borders originated in The Netherlands. By 1997, however, countries in South and Central America, Africa, as well as China and New Zealand, had taken strong positions in the trade. Much of the production of cut flowers in the USA had moved to Colombia - now the world's second-largest flower exporter after The Netherlands, followed by Ecuador, Costa Rica, and Guatemala exploiting the benefits of direct air flights, ideal climate, low production costs and skilled management. European production had moved for the most part to Kenya and Zimbabwe, but Côte d'Ivoire, South Africa, Tanzania, and Zimbabwe supplied significant quantities for export to Europe. Production of freshcut flowers in LDCs was expected to increase for the foreseeable future, whereas production in MDCs would decline, or stabilise at best. Colombian flower producers were concerned, however, that drug-related USA sanctions will affect the Andean Trade Preferences Act under which Colombian flowers enter the US market tariff-free until 2001. There were estimates of mourners purchasing 60 million cut flowers to honour Diana, Princess of Wales, after her death in August 1997.

An attack on farm subsidies and agricultural trade tariffs in Europe and Japan was launched by the 15-member Cairns Group of agriculture exporters in early April 1998. The Cairns Group, named after the venue of its first meeting in 1986, includes Argentina, Australia, Brazil, Canada, Chile, Colombia, Fiji, Indonesia, Malaysia, New Zealand, Paraguay, The Philippines, South Africa, Thailand, and Uruguay. It represents 550 million people and around 20% of global agricultural exports. The views of the Cairns Group in favouring trade liberalisation by lobbying against farm subsidies, export credits and trade barriers, are in accord with the tenets of the WTO. The EU has historically argued for exemptions in primary industries.

Competitive pressures from the WTO and the major agricultural exporting nations, together with the prospect of enlargement of EU membership to the east, have induced proposals to overhaul the CAP, which has been viewed with growing distaste by urban politicians and public alike. In an attempt to streamline the EU and to help revamp structural funds for improving regional infrastructure, the Agenda 2000 proposals launched in March 1998 by Franz Fischler, the EU's Agricultural Commissioner, envisaged cuts of up to 30% in guaranteed minimum prices for cereals, beef, and dairy produce. It also foresaw removal of production limits for cereals, together with compensation payments for loss of farmers' incomes. That the annual cost of CAP would increase by 10% if the reforms are implemented weakens the prospects of full acceptance. Europe's trading partners will press for more open access to the EU market, and difficulties will be likely with the WTO. Failure to reform the CAP has severely disadvantaged EU agriculture in the last round of farm trade talks in the early 1990s (see previous SCRI Annual Reports), and could well lead to the collapse of the CAP.

#### Cereals

The USDA issued a forecast that global cereal stocks in 1997-1998 would represent just 16% of world cereal consumption, an improvement of 1% over the 1996-1997 figure but substantially lower than the 28% figure recorded in the mid-1980s. A considerable proportion of the stocks was held in countries such as China and was not available for world trade. The insecurity of the subsequent absence of strategic reserves, coupled to increased demand for livestock feed, was offset by record grain harvests (wheat, coarse grains, and milled rice) in 1996-1997 (1,867 mmt)

and an expected record in 1997-1998 (1,874 mmt), although coarse grain production was expected to decline.

Most of the increased cereal production in the last two years was recorded in China and the grain-exporting countries of Argentina, Australia, Canada, the EU, and the USA. For 1997-1998, China was expected to produce a 9% increase in the wheat harvest but would experience a 16% decrease in coarse grains, placing pressure on reserves to sustain livestock production. A major increase of around 25% was anticipated in cereal production in the former Soviet republics.

A decline in the intake of selenium of Europeans was noted in several studies, attributed to a fall in imports of high-selenium, high-protein, bread-making wheats from Canada and the USA. Selenium plays a pivotal rôle in various metabolic functions, and there were therefore calls for selenium supplements in flours, and for selenium to be incorporated into fertilisers as is now the case in Finland.

#### **Oilseeds**

As a result of a rapid growth in demand for oilseed products (meal and vegetable oils), the prices of oilseed products rose in global trading markets, leading to a decline in ending stock levels in 1996-1997 (16.2 mmt) from the 1995-1996 levels (22 mmt). Although the USDA forecast an 8% increase in global oilseed production for 1997-1998, sufficient to exceed world consumption and restore year-end stocks, recent data point to a short-fall in production principally because of the effects of drought in South-East Asia and poor production levels in Argentina, China, India, and the former Soviet Union.

In order of predicted production levels in 1997-1998, the seven main oilseeds were soy(a) beans (149 mmt), cottonseed (35 mmt), rapeseed/canola (33 mmt), peanuts/groundnuts (26 mmt), sunflower (25 mmt), copra/coconut (5 mmt), and palm kernel (5 mmt). Edible vegetable seed oil production amounted to 75.6 mmt, excluding palm oil (17.2 mmt) and olive oil (2.6 mmt) which are not regarded as seed oils. In world trade terms, sesameseed (2.7 mmt), linseed/flaxseed (2.3 mmt) and castorseed (1.2 mmt) were three important additional oilseed commodities. Castorseed crops in Brazil, the world's third largest producer, fell from 0.15 mmt to 0.05 mmt. India, which produces an average three-quarters of the world's castor crop, forecast diminished cropping, and China, the second largest producer, intended increasing its imports of castor oil, as did Brazil. Castor oil prices were expected to increase sharply.

FAO estimated late in 1997 that in 1997-1998, 11%-14% (3 million - 4 million hectares) of the US soyabean crop will be of genetically modified soyabeans, and about 20% (1.5 million hectares) of the Argentinean crop. In 1998-1999, these levels are expected to double, while in Canada, 25% of total soya-bean plantings will be of genetically modified seed.

Production in 1997 of the so-called oil-meals (147 mmt) included residues from the ten main oilseeds listed above, except castorseed, as well as fish meal, and maize (corngerm and corngluten) meal. Oils and fats (89 mmt) included the oils from the ten oilseeds above, plus maize (corn) oil, olive oil, palm oil, fish oil (all marine oils except from mammals), butter, lard, tallow, and greases.

#### Sugar

As in previous years, about 70% of the world's sugar supply was derived from the monocotyledonous sugar cane, and the remainder from the dicotyledonous sugar beet. A projected increased production level of 125 mmt centrifugal (freed from liquid) sugar in the crop year 1996-1997 did not materialise, leading to a production level of 122 mmt, closely matching consumption levels. Preliminary projections from the USDA for 1997-1998 indicate that sugar production would remain constant despite growing demand, and would lead to a slight decline in stocks to 26 mmt (21% of consumption). Other commodity analysts expect 1997-1998 levels to reach 126mmt, and 1998-1999 levels to rise to 128.5mmts.

Brazil (15 mmt) was forecast to displace India (13 mmt) as the world's largest producer nation in 1997-1998 in view of the fact that Brazil's production was aimed at export markets whereas Indian farmers substituted sugar crops for more-profitable crops. Production increased in Argentina, Australia, Brazil, Central America, China, the EU, Mexico, Pakistan, South Africa, Turkey, and the USA. Decreases in production were noted in Colombia, Cuba, India, Indonesia. Poland. Russia. Thailand, and Ukraine.

Population growth and increasing wealth in the LDCs were expected to increase demand for sugar and sugar-based products. In the MDCs, however, slow population growth and the greater use of sugar substitutes quelled demand.

By the end of March 1998, the US Food and Drug Administration (FDA) approved the use of sucralose, the only artificial sweetener made from sugar and 600 times as sweet as sugar. It was the first new artificial sweetener in ten years and became one of only four artificial sweeteners available for use within the USA, and one of only two without any substantive health warnings or usage restrictions. A rival sweetener, acesulfame potassium, still awaits FDA approval. Nearly 73% of adult consumers (144 million) in the USA are classified as users of low-calorie, sugar-free products, according to the 1998 Calorie Control Council/National Consumer Council.

#### Coffee

World green coffee production in 1997-1998 was forecast by the USDA to increase to 103 million 60kg bags, compared with 100 million 60-kg bags in 1996-1997. In view of record domestic demand in producer countries and enhanced exports, the 1997-1998 coffee market will be tight, and demand at a record high. Year-end stocks were set for a further decline. In order of production (million 60-kg bags) of both arabica and robusta coffees, the eight major producer nations were Brazil (28-35 million), Colombia (11.3 million), Indonesia (6.8 million), Mexico (5.7 million), Vietnam (5 million), Guatemala (4.2 million), Ethiopia (4 million), and Uganda (4 million). Costa Rica, El Salvador, Hondurus, Ecuador, Peru, Cameroon, Côte d'Ivoire, Kenya, Zaire, and India each produced between 1 and 3.8 million 60-kg bags in 1997-1998. There is some evidence that Vietnam will maintain its position as the biggest exporter of robusta in the Asia-Pacific zone in the 1998-1999 season, having overtaken Indonesia.

Worries about the power of Brazilian coffee producers surfaced when the Brazilian coffee exporters sought to have their washed arabica beans delivered against the benchmark 'C' contract at the Coffee, Sugar & Cocoa Exchange in New York, potentially flooding the market and causing a fall in prices. Rival exporting nations were concerned that Brazilian producers would switch from unwashed arabicas - coffee beans dried in the open air in a process peculiar to Brazil among the big producers - to washed arabicas. Arabicas are regarded as superior to robustas which tend to be used for soluble and instant blends. The competing nations already claim that Brazil dominates the International Coffee Organisation and the Association of Coffee Producing Countries, the two main international bodies.

#### Cocoa

An 8% decrease in world cocoa production in the crop year 1996-1997 to 2.71 mmt led to a substantial decline in carryover stocks; for 1997-1998 the USDA forecast an increase in production to 2.75 mmt but the International Cocoa Organisation (ICO) forecast in February 1998 that production would decline whilst consumption would rise to record levels. At 1.18 mmt, the Côte d'Ivoire accounted for more than 40% of world production but government action to inhibit expansion into virgin forests will impede further expansion. Substantial increases are expected in Ghana to 0.350 mmt and in Indonesia to 0.325 mmt despite the drought. Disease problems in Brazil (0.152 mmt) and replacement by other crops in Malaysia (0.115 mmt) have reduced the relative importance of the cocoa crop in both these countries. Nigeria (0.145 mmt) and Cameroon (0.120 mmt) remained important producers. It was projected by the ICO that stocks would decline in the 1997-1998 year to 1.19 mmt, their lowest level since the 1980s.

According to a survey by Datamonitor, the average consumer in the UK consumed almost 14 kg of chocolate *per annum*, those in the Irish Republic consume 13.72 kg a year, and consumers in Belgium and Switzerland, countries renowned for the quality of their chocolate, ranked numbers four and eight in the league table. The total chocolate market in Europe was worth \$18.5 billion. Therapeutic properties have been claimed, including aphrodisiac effects such that three out of four American women prefer chocolate to sex, and the prevention of heart attacks by consuming chocolate. Such claims will no doubt ensure an expanding market.

#### **Pineapple**

Around 75% of pineapple production was located in Indonesia, the Philippines, and Thailand; Kenya was becoming a large-scale producer but drought in Indonesia and Thailand, and drought followed by floods in Kenya, hit production sufficiently to cause a 33% rise in juice concentrate prices in less than two years. The USA is the world's largest market for tinned pineapple and juice.

#### Cotton

After dipping to 89.1 million 480-lb bales in 1996-1997, world cotton production was forecast by the USDA to increase to 90.2 million 480-lb bales in 1997-1998. The major producers were the USA (18.8 million 480-lb bales), China (18.0 million 480-lb bales), and India (12.9 million 480-lb bales). Africa

and the former Soviet republics were expected to benefit from greatly increased production, whereas declines were expected in the USA, China and India.

With the exception of South-east Asia, consumption of cotton was expected to increase globally at a trend of between 1%-2% per year. In regard to world cotton stocks, thought to be equivalent to approximately 40% annual world consumption, and about 7% above those of 1996-1997, a review is needed of the impact of China which holds around 40% of total stocks and is expected to reduce its stockpile to lessen importation needs.

Although consumption of cotton world-wide in 1997 increased by 2.1% to 19.2 mmt, inroads were made into its traditional markets by synthetic fibres, particularly polyester. Global cotton production fell by 1.6% to 18.9 mmt, with the USA, China, India, Pakistan, and Uzbekistan as the five major producer nations.

Genetically engineered cotton was increasingly important, as producers gained benefit from the reduction in inputs, most notably of pesticides. In the USA, five herbicide-resistant cottons were available commercially, as well as several short- and medium-staple cotton cultivars resistant to certain types of butterfly, moth and virus.

#### Jute

Uniquely in the UK broadsheets, reports on jute fibre prices and production appeared regularly in The Courier & Advertiser, one of Scotland's major daily newspapers and based in Dundee, formerly the thriving centre of jute processing in the UK. Jute prices collapsed early in 1998 as India (circa 10.5 million 180-kg bales) and Bangladesh (circa 6.5 million bales) harvested bumper crops in the 1997-1998 season. The total world trade in raw jute in 1997 was more than 2 million bales; the main importers were Belgium, Brazil, China, Cuba, Côte d'Ivoire, Pakistan, Russia, and the UK. The price of TD-4, the Indian benchmark grade fell to Rs700 a quintal (100 kg) against Rs1,100 a year before.

#### Rubber

With the exception of parts of Southeast Asia, natural and synthetic rubber markets expanded in 1997. Tyre manufacture for passenger cars, light-trucks, heavygoods vehicles, and agricultural vehicles dominated the market. A combination of an abundant supply of natural rubber, a relatively low-paid workforce, manufacturing overcapacity, and currency devaluations

ensured that Southeast Asia remained the world's largest producer of raw and manufactured rubber.

Legislation was introduced in Europe and the USA recognising allergies associated with latex products. Natural latex contains antigens to which more than 1% of workers are allergic. In concert with powders such as maize starch commonly used inside examination gloves, it was estimated that up to 10-12% of US healthcare personnel were affected by latex-glove-related allergic reactions.

#### **Tobacco**

According to World Tobacco File, global sales of cigarettes in 1997 rose by 0.9% to 5,370 billion, directly in the face of legislation designed to impede smoking. The demand was met by the increase in tobacco leaf production to 7.5 mmt, the highest since 1993. Decreasing sales in nearly all EU countries and the USA were more than fully compensated for by increasing sales in the Middle East, Southeast Asia, and former communist countries in Eastern Europe. To address well-publicised health concerns, cigarette manufacturers produced more filter-tipped cigarettes, as well as light and ultralight cigarettes with lower tar and nicotine levels, and attempts were made to make them less socially offensive.

In the USA, tobacco manufacturers sought immunity from tobacco liability court actions in exchange for payments approaching \$370 billion over 25 years and restrictions on cigarette manufacture and marketing.

Given the EU's attitude to tobacco advertising and awareness of health risks, it was surprising to note that the EU paid subsidies of Ecu 995 million in 1997/1998. Italy was the largest producer, with 40% of EU tobacco, with Greece close behind at 36%, Spain at 13%, and France at 8%. The European Commission proposed in February 1998 to persuade growers to shift production to higher-quality tobacco. It was even more surprising to find that in the USA, the \$49 billion 1998 Agriculture Spending Bill continues subsidies to tobacco as well as to peanut/groundnut and sugar crops. The tobacco crop insurance subsidy amounts to about \$34 million per year directed to about 89,000 tobacco farmers. More than \$177 million were spent on anti-smoking programmes.

#### Wood, Paper and Pulp

In common with market trends in recent years, the US and European wood products industry in 1997 experienced healthy growth of demand and prices in the first half of the year, but witnessed a decline in the latter half in response to increased supplies. Some uncertainty in the market was created by the operation of the Canada-US lumber quota agreement which limited duty-free access of Canadian lumber to the US market, but it was not expected to impact to any great extent on the volume of trade.

After Austria, Finland, and Sweden joined the EU in 1995, the EU moved into a state of self-sufficiency for forest products. Dull markets in France, Germany, and Italy, coupled to a depressed export market in Japan, led to oversupply and weak prices by the end of the year. This weak demand from Japan, and from Taiwan, South Korea, and other consuming countries led to a depleted trade for the main Southeast Asian exporting countries. Competition from softwood supplies from Russia and New Zealand, and hardwood supplies from Africa and South America, compounded the difficulties for the producer countries in Southeast Asia. The UK and US pulp and paper mills struggled against the impact of strong currencies, made even more difficult by the devaluation of southeast Asian currencies. Market rates for Northern Bleached Softwood Kraft - the industry benchmark fell from \$610 per tonne in December 1997 to below \$500 in early 1998 to reach \$550 by spring following a reduction in North American and Scandinavian pulp stocks.

At 282 mmt, paper and board production reached a record high in 1996, the last year of reliable data. The main producing areas were North America (100.2 mmt), Asia (82 mmt), and Europe (81 mmt). In line with waste paper replacing pulp as a raw material, total pulp production declined by about 4% in 1996 to 174 mmt; the USA (58.2 mmt) and Canada (24.3 mmt) were the major producer countries. The USA was also the world's leading exporter of surplus recovered paper and Asia the world's leading importation area.

Clear-felling operations caused conflicts with indigenous populations and environmentalists. There were few examples of genuine sustainable forestry and habitat regeneration. According to the UN Economic Commission for Europe, there was little impact on the market of timber certified to be from sustainably managed forests, with availability of such products limited and, in most cases, unable to command a price premium. The elimination of slow-growing, forests in the former Soviet Union was a special concern. On the basis of phytosanitary risks to US forests, imports of unprocessed logs and wood chips were halted from

Chile, New Zealand, and Siberia, but tropical hard-woods and products from the borders of Mexico and from Canada were not included in this ban.

#### Food Processing, Retailing and Consumer Issues

Processed ready-to-eat meals, convenience foods, socalled 'fast foods', low-fat and low-calorie foods, soft drinks, alternative meats to beef, and chilled, frozen and fresh fruit and vegetables continued to represent growth sectors in consumer markets in 1997-1998. North Americans and Japanese consumers made the most frequent use of restaurants and non-domestic eating establishments. Euromonitor in March 1998 highlighted the change in global lifestyles, with an internationalisation of food markets. Noting the surge in sales of breakfast cereals, ethnic food products from the Far East, hamburgers, pasta, and pizza, Euromonitor considered world trends. The rest of the world lags behind the USA in fast-food consumption where there were 25 burger outlets and 11 pizza parlours per 100,000 people. Japan had five burger restaurants and two pizza parlours per 100,000 people, and the UK was close behind. Other European countries had less than two burger outlets per 100,000 people, and were seen to have the most potential for future growth. Vegetarianism and exotic meats (e.g. crocodile, emu, ostrich) grew in popularity. Again, topics including dietary fibre, functional foods, fish oils, ethnic food, food labelling, food contamination, organically produced agricultural and horticultural foodstuffs, and genetically modified (or manipulated, enhanced or engineered) organisms (GMOs), appeared frequently in the food-related popular media.

Food-poisoning incidents were reported world-wide. Improved diagnosis techniques have highlighted the need for educating those involved in commercial and domestic food preparation and serving. At the beginning of 1998, the UK Government announced a ban of the sale of beef on the bone. By that time, the death toll of those believed to have contracted the new variant of Creutzfeldt-Jacob disease had reached 23. Reports of food adulteration became more prevalent, involving fruit juices, olive oil, honey, coffee, cheese, yoghurt, and other milk products.

Major investments were made in 1997 by several international food companies in South America. Japan remained the world's largest food importer, worth more than \$60 billion, consuming food and drink at more than \$3,000 per person. The country was able to supply only 46% of its total food requirements. Rice imports of 0.5 mmt in 1997, however,

appeared to go into processed foods, or was re-exported as aid, or stockpiled, thereby protecting Japanese domestic rice production.

In the EU, there was substantial growth in chilled foods, own-label products, and herbal teas. Beer sales grew by 1%. In the UK, the expansion in sales of alcoholic soft drinks ('alcopops') continued.

Technological developments included the widespread application of electronic aroma-sensing instruments to detect food-product degradation, adulteration, and contamination. The number of countries that approved the use of ionising radiation (gamma-irradiation) for helping the preservation of one or more food items - typically spices, fresh fruit and vegetables - reached 39, and 29 of which were using this valuable but undeservedly much-berated technology. In December 1997, the US Food and Drug Administration (FDA) approved the use of ionising radiation to control disease-causing micro-organisms in meat and meat products. The procedure was declared to be safe, and to have no effect on nutritional value, taste, or appearance of fresh and frozen meat, including beef, lamb and pork. In October 1997, the US government announced that it would be undertaking new steps to ensure the safety of imported as well as domestically grown fruit and vegetables.

Advances were made in packaging technology with the development of edible protein- and carbohydrate-based water-soluble packaging films which become part of the enwrapped foodstuff. A wider range of flexible packaging systems was developed for microwavable food products. Japanese R&D showed that barrier properties and transparency of food packaging were improved by the addition of a thin silicaglass layer on plastic packaging film. Improvements were made in metallocene and multi-layer linear low-density polyethylene to help control water and gas transmission in foods. Ethylene-vinyl acetate stoppers were developed to replace corks in wine bottles.

In March 1997, EU regulations were introduced to oblige companies to recover and recycle specific tonnages of packaging waste. In May 1997, EU regulations came into effect requiring labelling for novel foods and ingredients, and those containing genetically modified organisms. (See section on **Plant Biotechnology**).

Following the UK General Election in May 1997, the Government announced the establishment of an independent Food Standards Agency, based on the report

and proposals by Professor W Philip T James, Director of the Rowett Research Institute, a sister Scottish Agricultural and Biological Research Institute (SABRI) located in Aberdeen. Thus, an 81-page White Paper entitled The Food Standards Agency, A Force for Change was released in January 1998, describing the establishment of an independent body with the power to monitor and supervise enforcement of food safety from 'plough to plate' and scheduled to assess the safety of new foods including those that have been genetically modified. The Agency will also have the final word on advice given to Government ministers about the safety of pesticides and veterinary medicines, and will be responsible for licensing all abattoirs and drafting legislation on the labelling of food. It will share with the Department of Health the task of advising the public on a healthy diet. Policy advice will be provided to ministers, and technical advisors will be supplied to assist ministers during EU and other international negotiations. Both the Veterinary Medicines Directorate and the Pesticides Safety Directorate will remain attached to the Ministry of Agriculture, Fisheries and Food (MAFF). A research budget of £25 million will be available to the Agency. With anticipated costs of more than £100 million a year, most of the funding will be borne by a charge on the food industry. The staff of the Agency will be drawn from MAFF and the Department of Health, and the Agency will be answerable to the Secretary of State for Health.

Per capita beer consumption in litres is dominated by Europe; in 1995, the World Drink Trends report noted that the Czech Republic (160), Ireland (138), Germany (120), Denmark (116), Austria (114), Belgium (103), the UK (102), and Luxemburg and Hungary (99), were relatively thirstier than New Zealand (95), Australia (88) and the USA (86). Market segments involving the low-volume craft beers and microbreweries were still coming under pressure from the major market brewers, leading to the first signs of a shake-out in the brewing industry.

A merger of Guinness plc and Grand Metropolitan plc, with the participation of LVMH Moët Hennessey Louis Vuitton, gave rise to a new conglomerate, Diageo plc, that has the potential to dominate the global spirits industry for the foreseeable future. New brands of vodka were launched in Canada, France and Poland.

In the wine industry, where inbibition fashions and marketing strategies have been inordinately influenced by the opinions of a few journalists, the quality of the 1997 vintage world-wide was judged to be reasonably good. With the exception of California, there were concerns world-wide about harvest yields of grapes. Consumption patterns in the non-wine-producing countries of the western world remained experimental, accessing wines from most of the wine-producing countries and placing pressure on the market share of traditionally sourced wines.

The major soft drinks in terms of economic value and volume during 1997 were cola drinks and fruit juices. Innovative new drink launches included several products aimed at the health and sports markets. The teas market did not experience an upsurge in sales.

International issues considered by consumer groups in 1997 related to the sustainability of production and consumption, and the widespread privatisation of public utilities. In July, the UN Economic and Social Council agreed to establish an expert group to develop consumer protection guidelines with reference to sustainable consumption, extending the *Guidelines for Consumer Protection*, produced in 1985 and revised in 1995. The new guidelines would target product pricing that takes environmental costs into consideration, education, and misuse of 'environmentally friendly' labelling.

World Consumer Rights Day in March 1997, was celebrated by Consumers International, a federation of 215 member organisations in over 90 countries. A booklet was produced entitled *Consumers and the Environment: Meeting Needs, Changing Lifestyles.* Consumer activists campaigned at the WTO to be permitted to provide inputs into dispute decisions.

Sensitivities over food safety and the genetic manipulation of food products were even more evident in 1997 than in 1996. Western European consumer organisations expressed concerns about BSE, and an EU-wide ban on the export of British beef remained in force throughout 1997 although there was strong lobbying by the UK beef producers to have the ban lifted at the earliest opportunity. Consumer-group pressure on the Codex Alimentarius Commission, the international body that sets food standards, led to a postponement of the vote to permit the use of a genetically engineered growth hormone to increase milk production in cows. The Commission was also lobbied to permit even more representation by nongovernmental organisations; in 1997 the approved list of 111 organisations included 104 industry-funded

groups, 6 health and nutrition foundations, and Consumers International. Reverse pressure by biotechnology companies and sympathetic governments could induce Codex Alimentarius to prohibit the world-wide labelling of GM foods, and, if achieved, then the WTO will adopt the ruling as the global standard. Countries and trading blocs that permit or insist on labelling GM foods may be subject to punitive sanctions. Labelling would only be justified where there is a demonstrable safety issue.

Deregulation and privatisation were lively topics in Latin America and Asia; consumer organisations sought to promote consumer inputs in all the public utilities. In some Asian countries, deregulation and privatisation lacked proper monitoring leading to corruption, anticompetitive practices, and waste.

The rôle of dietary fibre from plants was considered in detail by an American Heart Association nutrition committee who suggested that various cereal grains, beans, other vegetables, and fresh fruit be included in the diet. Australian research suggested that the risk of breast cancer was lower in women who had a high intake of phytoestrogens, diphenolic compounds (e.g. certain flavonoids, isoflavanoids, lignans, coumestans and chalcones) in plants whose chemical structures and biological activity are similar to estrogens in mammals. Phytoestrogens are found in soy products, grains, fruits, and vegetables.

#### **Environment**

More than 1500 dams were reported to be under construction world-wide in 1997, nearly all of which were being built in seven countries: India (650), China (280, including the enormous 600 km-long Three Gorges Dam), Turkey (173), South Korea (131), Japan (126), Iran (49), and Brazil (42). Population growth stimulated increased demands for agricultural and horticultural irrigation, the need for potable water supplies, industrial needs, and the rapid growth in demand for electrical power. Thus, regardless of their environmental and sociological impacts, the numbers of dams under construction will increase in the years ahead. In an effort to establish common standards and principles for dam building, the World Commission on Dams, a joint effort by the World Bank and the International Union for the Conservation of Nature (IUCN), the World Conservation Union, was launched in South Africa in February 1998. Its remit includes the review of the effectiveness of dams, and to assess alternatives for water resources and energy development. About

35,000 large dams have been built since 1950. The International Finance Corporation, the World Bank's lender for private-sector projects, noted in October 1997 that by 2025 about 1 billion people could suffer water shortages unless radical changes were made to ensure reliable supplies. This would involve at least \$600 billion expenditure over the next decade. Because water supplies are so politically sensitive and companies are rarely allowed to finance investments through profits, water supplies will inevitably dwindle as populations increase.

Irrespective of the legally binding targets for the reduction in greenhouse gases agreed at the December 1997 UN Framework Convention on Climate Change in Kyoto, strong demand for coal was noted in 1997. This led to an all-time-high production level of 5.4 billion short tons in 1996 with preliminary data showing a combination of high demand for electrical power generation. Consumption was greatest in China, India and the USA. The Kyoto summit produced an outcome which would surely indicate higher demands in the future for natural gas, nuclear energy, and alternative energy.

The biggest fraud in the history of mining was exposed in March 1997, after Bre-X Minerals Ltd, a small Canadian exploration firm, had falsely claimed it had made one of the world's largest gold discoveries in Busang, Indonesia, possibly containing 3-4% of the world's reserves. Due diligence tests by the US-based Freeport-McMoRan revealed that Busang contained no significant gold reserves. Bre-X Minerals Ltd declared bankruptcy in November 1997.

Non-governmental organisations (NGOs), agencies, and affected local populations continued to exert pressures on mining companies to ensure proper environmental safeguards and remedial actions were put into place. Projects such as the open-pit nickel mine in Voisey's Bay, Canada, and the Century and Dugald River open-pit copper, cobalt, and zinc mine were delayed by concerned indigenous groups.

Early in 1997, the International Atomic Energy Agency reported that in 1996 there were 442 nuclear units operating in 33 countries, with a total operating capacity of nearly 351,000 MW and producing a total of 2,312 TWh. There were 36 nuclear units under construction in 14 countries; five units were scheduled to begin production in 1997. Political commitments in certain countries such as Sweden to phase out nuclear power presented the authorities with the

dilemma of developing energy-generating strategies to meet the Kyoto commitments. Partial progress was made in the international effort dealing with the deteriorating Chernobyl 4 sarcophagus - the Shelter Implementation Plan - a project phased over the next 9 years.

Major investments were made in the alternative energy sector by British Petroleum and the Royal Dutch-Shell Group. A Shell study predicted that alternative energy - principally solar, wind, and biomass but also geothermal and hydro - would provide 5% to 10% of the world's energy needs within 25 years, and could account for half of global energy consumption by the middle of the next century, especially if one or more new technologies were developed. A White Paper from the EC in 1997 argued that member states urgently needed to draw on renewable energies to meet the Kyoto targets. In 1995, renewable energy accounted for less than 6% of total energy demand in the EU, according to Eurostat, the EU's statistical agency. There were substantial variations between countries, from Sweden's 25.4% to the UK's 0.7%. Addressing renewable energy provision has the potential to assist in the solution of the EU's CAP problem in respect of biomass cultivation, wind- and solarenergy farms, and forestry.

Atmospheric and oceanic patterns across the tropical Pacific Ocean in early 1997 were indicative of the rapid warming episode referred to as El Niño. Originally, El Niño was defined as the occurrence of warm, southward ocean currents every few years near the coasts of Ecuador and Peru during the Southern Hemisphere summer when the winds are at their weakest. It signalled both a shift in local weather and a change in the biology of the ocean waters. It is now recognised that the strong episodes involve climatic anomalies that may begin in the tropics but ultimately extend over the entire Pacific Ocean and beyond. Such large-scale events are now known as El Niño, strictly speaking the term for El Niño - Southern Oscillation (ENSO). The ability nowadays to monitor the ENSO changes has permitted governmental agencies to plan rationally to counteract possible adverse effects of the episode. In the months following the outset of ENSO, monitored by instrumented buoys, some of the largest El Niño effects of the 20th century developed. Severe flooding, tornadoes, and torrential rains were recorded in the USA. Severe hurricanes were recorded in the Pacific; above-average temperatures were noted in South America and northern Africa; and perturbations to the normal weather patterns occurred in much of Asia. Intense dryness, regarded as one of the effects of El Niño, affected Indonesia, leading to the worst episode of air pollution in 50 years. Starting in mid-September, a photochemical smog and dense smoke from forest and peat fires settled over Indonesia, Malaysia, Singapore, Brunei, and spread to a lesser extent to Thailand, Hong Kong, the Philippines, and even reached the edge of northern Australia. By early October, four people in Indonesia had died and at least 32,000 had been treated for smoke inhalation. The polluted air was worsened by smog created by industrial and traffic emissions. The Australian office of the World Wide Fund for Nature claimed that between 485.000 and 610,000 hectares had been incinerated in Indonesia, some of which had been deliberately burned to provide low-quality, short-term grain cultivation to feed poultry to supply the 20% per annum rise in consumer demand in Asia. Nonetheless, the impact of ENSO on global food supplies in 1997 was minimal.

Environmental and ecological problems affected the textile industry in 1997, especially with regard to the toxicity of synthetic dyes and the degradation of products used in textile manufacture.

In February 1997, at the annual meeting of the governing council of the United Nations Environment Programme (UNEP), held in Nairobi, the MDCs charged that this UN agency had failed to deliver its main task - translating the findings of scientific organisations into policy proposals. The UK and USA refused to pay their 1997 subscriptions after several Asian LDCs blocked the formation of a task force to devise reforms.

In May 1997, the WHO published the results of an assessment of 12 toxic organic pollutants by the International Programme on Chemical Safety. There was sufficient evidence to merit international action to reduce or eliminate the discharge of these pollutants, namely, aldrin, chlordane, dichlorodiphenyltrichloroethane (DDT), dieldrin, dioxins, endrin, furans, heptachlor, hexachlorobenzene, mirex, polychlorined biphenyls (PCBs), and toxaphene. All 12 chemicals can be readily transported from their source by air or water.

In early June 1997, the World Bank released its *Green Top 10 Plan*, a list of proposed and desirable actions to address the world's most pressing environmental

problems. In noting that the \$800 billion per annum energy-related subsidies world-wide rarely benefited the poor, that global carbon dioxide emissions had increased by nearly 25% since the 1992 Rio Summit, and that 1.3 billion people were affected by polluted air, the Plan proposed two obvious actions: the phasing out of leaded petroleum spirit, and a marked reduction in the manufacture and use of chlorofluorocarbons (CFCs). More controversially, the Plan supported the market-related concept of countries trading greenhouse-gas emissions so that those countries unable to meet their targets could purchase permission to pollute from those countries whose emissions were below target. Any international programme for trading, however, would need to devise an initial allocation of emissions that would be acceptable to LDCs and MDCs, and the allowances would need to be reviewed regularly. Any scheme would face tough challenges in enforcement of regulations and shifting benchmarks.

Later in June 1997, 'Earth Summit+5', a special session of the UN General Assembly, was held in New York to review the pathetic progress made in the 5 years since the UN Conference on Environment and Development in Rio de Janeiro (The Earth, or Rio, Summit). Pressure was placed on the USA to join the EU in setting specific targets and dates for cutting greenhouse-gas emissions which had continued to rise despite a voluntary, but clearly empty agreement, to reduce emissions to 1990 levels by 2000. No progress had been made in curbing the depletion of ocean fish stocks, or combating deforestation and desertification.

Moreover, the scope of the Global Environment Facility, an international fund to support environmental projects in LDCs, was rendered ineffective because of sharp decreases in aid from the donor countries. The summit ended without agreement on the production of a political statement indicating how the Rio objectives might be met. Rather, the participants debated a programme for implementing Agenda 21 of the Rio Summit, a blueprint for sustainable development.

Signatories to the United Nations Framework Convention on Climate Change met in Kyoto during December 1997. Proposals by the USA, including the Global Warming Potential to rank greenhouse gases on their level of destructiveness, pollution credits, and differentiation between MDCs and LDCs, were carefully debated. Economic considerations tended to overrule environmental concerns. On 11 December

1997, a treaty - the Kyoto Protocol - was signed, committing the industrialised countries to reducing the emissions of six gases by an average of 5.2% (below 1990 levels) by 2012. Ratification started in March 1998 but there is doubt as to whether there will be full compliance by all the signatories.

The tenth anniversary of the signing of the Montreal Protocol on Substances That Deplete the Ozone Layer took place in Montreal in September 1997 in the presence of representatives of over 100 signatory nations. CFCs received particular attention at this meeting with agreement to discourage illegal trade in CFCs by adopting a formal licensing system for their transport. Participants also agreed to ban most uses of the ozone-depleting pesticide methyl bromide by 2005 (with the exception of quarantine and pre-shipment uses) in MDCs and by 2015 in LDCs. About 70%-80% of the 70,000 tonnes used annually worldwide are for soil-sterilisation prior to planting a wide range of crops. Poorer nations would have access to a fund of \$18m to help farmers convert to alternatives, if any become available in time. Biotechnological solutions are urgently required to introduce resistance to soil pests and diseases in the major crops.

The London-based Environmental Investigation Agency reported that between 6,000-20,000 tonnes of ozone-depleting CFCs were being smuggled into the EU each year from factories in China and Russia. For the USA, it was estimated that between 1994 and 1996, around 10,000 tonnes entered illegally through Florida alone.

In Vienna during September 1997, the 62 member nations of the International Atomic Energy Agency agreed to more stringent rules on the handling of nuclear waste and spent reactor fuel. During the same month the 12 nations and EU signatories to the Ospar Convention (formerly the Oslo and Paris Commissions) met in Brussels to debate methods to eliminate pollution in the North Sea and Northeastern Atlantic Ocean. The UK announced that it would join the ban on dumping low- and intermediate-level radioactive waste into the Atlantic, and would effect a virtual halt to discharges of harmful chemicals into the ocean by 2020. Attitudes are changing to the old adage 'the solution to pollution is dilution'. Towards the end of September, parties to the International Convention of Pollution from Ships met in London. All 75 shipping nations belonging to the International Maritime Organization agreed to reduce air pollution from ships by setting a ceiling of 4.5% on the amount of sulfur permitted in marine fuel - a somewhat meaningless figure given that the current average is 3%. Lower units were agreed, however, for designated areas e.g. the Baltic Sea, where concentrations were limited to 1.5%.

By mid-April 1997, the USA became the 24th country to ratify the Antarctic Environment Protocol, but two key nations, Japan and Russia, had yet to pass the necessary legislation to enable signing.

A study entitled *State of the Arctic Environment* by 400 scientists from the eight member nations of the Arctic Council was released in June 1997. Concentrations of DDT, lindane, PCBs and other pesticides from the Ob, Yenisey, and Pechora Rivers in Siberia were much higher than in North American and Scandinavian rivers. The pollutants were shown to be concentrated in organisms higher up in the food chain. Potentially harmful blood levels of mercury were found in 16% of Greenlanders, mostly acquired from eating whale and seal meat.

New Ambient Air Quality Standards legislation was approved in the USA in June 1997. Under the new regulations, the 24-hour permitted standard for PM2.5 (particles up to 2.5  $\mu m$  in diameter) was set at  $65 gm^{-3}$  of air. In addition, factories that continued to exceed unacceptable levels of ozone emissions after their fourth citation would be fined, but municipalities would have exemption for at least 7-8 years.

Climate change reports attracted headlines throughout the year. The EU appeared to be on track to break its own target of reducing greenhouse-gas emissions to below 1996 levels by 2000. This was attributed to the switch from coal to natural gas for generating power in the UK, the closure of inefficient factories in Germany, enhanced nuclear-power production in France, and recession-induced decline in the demand for power. Satellite evidence indicated that photosynthesis increased by an average of 10% between 1981 and 1991 in regions between latitudes 45°N and 70°N; and that higher temperatures had lengthened the growing season by 8-16 days.

In June 1997, the problems caused by the trade in wildlife were discussed at a meeting in Zimbabwe of the 10th Conference of the Parties to the Convention on International Trade in Endangered Species of Wild Fauna and Flora. At that meeting, Botswana, Namibia, and Zimbabwe were given permission to export ivory to Japan, reflecting a profound shift in attitudes towards balancing species protection with the sustainable use of natural resources, especially in LDCs.

The first European Botanic Garden Conference, 'Eurogard '97', was held in April 1997 at our sister organisation, the Royal Botanic Garden, Edinburgh. Organised by the Botanic Gardens Conservation International and International Association of Botanic Gardens Joint Advisory European Botanic Gardens Consortium, the conference aimed to identify the priorities for botanical gardens in a European Botanic Garden Action Plan for the EU, and for the promotion of closer links and collaboration between botanic gardens throughout Europe. In June, the European Parliament passed a motion supporting enhanced recognition of the rôles of botanic gardens in the EU in conservation, education, science, and culture. Major investments for botanic gardens included funding for the Millennium Seed Bank project of the Royal Botanic Gardens, Kew; a new herbarium for the Irish National Botanic Gardens in the Irish Republic; funding for rain-forest research at the Fairchild Tropical Garden in Miami, USA; a new botanical garden at the Tam Dao National Park, near Hanoi, Vietnam; and a new major conservatory at the Kirstenbosch National Botanical Garden, Cape Town, South Africa. The most frequently used international genebanks and germplasm collections are located in institutes such as SCRI.

Habitat destruction, largely as a result of logging, was thought to be responsible for the loss of at least three animal species per hour in tropical forests alone in 1997. The 1996 Red List of Threatened Animals issued by the International Union for Conservation of Native and Natural Resources identified 5,205 species in danger of extinction. According to the first Red List of Endangered Plants published in early 1998 by IUCN, 33,798 of the 270,000 plants thought to exist are threatened with extinction. The number of plants at risk amounts to six times the number of threatened birds, mammals, fish, amphibians and measurable invertebrates. One in 40 plants was listed as highly endangered or at imminent risk of extinction. In Turkey, one in five of the plant species is threatened compared with one in 10 in Greece and 1% in the UK. Many but not all of the threatened British species already have species-recovery plans devised for them under the Government's commitment to the 1992 Biodiversity Convention. According to Nature, the world's natural resources seem to be distributed in approximately inverse proportion to material wealth, e.g. the UK has around 1,800 plant species compared

with 18,000 in Peru. By way of contrast to the threat of extinction, *Takhtajania perreri*, assumed to be an extinct member of the ancient family Winteraceae, was found in Madagascar, 88 years after its only other sighting.

Only 6% of the Earth's forests were formally protected, leaving the remaining 3.6 billion hectares vulnerable to exploitation. Logging concessions rarely required habitat reconstruction, and LDCs are especially vulnerable to short-term financial gains from exploiting their natural resources. Research funded by the Brazilian government and released in January 1998, indicated that deforestation of the Amazon reached a record 2.9 million hectares in 1995, falling to 1.80 million hectares in 1996 and 1.3 million hectares in 1997. The deforestation resulted from increased local consumption, demand by international logging companies, expansion of agriculture - not least livestock grazing, and invasions by landless workers. The World Resources Institute in Washington reckoned that between 1960 to 1990, 20% of all tropical rainforest cover was lost, and around 10% of the world's coral reefs are now degraded beyond recovery. FAO estimate that 75% of the world's crop varieties have become extinct.

Newly established conservation areas in 1997 included the Bastak Nature Reserve in the Jewish Autonomous Region of the Russian Far East, the Hawar Islands in Bahrain, the Masoala National Park in Madagascar, and the Northern Truong Mountain Range in Laos and Vietnam. The UK Joint Nature Conservation Committee listed several areas for designation as Special Areas of Conservation recognised by the EU.

In addition to logging, farming, fishing, mining, and tourism exerted their toll on wildlife and their habitats. Industrial fishing fleets; unregulated trade in shark, tiger, leopard and tiger products; removal of young ostriches and their eggs for ostrich farming; construction of fish ponds and livestock paddocks in wild habitats; and conversion of natural habitats to crop production, were widely reported in terms of their deleterious effects on plant, animal, and fungal natural communities. Pollutants such as PCBs, DDT, and lindane were frequently identified in a wide range of wild animals. Road and rail accidents, electrocution, toxins from naturally occurring dinoflagellates, and poaching also exerted their distressing toll.

### **United Kingdom Perspectives**

Fundamental changes to the British constitution took place after the decision by the process of referendum in 1997 to establish by 2000 a Scottish Parliament and a Welsh Assembly. The Government's aim was to provide more accountable government in Scotland and Wales within the framework of the UK. The Scottish Parliament and Executive will be responsible for health; education and training; local government, social work and housing; economic development and transport; the law and home affairs; the environment; sports and the arts; research and statistics, and agriculture, fisheries and forestry. Reserved to the UK Parliament will be the constitution; UK foreign policy; UK defence and national security; the UK's fiscal macroeconomic and monetary system; common markets for UK goods and services; employment legislation; social security; and most aspects of transport safety and regulation. Relations with the EU will remain the ultimate responsibility of the UK Government but the Scottish Executive will be involved in decision-making on Europe. SCRI was one of the Scottish Public Bodies listed in Annex A of the July 1997 White Paper Scotland's Parliament (Cm 3658). These are deemed to have a remit which is concerned with matters to be devolved. Other such bodies include the other four SABRIs; the Royal Botanic Garden, Edinburgh; the Scottish Higher Education Funding Council; water authorities; health bodies; and various advisory bodies. The SABRI remits clearly extend beyond the UK, but they have a special relationship with Scotland. Any changes to the Annex A bodies will depend on ministerial decisions. The Scottish Executive will be able to alter the structure of or wind-up existing bodies, and create new ones. It will also be able to alter budgets of the Annex A public bodies to suit its own priorities.

Much debate ensued about taxation arrangements in the new devolved Scotland. Only 5.25% of employees in employment earn £30,000 or more in Scotland, compared with 7.25% in England, and 11% in South East England. The legislation proposed continuation of the 'Scottish Block and Barnett formula' system of funding, and the Scottish Parliament will be able to raise limited income by means of a defined but limited power to vary the rate of basic income tax in Scotland, and the Scottish Parliament will be responsible for local-government finance including local taxation.

According to Employment Conditions Abroad International (eca@ecaltd.com), spending taxes mean

that the UK, with the exception of Denmark and Japan, had the highest real cost of living in the world. This undesirable position was somewhat worsened by high prices for services and branded goods. According to the Advocate General of the EU, such differentials between the UK and the USA, for example, are justified by the 'principle of community exhaustion' whereby protectionism is approved. This contrasts with the operation of the doctrines of the international exhaustion of rights and of reciprocity. On the other hand, according to the WHO's 1998 World Health Report, the UK gained top placing in a survey of the proportion of people who survive to the age of 50, reflecting a combination of good health and low accident rate. Road deaths in Britain are lower now than they were in 1926, even though there are more than 30 times as many vehicles.

Similar to last year, the UK R&D Scoreboard 1998, produced by the Department of Trade and Industry, demonstrated the relatively poor performance of UK companies compared with international competitors. The top UK companies increased their R&D investment between 1996 and 1997 at a rate well below the average of those countries with companies in the top 300 international list. For 1997, the percentage increases in corporate R&D investment by the relevant top 300 international companies were as follows: Sweden 26, Denmark 23, Canada 18, USA 17, Germany 10, Japan 9, Switzerland 8, France 7, UK 5, The Netherlands 4, Italy 3, and Belgium -7. R&D intensity is a measure of the extent to which sales revenues are reinvested in R&D. When aggregated on a country-by-country basis, the R&D Scoreboard clearly shows that the for past 6 years the UK's aggregate R&D intensity lagged seriously behind that of the world's top 300 companies and the other G5 countries. This conclusion is supported by OECD data, although the aggregate intensity is weighted mostly by the intensities of those companies with the largest sales figures. If the average of the company R&D intensities is considered instead, then the UK appears to be more compatible with France, but still behind Germany, Japan, and the USA. When viewed at the level of individual sectors, then the UK pharmaceuticals sector showed significant R&D growth in 1997 taking it to the top of the international R&D league. Most of the other sectors which were engaged in international trade were significantly below the R&D intensity international average. Even though a survey by the Office of Science and Technology revealed that UK researchers have been the most productive and

cost-effective in the world, UK business was failing to exploit the science base. Too much of the UK economy is low technology, low wage and low aspiration, and a revitalised Foresight Programme is eagerly awaited to change attitudes on R&D investment. Spending on civil research by the UK public sector had, unlike other G7 nations, declined markedly over the last 10 years, whereas non-governmental spending had increased dramatically.

From the Internet site: http://www.cbi.org.uk/innovation, the latest survey of innovation trends by the Confederation of British Industry revealed depressingly that spending on innovation was down for the third year in succession. UK domestic companies, in particular, were innovation unfriendly, having the least positive attitude towards the exploitation of novel technologies. Investment in R&D was skewed, with most firms spending very little and a few large companies spending heavily. The innovation process was defined as a combination of activities including design, research, market investigation, process development, and organisational restructuring. British industry recently appeared unable to make productivity gains and reduce labour costs, perhaps related to an inability to exploit innovation.

A London School of Economics research report in September 1997 noted that regulation and inspection of public services by 'watchdog' auditors, ombudsmen, inspectors and the like, excluding the regulation of the privatised industries, had grown over the past 20 years into a £2.6 billion 'business' employing over 20,000, possibly 30,000 people. It was claimed that most regulators ('enforcers') did not attempt to assess the costs of compliance to their clients. Over 20 years, expenditure on regulation had risen between 3-6 times faster than overall public spending. Other reports complained of over-zealous enforcement, lack of transparency and consultation, lack of co-ordination between enforcing bodies, lack of consistency and failure to keep enforcement in proportion to the cost and risk of not taking action.

Historically, British agriculture has been regarded as highly competitive, efficient and forward-looking, but doubts were expressed by the National Farmers' Union as to its ability to compete when CAP-derived protection from foreign competition is removed. With a massive decline in profitability, asset values (mainly livestock) plunging, and no redundancy schemes or industry pension funds to reduce farming numbers, British agriculture will need to restructure

irrespective of the *Agenda 2000* proposals. The labour productivity of UK farming, which in the early 1980s showed a significant lead over other European countries, has been overtaken. For UK agriculture, the net value added (which measures how efficiently an industry converts inputs to outputs) was 90% above the EU average in 1980 but has now sunk to just 15% above the average, excluding the effects of currency valuation changes. In respect of EU agricultural productivity growth between 1980-1996, only Finland, Sweden, and Germany were lower than the UK. Denmark, Greece, Belgium, France, Portugal, and Spain recorded improvements of 20% to over 70%.

Data for UK agricultural production are readily accessible from MAFF on http://www.maff.gov.uk/, and in *Food & Drink Statistics 1998*, published by Euro PA & Associates, there is a sector-by-sector guide to agriculture, fisheries, food and drink statistics in the UK. Most of the information available at the time of preparing this review article referred to years no later than 1996.

For 1996, UK farm output was £18 billion, and food and drink manufacture was £62 billion. Consumers spent £52 billion on food and non-alcoholic drink, £28 billion on alcoholic drinks and around £38 billion on catering outside the home. To the £643 billion UK GDP, agriculture, hunting, forestry, and fisheries contributed £11.8 billion, and food and drink manufacturing £16 billion. The overall food trade gap was at least -£7.7 billion, of which -£0.8 billion was for commodities covered by the Lomé IV agreement (cane sugar and bananas), -£2 billion was for non-indigenous commodities that cannot be grown or processed in the UK, or production is limited (e.g. rice, citrus fruit, unmilled maize, coffee, cocoa etc.), and -£2.4 billion for non-substitutable processed products such as wine, juices, certain animal fats and oils, leaving -£2.5 billion at least as the 'realisable' domestically substitutable food and drink trade gap. Of the total food expenditure of £52 billion, 21% of this was spent on fruit and vegetables including potatoes. Provisional figures for 1997 from MAFF indicate that UK wheat production was 15.1 mmt, a drop of 1 mmt from 1996; 85% of wheat for flour milling was home-grown; barley production was 7.85 mmt, a slight rise above 1996 levels but less than production levels during 1982-1990. Oat production dropped slightly to 0.54 mmt. Rapeseed production rose to 1.5 mmt, linseed was 0.1 mmt. Sugar beet production rose from 10.4 mmt to 10.5 mmt. Potato production was affected by disease and declined to 7.1 mmt, 0.4 mmt of which were earlies. Processed and

raw potatoes accounted for 0.5% of all consumer spending as measured by the basket of goods and services within the Retail Prices Index (RPI). All other vegetables combined make up only 0.7% of the RPI. A steep decline in potato production area was more than compensated for by a rise in yield. In 1996, field vegetables, i.e. grown outside and including carrots, parsnips, onions, cabbage, brussels sprouts, cauliflower, calabrese, beans, and peas, amounted to 2.7 mmt with a farm-gate value of £666.7 million. Protected vegetables, e.g. peppers, celery, tomatoes, mushrooms, lettuce, cucumbers, etc., amounted to 352,000 tonnes with a value of £346.3 million. Fruit production was 364,000 tonnes with a value of £231.9 million. By proportionate value, the most prominent fruit were strawberries (23%), dessert apples (19%), raspberries (15%), culinary apples (13%), pears (7%), blackcurrants (5%), and plums (3%). Imports of vegetables from outwith the UK and Channel Islands were around £890 million, and comprised by value, tomatoes (22%), dried vegetables (12%), mushrooms (10%), cauliflower and broccoli (8%), lettuce (8%), peppers (8%), cucumbers (4%), and onions (4%). Fruit to the value of £1.47 billion was imported into the UK in 1996, and comprised by proportionate value, bananas (22%), apples (19%), grapes (10%), oranges (8%), mandarins (8%), melons (6%), peaches and nectarines (5%), and pears (4%). Analysis of household consumption patterns of fruit and vegetables in 1996 show that 22.1 mmt of fresh vegetables excluding potatoes and potato products, 11.8 mmt of processed vegetables excluding potatoes and potato products, 21 mmt of fresh fruit, and 10.3 mmt of processed fruit, were consumed in the UK. Expenditure on prepared salads rose to £100 million, and the UK total organic fruit and vegetable market rose to around £200 million. The frozen ready-meal market reached £875 million and the prepacked sandwich market £1.9 million.

#### **Plant Biotechnology**

Mankind has already entered the era of genetic commerce, but agricultural biotechnology (agbiotech) markets in Europe have been suppressed compared with other regions of the world. The main reasons for this situation are fivefold. (i) The CAP favours the inefficient with a mixture of production and marketing subsidies and compensation payments, leading to rural social therapy and a bloated EU-related bureaucracy. R & D programmes become relegated to policy-impact studies. (ii) A stable but rapidly urbanising population is becoming divorced from the realities of rural enterprise and demands a risk-free existence. (iii)

Bovine spongiform encephalopathy and food contamination issues have confused regulatory failure with scientific advances, and there has been a loss of confidence in scientists. (iv) Ignorance of biotechnology generally is profound in the population at large, and in most political and decision-making groupings. (v) There is a persistent suspicion of technology, profitmaking, and multinational companies, aided by largely antibiotechnology news media dependent on sound-bites. The careful incremental advance of science using complex concepts, specialist terminology and natural tendency to avoid absolute answers to simplistic questions have made science mediaunfriendly. Not surprisingly, these factors have induced new legislation, burdensome regulation, potentially pejorative labelling, industry-wide codes of practice, and prolonged monitoring systems within the EU, mainly to satiate wantonly poorly informed, often paternalistic, political and pressure groups. Resources that could be spent on R&D and market development are being diverted not only to overbearing regulation and resource- and time-sapping bureaucracy in a zealous application of the precautionary risk principle, but also to security and protection from attacks against technology centres and GMO crops.

Nevertheless, the agbiotech market and underpinning R&D activity will grow for at least a dozen reasons. (i) WTO rules will overcome contrived trade barriers to GMO products. (ii) EU enlargement and dissatisfaction with costs will bring about a radical change or collapse of the CAP. (iii) Improved yield efficiency and quality of agbiotech crops and livestock will force acceptance by European producers and processors. (iv) New niche markets which can be generated by biotechnology are desperately required by a loss-making agricultural sector. (v) Agbiotech provides improved intellectual-property protection, improving returns on investments and giving competitive advantage. (vi) Despite the success of those individuals and organisations propagating misinformation, and regardless of set-backs from time-to-time as occurs to all technologies, the truth of the advantages and disadvantages of agbiotech will eventually become manifest, aided by industry bodies and independent scientific organisations. (vii) Gradually, the body politic and investment managers are realising that global opportunities for agbiotech will greatly assist in wealth creation and improving employment prospects in Europe. (viii) Several international technology foresight programmes set up to advise public- and privatesector R&D sponsors have highlighted the pivotal rôle of biotechnology for future wealth creation, improved quality of life and enhanced industrial competitiveness. (ix) Agbiotech has the ability to address major environmental problems (e.g. replacing conventional and low-efficiency agriculture that threaten natural habitats; bioremediation of polluted land, water and air; improving visual amenity and biodiversity etc.). (x) Constraining agbiotech will drive away the most innovative young life-scientists from Europe. (xi) Rapid development of new biotechnology companies in the EU - seen especially during the past year in Germany and France - will help bring about attitudinal changes. Scrutiny of UK parliamentary reports since the 1890s reveals remarkable attitudes to the introduction of new technologies. For the most part, there has been deep suspicion and often open hostility to virtually all the advancements that are now taken for granted.

A synopsis of the agbiotech industry world-wide in the financial year 1997-1998 shows it to be growing at a rate of around 20% per annum, and receiving massive investments. It was also supporting huge research programmes in molecular genetics and proteomics. Between 40%-50% of all US crops will be transgenic by 2000; the global area of transgenic crops in 1997 was about 31.5 million hectares. In the period 1986-1997, there were 25,000 transgenic crop trials using more than 60 crops in 45 countries. At the end of 1997, 48 transgenic crop products involving 12 crops and 6 new traits were approved for commercial-scale release and commerce. A useful reference is Global Status of Transgenic Crops in 1997 by C James, published by the International Service for the Acquisition of Agri-biotech Applications. To date, the technology has proved to be stunningly safe, especially in respect of human health and the environment. Many technological advances have been made since the early phases of deploying antibiotic-resistance marker genes. Ancillary non-transgenic agbiotech advances were made in (i) diagnostics for pests, diseases, and quality traits; (ii) selection of parental material for rapid conventional breeding; (iii) techniques to measure and quantify biodiversity and gene flow; and (iv) development of novel approaches to produce pharmaceuticals, carbohydrates, oils, plastics, etc. A common feature of GMO crops has been a reduction in inputs.

Intellectual property in biotechnology can be protected, providing the basis of competitive advantage and the basis of investment. At present, the enabling (platform) technologies and techniques are mainly in the

hands of a few major companies, and as public-sector investments in research decline then there will be a gulf between publicly available generic technologies and those controlled by the major companies who are in a phase of mergers and take-overs. The disparity is aided by attitudes that prevent the use of public funds for what is deemed to be "near-market" research. Fortunately, biotechnology is still a young technology, where there are still endless opportunities for the astute and/or lucky, and the costs of biotechnology are declining rapidly with the advent of new automated systems. Concerns about LDCs - and some MDCs accessing the products of biotechnology can be addressed to some extent by those countries investing in the exploitation of biotechnology generally, and agbiotech in particular, to suit their own priorities.

New types of agronomy will have to be developed to respond to the disquiet of environmentalists. Release and monitoring trials will need to be approved on a case-by-case basis. GMO crops will require proper segregation to lessen the possibility of gene flow ("genetic pollution"). Refugia and dispersal corridors (e.g. wide hedgerows) for the natural flora and fauna, need to be safeguarded and developed, a point which I have advocated for several years and which I included in the Delphi questionnaire for the Agriculture, Natural Resources and Environment Sector Panel of the UK Technology Foresight exercise. Monitoring systems should be in place anyway, not only to check on the performance and impact of GMO crops but to determine the impacts of new pests, diseases, plants, and animals directly and inadvertently introduced most frequently by domestic horticulturalists. Any new technology, (e.g. aircraft, motor cars, weaving machines, pesticides etc.) may be expected to have failures and so the environmental and human health impacts of agbiotech as much as conventional agriculture and horticulture will constantly be assessed, but this does not imply overly heavy-handed regulation. No company in a litigious society would wish to face the financial consequences of failure; likewise, the marketplace will determine the need for a product, not socio-political panels.

In a supplementary and on-going survey complementary to the UK Technology Foresight and Foresight exercises, I have sought the views of leading-edge successful agriculturalists in Australia, Canada, France, Germany, South Africa, Spain, UK and the USA. Over four years, it has been remarkable to see a relative unanimity of views that point to the main needs of agriculture. For crops, breeding is regarded as a piv-

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otal requirement, with access to improved cultivars for enhanced pest and disease resistance, enhanced quality and ability to grow over longer periods. Automation, involving precision systems, decision-support systems, and robotics are seen to bring huge benefits in costeffectiveness. Sustainability relating to profitability is of increasing importance in respect of water- and nutrient-use efficiency, fewer pesticides, and soil remediation. Biotechnology is seen very positively as the primary process to deliver breeding systems, new products and markets, health and disease control, and new opportunities. Animal welfare seemed to be a peculiarly British phenomenon. Concern is expressed about accessing new technologies and markets and how to achieve on-farm added value, but there is the realisation that family farms are under pressure as agricultural and horticultural industrialisation is underway, and vertical and horizontal integration of agriculture and other related industries takes place. In due course, I shall publish the survey.

Simply put, modern-day agriculture, if allowed to flourish as a proper business, will allow crops and livestock to be grown where they produce the most efficient yields. Huge post-harvest losses suffered in the LDCs will be largely eliminated. Herbicides, pesticides, chemical fertilisers, new varieties, and automation, collectively were responsible for the tripling of global crop yields between 1960 and 1992 on more or less the same area of cultivated land (circa. 1.56 billion hectares). Without agricultural improvement, an additional 2.87 billion hectares would have been needed, all derived from natural ecosystems. Thus, the natural habitats have been protected. Our new challenge is to improve yields on existing land to meet a doubling of the present global population in 47 years, if current population trends are to continue. The challenge may be too modestly presented were the populations of LDCs to demand a diet rich in animal products as in MDCs.

Funding for agricultural research has diminished as rapidly as the population grows. The International Food Policy Research Institute estimated that global spending on relevant research was only \$15 billion to support a multi-trillion-dollar food industry. Over the last 30 years, cuts of around 30%-65% have been made in public spending on agricultural research in the MDCs. Farm subsidies and trade barriers, however, have risen enormously. Biotechnology and newgeneration agro-chemicals are now revolutionising agriculture and preserving biodiversity. Pesticides have not yet caused the extinction of a single known

species, despite their use over 50 years. A crisp and pithily accurate review of agriculture entitled *Saving the Planet with Pesticides, Biotechnology and European Farm Reform* by Dennis Avery was presented as the 24th Bawden Lecture at the 1997 Brighton Conference organised by the British Crop Protection Council.

After long deliberation, the EU farm commissioner, Franz Fischler warned at a meeting in Vienna in October 1997 that the hostile attitude in the EU towards biotechnology could cost up to 200,000 jobs as agbiotech companies will only invest in countries where a friendly environment exists.

In 1997, the US Environmental Protection Agency (EPA) announced its intention to regulate (EPA Plant Pesticide Proposed Rule) biotechnological alternatives to pesticides in the same way as conventional pesticides. This would therefore include all transgenic plants that have been engineered to be resistant to pests and diseases. A chemically identical plant produced by conventional breeding would remain unregulated. Thus, a GM plant with thicker epidermes or hairs to repel pests and diseases could be classified as a pesticide. The sheer costs of such a scientifically incoherent proposal would mean that both minor crops and small companies would suffer. Only the large transnational companies would be able to address the regulatory burden. Eleven scientific societies contested the logic of the proposal.

In the UK, the Department of the Environment, Transport and the Regions proposed the introduction of pesticide taxes in its consultation paper *Economic Instruments for Water Pollution*, and a follow-up commissioned paper *Private Costs and Benefits of Pesticide Minimisation*, suggesting that tax levels of up to 125% might be required to bring about a significant reduction in pesticide usage. The latter report was commissioned in 1996 and used out-of-date grain prices and did not take account of new pesticide-use agronomic technologies, nor costed accurately the limited potential benefits to growers if all available techniques are used to minimise pesticide usage.

Plant genetic engineering in 1997 addressed a phenomenal range of problems, including tolerance to drought, frost and aluminium toxicity. The US Congress announced a \$40 million plan to analyse the maize genome which comprises three billion base pairs and 30,000 genes, and the Japanese government pledged to map and sequence the rice genome, a sixth

the size of the maize genome. Important advances were made in the genetics of resistance mechanisms to pests and diseases, and in physiological processes downstream from the genes.

In my review article in the 1996/1997 SCRI Annual Report, I described briefly the pioneering work of Ian Wilmut and his team at the Roslin Institute in Edinburgh. They reported in early 1997 the first clone of an adult mammal using the nucleus of a differentiated somatic cell from the mammary gland. The birth of the Finn Dorset ewe named Dolly both dispelled the presumption that adult mammals could not be cloned, and raised globally the whole profile of the ethics and morality of mammalian cloning technology. Strictly speaking, the term 'cloning' (etymologically from the Greek Klon, meaning twig) refers to the taking of plant cuttings or offshoots for onward propagation, a practice thousands of years old. It has now come to mean the production, typically by means of a nuclear transfer as opposed to naturally occurring clones such as monozygotic twins, of genetically identical animals. Nuclear transfer involves removal of chromosomes from an unfertilized egg and replacing them with a nucleus from a donor cell. The fact that the donor nucleus comes from an adult cell is remarkable for it had long been supposed that, because of differentiation, the cells had lost their totipotency or embryonic characteristics. There are fascinating new research lines on X-chromosome inactivation, cytoplasmic effects, applicability to a wide range of species (there are now cloned mice in Hawaii), introduction of precise genetic characteristics, genotype x environment interactions etc.

Patenting of plants and animals in Europe remained unclear even after the EU Directive on the Legal Protection of Biotechnological Inventions was passed by the European Parliament in Strasbourg on 12 May 1998, after 10 years of debate and even a rejection by the European Parliament in 1995/1996. The Directive is binding only on member states of the EU, not on the European Patent Office (EPO). Article 4.2 ('Inventions which concern plants or animals shall be patentable if the technical feasibility of the invention is not confined to a particular plant or animal variety') was intended to supplant decision T356/93 (Greenpeace versus Plant Genetic Systems (PGS)) of the EPO Board of Appeal. This decision in effect invalidated PGS claims because they embraced plant varieties which are statutarily impatentable subject matter, even though the claim did not define plant varieties nor was the invention confined to a particular variety.

Should the EPO Enlarged Board of Appeal not reverse the PGS decision then such patents would have to be obtained through national patent offices.

The essential features of the new EU Directive are sixfold. (i) Inventions concerning plants and animals are patentable only if the inventions are not confined to a particular plant or animal variety. (ii) Mere discovery of a gene is not patentable, but an element produced by means of a technical process (whether or not a gene or partial sequence) is patentable. (iii) At the outset, the industrial/commercial application of a gene sequence or partial sequence must be disclosed in the patent application. (iv) There are complicated provisions relating to whether patent protection extends to material propagated from a patented product. (v) In some circumstances, plant breeders may seek compulsory licenses under plant patents. (vi) Four areas of potential inventions are unpatentable: methods of cloning humans; modifying the human gene line; using human embryos for industrial or commercial purposes; and genetically modifying animals if likely to cause them suffering without any substantial benefit to man or animal.

In an attempt to impede the Directive which harmonises patent law in Europe, 'green' members of the European Parliament donned black pirate costumes and waved a banner in the European Parliament denouncing 'biopiracy'. The new Directive will not substantially change UK patent law and will at least remove the threat of pharmaceutical and biotechnology-based companies moving outwith the EU. Even though the Directive still lacks clarity in many areas, and because it falls within the framework of the Single Market Initiative, there is potential for erratic implementation across the EU. EU patent law still remains vastly inferior to that operating in the USA, with the possible exception of the far-too-narrow 'experimental use exception' in the USA for plant varieties.

Protection of plant varieties (plant variety rights) and plant breeders' rights are covered in Europe not only by national law but also by an international convention, the International Union to Protection of New Varieties of Plant (UPOV, 1961). Unlike patent protection, competing plant breeders have special privileges (breeders' rights or breeders' privilege or research exemption) in using protected varieties in their own breeding programmes, and farmers can use harvested seed for subsequent saving on their own farms (farmer's privilege), subject to safeguarding the interests of the holder of the plant variety rights. In time,

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the UPOV regulations which were designed for conventional breeding systems, and patenting will have to interrelate more closely. At present, the currently operative text is UPOV 1978. A further revision was made in 1991 but awaits ratification by member states. There is also the European Council Regulation on a community-wide system of plant variety rights agreed in 1994.

In *Love à la Mode, II.* i, Charles Macklin, the Irish actor and dramatist (*circa* 1697-1797), wrote that: "The law is a sort of hocus-pocus science, that smiles in yer face while it picks yer pocket; and the glorious uncertainty of it is of mair use to the professors than the justice of it". Patent law is very complex and expensive.

To bring a GM crop and its products to the marketplace in the EU involves a frustrating jungle of legislatory and regulatory procedures, causing uncertainty and risk to companies, investors and employees. The whole approval process is at least twice as long in the EU as in Japan and the USA. In theory, the principles of relevant legislation are (i) not to prevent the supply of safe, wholesome foods; (ii) to permit the free movement of products in a single market; (iii) to reflect changing consumer demands; (iv) to be based on sound science; (v) to be enforceable, and (vi) protect human health and the environment from possible undesirable effects of GMOs.

Two EU Directives are critical for GMO regulation, according to Nick Tomlinson of the Joint Food Safety and Standards Group based in MAFF. (i) Directive 90/219/EEC covers all GMOs in containment, and includes GMOs used to produce food additives or processing aids. In the UK, this Directive is implemented under the Health and Safety at Work Act through the Genetically Modified Organisms (Contained Use) Regulations, administered by the Health and Safety Executive (HSE) which in turn is advised by the Advisory Committee on Genetic Modification (ACGM). (ii) Directive 90/22/EC covers the deliberate release of GMOs into the environment, and, unless and until covered by other EU legislation, includes the marketing of GMOs. Originally published in May 1990, the Directive has been amended twice and is still not implemented in all member nations. The Directive is implemented in the UK through the Genetically Modified Organisms (Deliberate Release) Regulations, made under the Environmental Protection Act, and administered by the Department of the Environment, Transport and the Regions (DETR), which in turn receives advice from the independent Advisory Committee on Releases to the Environment (ACRE). All releases are advertised locally, the details are made available on a Public Register and posted on the Internet, and release sites are inspected by the Health and Safety Inspectorate. Risk assessments must demonstrate no significant risk to humans or the environment. With openess comes vulnerability to attacks by 'eco-warriers' who are impervious to reason.

Applications for a marketing consent are scrutinised by all 15 member nations. In addition, there is a tranche of sectoral legislation at EU level relevant to GMOs, including in the food sector the May 1997 EC Novel Foods Regulation (EC 258/97). For GM crops intended for food use, the marketing of seed for cultivation in the EU will require marketing consent under 90/220. To this must be added specific approval for food products derived from the GM crop required in accordance with the Novel Foods Regulation. This regulation which went through 14 redrafts and three EU Parliamentary Readings, introduces a mandatory EU-wide pre-market approval process, which involves the EC and Standing Committee for Foodstuffs, consulting if necessary the EC Scientific Committee for Food.

Novel foods are defined as those foods that hitherto have not been consumed to any significant extent in the EU. In the six categories identified are included both food containing or consisting of GMOs (as defined in the Directive 90/220) and food produced from but not containing GMOs. Approval depends on the novel food not (i) constituting a danger to the consumer, (ii) misleading the consumer, and (iii) differing from foods or food ingredients which they are intended to replace in such a way that their normal consumption would be nutritionally disadvantageous.

In the UK, the safety of all novel foods, including GM goods, is assessed by the 18-member independent Advisory Committee on Novel Foods and Processes (ACNFP), formerly chaired by Derek Burke and currently chaired by Janet Bainbridge. ACNFP adopts the authoritative approaches developed by the WHO and OECD in assessing the safety of novel foods, and is based on the concept of substantial equivalence. According to the WHO "substantial equivalence is established by a demonstration that the characteristics assessed for the GMO, or the specific food produce derived therefrom, are equivalent to the same characteristics of the conventional comparator. The levels and variation for characteristics in the GMO must be

within the natural range of variation for those characteristics considered in the comparator and be based upon an appropriate analysis of data". Where there is not a proper comparator does not imply that the GMO and its products are unsafe, only that extensive analytical data will be required to verify safety.

Specific labelling requirements were introduced by the Novel Foods Regulation, under the auspices of Directive 79/112/EEC which was implemented in the UK by the Food Labelling Regulations 1996 made under the Food Safety Act 1990. In June 1997, the UK Government stated that all foods containing GM material must be clearly labelled. The regulations therefore ensure that the consumer would be informed when a foodstuff is no longer considered to be equivalent to existing foodstuffs and ingredients, or when it contained allergens not present in the convention or equivalent, or if there are special ethical concerns. All live GMOs (those that would theoretically grow if planted) would require labelling. In July 1997, there was EU-wide agreement on regulation 1813/97 to apply these labelling rules to GM soya and maize which had been introduced to the market-place prior to the Novel Foods Regulation.

The EC Proposal (COM[1998]1999 final) exempted additives, flavourings and extraction solvents from labelling, and specified that labelling should apply to a GMO and its produce, and would be triggered by the presence of DNA or protein resulting from genetic modification. The 'may contain' labelling proposal was shelved in the face of opposition of all EU member states except Denmark, Italy, and Sweden. Some EC officials believe that the labelling policy will be unworkable, and the USA has submitted objections to the WTO.

In a revealing and acerbic analysis of pseudo-environmental and related pressure/activist groups, Mark Neal and Christie Davies detailed the tactics used to attack the business world and free enterprise in their book *The Corporation under Siege*, published in 1998 by the Social Affairs Unit, London. Originally focusing on the pharmaceutical, drinks and tobacco industries, the pressure groups now include food, farming, forestry, mining, water and other utilities, chemicals, toys, and tampons in their sights. Realising that medically related biotechnology enjoys public support, the groups have focused particularly on agricultural and food biotechnology. Aided by a mixture of uncritical, ignorant or sympathetic journalists, and by bureaucrats who impose tough regulations even when the

evidence to justify them has not been provided, there has been cynical and mendacious manipulation of public opinion; in some cases, there is evidence that the groups believe their own utopian pseudo-ethical propaganda. Parenthetically, some of the most accurate and balanced scientific reporting is carried out by agricultural journalists and correspondents. Techniques used successfully by the pressure groups to make allegations and generate fear include (i) exaggeration, so that when claims are later scaled down, some of the 'mud sticks'; (ii) identification of 'clusters' of disease and blaming environmental causes without sound statistical analysis, frequently using selective citations to infer a consensus; (iii) mistaking coincidence for causality; (iv) claiming that small amounts of a substance are dangerous just because large amounts are; (v) ignoring real levels of risk but emphasising relative risks which sound more newsworthy; (vi) launching vexatious litigation; (vii) using selective citations of reports of laboratory experimentation on GMOs to condemn agbiotech; and (viii) ignoring the benefit, pleasure or necessity of a product or process but emphasising the harm it causes. Exaggerated and irresponsible scare stories are made, damaging companies as well as independent scientists who are without recourse to claim damages. We all acknowledge that there must be freedom for the public responsibly to express concern and seek explanation. To date, corporations have tended to appease the activists by avoiding public debate and issuing 'corporate-responsibility' reports. Furthermore, perfectly valid environmental and dietary concerns are submerged beneath distorted propaganda.

Classification of research activity is fraught with rigidities and complications, given that the research process is neither linear nor unidirectional. Applied research can generate the raison d'être for basic research - witness, for example, studies on photoperiodism and phytochrome. The widely applied Frascati Analysis is an OECD Coding and is in three major categories: oriented basic research is carried out with the expectation that it will produce a broad base of knowledge to the solution of recognised or expected practical problems or possibilities; strategic applied research is defined as applied research where the work, although directed towards practical aims, has not yet advanced to the stage where eventual applications can be clearly specified; and specific applied research is not strategic in nature and has as its aims specific and detailed processes, systems and the like. All of the UK institutes involved with the life sciences operate within

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these major categories, which intimately cross-link. Crucial scientific work (e.g. long-term plant breeding, plant gene promoters etc.) is regarded as 'near-market' and in Scotland at least is no longer funded by the Government. The broad church of biotechnology covers all the Frascati sectors as well as crucial scientific work. There were views expressed by Graham Hills and others during 1997-1998 that assumptions on the linear relationship between science and innovation are flawed, and that industrial innovation often stems from improved technology. Thus, more encouragement is required to switch more of the best researchers into interacting with industry, rather than adopting a condescending attitude towards matters applied, and regarding blue-skies research as the acme of achievement.

#### **Careers in Science**

Sad to say, science now represents a poor and insecure career choice for young people with talent, a point made during the year by many senior scientists, not least Harry Kroto, the Nobel Laureate. Compared with other professions, scientific careers likewise require specialist knowledge but may last just a few The rate of attrition is staggering. Measurements in the USA by Art Sowers (see newsgroup <sci.research.careers>), using Current Contents, would indicate attrition rates of 29%-40% for biologists. The situation was no better for authors based at pharmaceutical and biotechnology companies than in universities. Statistics generated by the US Government show that only 40% of those with PhDs in science are actively involved in research. My own provisional analyses indicate that the picture is much bleaker in the UK, descending to a figure of less than 20%, raising questions about the value-for-money of PhD training - fundamentally, it can be inordinately expensive to the individual in terms of career development let alone to the public purse. The bulk of university research is conducted through the medium of training PhD students, possibly indicating a demeaning of the priority status of the research. If it is worth doing, then it should be done properly, not on the 'cheap'. Training PhD students is an honorable profession in its own right, but is there a 'need' for so many to be produced? The present trend in the public sector involves an expectation of appointing graduates with a first or upper second-class honours degree to low-paying postgraduate studentships. They are then expected to complete a successful PhD in less than 4 years, produce several refereed papers, and complete two or more poorly paid fixed-term postdoctoral posts in first-rate laboratories, thereby becoming technologically and conceptually competent to conduct free-thinking research, accurately employing specialist terminology, writing fluently for high-impact journals, and presenting the research at international meetings. The young scientist also has to be entrepreneurially alert and be aware of generating and protecting intellectual property, and must have good abilities to attract highly competitive grants and contracts.

Security of tenure in public sector research institutes is weak, and is becoming so in universities. Projects are typically of three or fewer years in duration, and the work is increasingly policy-driven rather than being intellectually 'entrepreneurial'. Frequent reviews of individuals, research projects (ex-ante, ongoing and expost), and the organisation, in parts or as a whole (periodically these are of a profound nature such that a poor report can lead to closure of organisations classified as 'quangos'), and severe recurrent and budgetary constraints over many years merely compound the effects of pay agreements which are and have been consistently below the rate of inflation. That said, all those that sponsor research in any organisation must have the right to conduct their own reviews and audits, not least the public sector. Clearly, however, a wholly fresh approach to the commissioning and reviewing of public-sector research in the UK is required. To what extent should the public sector be involved in funding research? How else will the public and governments have access to intellectual property, an understanding of science, and trained personnel? Should public-sector science be governed by those that are more interested in processes of administration than in output? There is an incompatibility between the tight control (accountability) of public spending and the desperate need for patience and flexibility in conducting scientific research, as opposed to policy-related enquiry founded on existing scientific knowledge. Science whether in the public or private sector demands fresh blood, continually, and new ideas crossing disciplines, and efficiency of operation. Resource limitations will always mean that only priority areas can be funded, but who decides the priorities? Should the market be the ultimate decider? The market can be blind to scientific discovery and invention. We must not, however, dismiss lightly our older scientists and frighten off the talented 'seed corn', as would appear to be the case with a long period of staffing cuts and the recent massive depletion of numbers of first-class students seeking a career in science. A lack of expertise in key

scientific areas (e.g. bioinformatics, plant breeding, plant pathology, plant physiology, biomathematics) and the dwindling numbers of high-quality students embarking on scientific careers caused concern in the UK Research Councils in 1997 and 1998, and some of these areas are stated in the conclusions of their SWOT analyses (strengths, weaknesses, opportunities, and threats) prepared for the Office of Science and Technology.

Through this prolonged period of more than 6 years of harsh financial retrenchment, though, SCRI has steadfastly sustained by its own efforts and the commitment of the Scottish Office Agriculture, Environment and Fisheries Department, a phenomenally productive, pleasant, and forward-looking research environment in the beautiful setting of the Tay valley. I congratulate my colleagues for their outstanding efforts.



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In 1996, genetically modified (GM) crops occupied 2.8 million hectares worldwide, and in 1997, 12.8 million hectares (Fig.1). The increase largely reflects the availability of new products, and these products are clearly popular. What benefits do they offer? Their proponents claim that they are a contribution towards a more sustainable agriculture, while the cynics say that they are being developed to provide greater profits for a few commercial companies. There is some truth in both views, and it is important that there should be open debate about the pros and cons of GM crops and about the ways in which they can most safely and effectively be deployed.



**Figure 1** Oilseed rape: the GM crop furthest towards commercialization in the UK.

There is no doubt that GM crops can contribute to sustainability by reducing inputs of agrochemicals. For example, insect-resistant GM cotton occupied only about 13% of the US cotton acreage in 1996, but is estimated to have eliminated the use of approximately 250,000 gallons of insecticide. At first sight, it seems likely that this reduction in insecticide usage will also have had a beneficial impact on biodiversity, but it is too soon to tell whether this is borne out in practice. Indeed, it is worth noting that there is an inherent tension between sustainability and biodiversity, because if the diversity of pest, disease and weed species is maintained, inputs that control them are going to be needed in any successful agriculture.

Many people fear that the novel technology involved in GM crops will have unexpected effects on health or

on the environment. On health, it is important to distinguish between possible effects of the crops themselves, for example in the form of allergies, and the safety of foods derived from those crops. In the environment, it is feared that we may be creating monster plants or 'superweeds'. However, given the many years of study that go into the development of any GM crop plant, they are much less likely to prove monstrous than alien plants that can be introduced quite casually. It is hard to imagine a GM crop plant becoming as much a problem as giant hogweed, Japanese knotweed or *Rhododendron ponticum* have been in Britain. The best answer to the fear of unexpected effects is to point out that the technology is better understood and more predictable than many people think. Globally, there have been about 25,000 field trials, i.e. experiments in the open field, which have provided a vast amount of observations and data on GM crop plants and risks that might be associated with them.

Some people have ethical objections to GM plants, or more often to foodstuffs derived from them. Everyone, including scientists, has moral views and concerns, many of which we hold without really knowing why: for example because of upbringing or the influence of friends and family. Ethics describes the more philosophical process by which a group, a community, or a society decides how to scrutinise its moral beliefs and develop standards by which to regulate future actions or behaviour. Because genetic manipulation requires premeditated, skilled and, to the layman, poorly understood processes, albeit involving extremely minor alterations to the genetic material of an individual organism, it raises individual, group and public concerns about immediate or longterm effects, the speed of change, and society's view on living matter as a commodity or human construct. There are some for whom firm intrinsic beliefs (moral codes) dictate that certain scientific or technological advances and applications are wrong. Yet, they may drive a car, fly in a plane or accept modern medicines (many of which are products of genetic engineering). For others, a more analytical cost-benefit approach is required. What are the detrimental versus the beneficial consequences of a given technology? Is it consistent to denounce certain developments while

accepting and benefitting from others? Where these concerns are based on sound information and deeply held conviction, they must be respected.

At a practical level, GM technology is, if anything, more precise and predictable than all the methods which humankind has applied in selective breeding for domestication of food crops and companion or farmed animals over 10,000 years. Moreover, releases of all GM plants are very heavily regulated, at least in the UK and Europe. The aim of this article is to describe how GM crops are developed and released, how they are regulated, and the ways in which these

processes interact with one another. The perspective is as seen from SCRI, under the UK regulatory regime, with examples drawn where possible from our own work.

What is a GMO? Genetic modification is defined as the insertion into an organism, either by means of a natural vector (e.g. Agrobacterium) or otherwise (e.g. by particle gun bombardment), of heritable genetic material (i.e. DNA) prepared outside the organism. It also includes protoplast or cell fusion when the parents are plants from different botanical families, but not when they are from the same family. It does not

include techniques such as mutagenesis or manipulation of ploidy unless the starting material has previously been genetically modified. A genetically modified organism (GMO) is an organism that has been produced by genetic modification as defined above, or an organism containing genetic material derived or inherited from such a modified organism. Thus, in addition to the original modified plant, any progeny plants derived from it through seed or vegetative propagation, or crosses bred from it, are considered to be GMOs.

**Development of GM plants in containment** The appearance of a GM crop on the market is the culmination of a long process of development. Much work is done under conditions of containment in laboratories, growth cabinets, glasshouses and plastic tunnels. This phase includes development of the methodology

required to transform the plant species in question (Fig. 2), and experiments designed to test whether the genetic constructs really do what they are designed to do. Experiments at this stage often involve model systems; for example, constructs designed to be used in potatoes may first be tested in tobacco (both are members of the family *Solanaceae*), because the experiments can be done more quickly and easily. It should also be remembered that GM plants are valuable research tools in their own right, and many experiments in containment are using them to investigate something else. Thus, it should not be assumed that every GM plant that scientists produce is the precur-

sor of a product intended for the marketplace.

The key element in the regulation of experiments with GMOs in containment is risk assessment. Even before starting the work, the scientists have to assess what risks the experiments they are proposing to do might pose to their health or that of their colleagues or members of the general public, and what risks there might be of harm to the environment. One difficulty here is in defining what constitutes harm to the environment. A major effect, such as the extinction of a species, is obviously harmful, but

where the likely effects are small, there is no clear baseline for comparison in deciding whether they are harmful. For example, in considering whether a GM insect-resistant crop is harmful to non-pest species, the obvious comparison is with the effects of the pesticides that would be used on a conventional crop. However, agriculture itself is artificial, and the decisions to cultivate the land, and to grow one crop rather than another or to put the field into set-aside have very large effects on the insect fauna.

Methods of Risk Assessment The first step in risk assessment is identification of hazards, which are characteristics of the GMO that could give rise to harm. This involves imagining the possible scenarios both of what the experimenters expect to happen, and of the plausible alternatives, including what might go wrong. Subsequent stages of risk assessment involve estimat-



**Figure 2** One of the first steps in production of a GM plant involves selection of transformed cells in tissue culture and their regeneration into whole plants.

ing the likelihood of the harm occurring and the magnitude of harm if it did occur, for each of the hazards that has been identified, and bearing in mind the conditions under which the experiment will be done. It is essential to remain open-minded at the stage of hazard identification, and not to discard any possibilities prematurely because they are unlikely or inconsequential. In this way, the written record of the risk assessment will show the reasons why some risks have been discounted. It is generally impossible to quantify risks in numerical terms, and resort is had to terms like 'high', 'medium', 'low', 'negligible' and 'effectively zero'. Finally, an overall risk for the experiment is estimated, which is usually equal to the greatest of the individual risks arising from the list of hazards. If the assessment shows that there is any significant risk, it indicates that the containment conditions need to be tightened up so as to eliminate that risk or, if that is not possible, that the experiment should not be done at all. For example, consider a GM swede plant, modified by insertion of a gene for resistance to fungal attack, perhaps aimed at improving the storability of the swedes. One of the hazards that would be identified would be the escape of pollen of the GM swede, which could fertilize not only swedes but also any oil seed rape that might be flowering nearby. Seed produced from such a fertilization event would carry the fungal resistance gene, and if this was expressed in the seeds themselves, it might make them less susceptible to fungal attack, more long-lived in the soil, and consequently even more of a weed problem than rape is at present. In the absence of any data indicating the contrary, it would have to be assumed that such a scenario is possible, and the potential harm to the environment would have to be assessed at least as 'moderate'. It would therefore be important to minimize the likelihood of such events occurring. One way of doing this would be to prevent the GM swedes from flowering, and in many experimental situations this would be acceptable. But at some stage, it would probably be necessary to produce seed from the plants for future propagation. This would need to be done under conditions of high security, outside the main flowering season for rape. However, it is not possible to choose a time of year when it can be guaranteed that no feral rape is in flower, so the escape of pollen from the swede flowers would have to be prevented by containing them within bags, as is done by breeders to achieve controlled self pollinations.

The Institute is legally required to have a Committee (the GM Safety Committee) to advise on these risk

assessments. In practice, this means that the scientists who propose to do each GM experiment have to satisfy a representative selection of their colleagues that they have not neglected or underestimated any potential risk. It also ensures that each proposal is examined by scientists with a wide range of different kinds of expertise.

Collection of data on risk The risk assessment will often highlight areas where the magnitude of harm that the GMO might cause in the environment is uncertain. In such cases, the worst-case scenario has to be assumed, and rigorous measures to prevent escape of the GMO into the environment may be required. Meanwhile, further experiments may be planned to clarify the uncertainties about the potential harm. Such experiments will allow the risk assessment to be revised and may generate important data to support a subsequent proposal deliberately to release the GMO. For example, with plants genetically engineered to resist attack by insect pests (e.g. by expressing antiinsect toxins; Fig. 3), it may be unknown whether the modifications have any effects on non-target insects, especially beneficial natural enemies of pests. Recent studies at SCRI and the Swiss Federal Research Station for Agroecology and Agriculture in Zurich have shown the potential for genes, designed to provide resistance to pest insects, to have adverse effects on predatory beneficial insects (ladybirds and

lacewings) via the food chain. Both these studies were conducted under laboratory conditions and each tested a specific anti-insect gene (GNA lectin



**Figure 3** Strawberries damaged by vine weevils (adult weevil, inset) are a target for GM insect resistance.

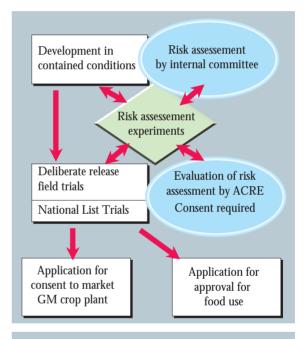
and Bt toxin respectively). The wider ecological implications for these and other anti-insect genes remain to be investigated under more natural field conditions, where the interactions between species are much more complex and, in particular, predators have a choice of prey. The likelihood is that after each of these careful 'case-by-case' risk assessment studies, strategies can be developed which will minimise any adverse effects on beneficial insects to a level below that caused by many widely used pesticides. However, each new promoter/gene/plant/pest combination will need to be carefully assessed under a range of environmental and agricultural conditions, so that we can ensure the new technology is effective and compatible with future environmentally-benign Integrated Pest Management systems. This will include checking for more subtle (sublethal), longerterm effects on the target pests, on other secondary pests of the crop, on beneficial insects, the GM crop and on wild / volunteer plants in the agro-ecosystem.

Another example where risk assessment is hampered by lack of data is with plants modified by insertion of a gene or non-functional sequence derived from a plant virus in order to make them resistant to that virus. Here, one perceived risk is that the inserted gene may recombine with the genome of another virus that happens to infect the plant, so as to create a novel pathogen. It is already clear that such events are extremely rare but not impossible, and also that most novel viruses that might be created in this way are feeble pathogens compared with those that have been refined by eons of natural evolution. However, research is continuing in order to elucidate the circumstances in which such risks might be important. Indeed, a substantial proportion of contained experiments with GM plants are concerned with the collection of risk assessment data of one kind or another (Fig. 4). It is important that scientists remain openminded enough to accept that the data they are collecting may show that their 'pet idea' has drawbacks which mean it has to be abandoned.

Often, the biggest and most uncertain step is to assess the risks at the scale of the agricultural ecosystem. A process of 'scaling-up' is needed in which the results of specific experiments on GMOs in contained environments or small field plots are placed in a realistic context. Some types of research aimed at tackling ecosystem risk directly need not even require the use of GMOs themselves. This summer (1998), for instance, SCRI has deployed populations of oilseed rape 'bait' plants in the Tayside area in order to assess

the likelihood of GM crops cross-pollinating feral and wild brassicas. The bait plants are non-GM cultivars, since there is no reason to suppose that GM plants will behave any differently in this respect. By combining plant-scale and ecosystem-scale knowledge, advanced mathematical modelling is then used to assess the potential for the spread and impact of GMOs on a regional scale.

Scaling from small plots to systems will nevertheless remain an area of scientific uncertainty and public interest, especially when the genetically modified trait, for example insect resistance, might influence more than one layer in the ecological food chain. This is another area where the predictive power of mathematical models can be very valuable.



**Figure 4** The pivotal role of experiments to provide risk assessment data in the development of a GM crop.

Release of GM plants. The next logical step in the development of a GM crop plant, after studies in containment, is field trials, properly called 'deliberate release into the environment for research and development purposes'. The R&D purposes may include the validation of results from contained experiments under more realistic conditions; it is well known that the behaviour of plants in the glasshouse is not always a reliable indicator of their behaviour in the field. In particular, levels of expression of introduced genes can be very different under different environmental conditions. Moreover, some characters cannot be sensibly tested in contained conditions, including yield param-

eters and characters such as potato tuber quality. These characters need to be assessed whether they are the object of the modification or not. The advantages, indeed the necessity, of field trialling GM crops rather than simply relying on data obtained from glasshouse trials has become increasingly obvious in recent years. For example, glasshouse selection of potato germplasm for improved crisping potential can bear little resemblance to performance in the field. This applies as much to non-GM as to GM crops. There are also cases where no phenotypic effect has been seen with GM plants in the glasshouse, but distinct phenotypic effects have been very obvious in the 'real' growing environment. As with traditional selective breeding, plants showing unexpected or detrimental phenotypes occur, and are discarded from the programme. In the longer term, the successful application of biotechnology will require a more comprehensive understanding of how the expression of any introduced gene is regulated by prevailing environmental conditions. However, modified phenotype does not necessarily imply a risk associated with the gene that has been introduced. Risk assessement, for any gene or gene product, must be carried out on a case-by-case basis. What these examples do show is the absolute necessity for controlled, regulated field trials that address all relevant parameters.

Once the potential usefulness of a particular GM plant for a particular agricultural application has been confirmed, the remaining steps in its development into a marketable crop are very similar to those for a variety bred by conventional selection. The agronomic characteristics of the plant may need to be improved and its genetic stability ensured by a programme of crossing and back-crossing with conventional varieties, and sufficient stocks have to be accumulated through seed multiplication or vegetative propagation. The next step may be to enter the GM plant as a cultivar in National List Trials, following which it will, if suitable, appear on the National List and be ready for the commercial farm market.

Throughout these development stages, up to and including National List Trials, work is regulated through a system of consents. It is an offence deliberately to release any GMO into the environment unless a consent to do so has been granted by the Secretary of State (consents are granted by the Secretary of State for the Environment, for Scotland, or for Wales depending on where the release will take place). A consent may contain conditions concerning the conduct of the release. Application for the granting of a

consent involves compiling a dossier of data on the plant, the way it has been modified, the characteristics of the modified plant, the site or sites at which it will be released, the way in which the release will be conducted and managed, plans to monitor the effects and outcome of the release, the subsequent treatment of the site and emergency plans to cope with any unexpected events. A risk assessment must also be prepared, similar in concept to the ones for contained use, but dealing in much greater detail with all the possible interactions that may occur when the plant is introduced into the environment. When the application is submitted to the Department of the Environment, Transport and the Regions (DETR), a Public Notice must be placed in a local newspaper to inform the general public of the proposed release. The risk assessment and a summary of the data dossier are placed on a Public Register that is open for inspection, and information is also made public on the Internet. A summary (the Summary Notification Information Format or SNIF) is circulated to all other EU governments. The application is scrutinized for conformity with the regulations and for completeness of the data, and the risk assessment is analysed in detail. In this, officials are assisted by an expert committee (the Advisory Committee on Releases to the Environment; ACRE), which advises the Secretary of State whether and under what conditions a consent should be granted. The conditions always include a requirement to report on the outcome of the release and any effects it may have had on the environment. In addition, there is a general obligation on all consent holders to keep themselves informed and report to the Secretary of State any new information that might affect the risk assessment. Furthermore, releases are inspected (at the releaser's expense) by Specialist Inspectors from the Health and Safety Executive to ensure that practice conforms with the proposal. As with contained uses, where there are uncertainties about environmental effects, the worst-case is assumed, and R&D releases may often include the collection of data to resolve the uncertainties.

When it comes to marketing, a similar application format is required, but the degree of uncertainty permissible in the risk assessment is minimal, and there are some additional requirements about packaging and labelling of the product. In this case, the government that receives the application makes a recommendation to the European Commission as to whether consent should be granted, and the application is then circulated to all the other EU member governments.

Because a marketing consent is valid throughout the EU, the final decision is taken at Community level.

Eight Scientific Committees, involving independent scientific experts have been set up by the European Commission, grouped under common management within DG XXIV - 'Consumer policy and consumer health'. The Committees advise the Commission on questions such as food safety, animal nutrition, animal health and welfare, veterinary public health, plants, cosmetics and other non-food products, toxicity and eco-toxicity and the environment, medicinal products and medical devices. To ensure consistency and relevant information flow, members of any specific Scientific Committee also attend, on a case-by-case basis, workgroups organised by other Committees. For example, members of the Scientific Committee on Animal Nutrition and the Scientific Committee on Food provide inputs into assessments made by the Scientific Committee on Plants. Highly relevant to the acceptance of GM crops within the EU is the role of the Scientific Committee on Plants. Under Community Directive 90/220, this Committee is required once again to assess the risks to human and animal health and to the environment which may be caused by a commercial release of a GM crop into the environment. The Committee, composed of independent experts from several member states, advises the Commission using information based on current scientific knowledge rather than on ongoing politics within any specific member state. The Committee is not legislative; if a vote is required on a proposed decision, it is taken on a qualified majority basis in the 'Article 21 Committee', which comprises officials representing each of the member governments. Basically, the Scientific Committee on Plants assesses the dossiers from companies wishing to release crops into the environment for commercial purposes. For example, the Committee must determine what DNA [gene(s)] from the vector has been incorporated into the crop and the potential risks associated with the gene(s) in the unlikely event that they are transferred from the GM plant to microbes in the soil or to animals fed on such crops. The impact on humans is also assessed in the case of accidental direct exposure to the plants, including the issue of allergenicity of the gene product(s), and potential hazards associated with the metabolites generated by the gene in question. On the environmental side, the Committee addresses issues such as wild species likely to cross-pollinate with the GM crop, the impact on such species should the gene be transferred (e.g. for herbicide resistance) and the potential impact on insect populations in the case of GM releases involving products toxic to specific insect pests. However, these assessments under Directive 90/220 do not cover directly the use of GM crops or derived products in the production of foods for human consumption; the regulation of these aspects is described below.

Other statutory hurdles The regulatory system described above considers only the safety issues involved in growing a GM crop plant, but of course most crop plants are destined for use as food. The safety of food products derived from GM crops is dealt with quite separately from the safety of the plants themselves, because quite different kinds of expertise are required. Another expert committee (the Advisory Committee on Novel Foods and Processes) advises MAFF. Those who wish to market food derived from GMOs have to provide another dossier of data covering such aspects as toxicity, allergenicity, nutritional value, wholesomeness and effects on the overall diet. There is also a European dimension. The Regulation of the European Parliament and Council on Novel Foods and Food Ingredients was published in February 1997 and came into force 90 days after publication. The Regulation applies to the placing on the market within the Community of foods and food ingredients which have not previously been used for human consumption to any significant extent within the Community. The Scientific Committee for Foods plays a key role in assessing safety issues related to this area, but again the Committee is advisory and not legislative. Clearly there are relevant links between assessments carried out under Directive 90/220 and under the Novel Foods Regulation. However, even if products have been accepted under 90/220, they may not be placed on the market as a food or food ingredient until authorised under the Novel Foods Regulation. This further emphasises the scrutiny that GM crops and foods undergo prior to acceptance at Community level.

The factors that have to be considered are rather different depending on the form in which the crop is likely to be consumed. For example, a GM strawberry will probably be eaten as raw fruit, which is a live GMO containing viable seeds. However, at least for the present, GM crops will be eaten most commonly in a processed form, for example as soya meal or rape oil incorporated into prepared foods. In these forms, the GMO is dead, and processing may have eliminated the distinguishing characteristic(s) imparted by the genetic modification. The inserted DNA itself will

probably have been destroyed. Indeed, it may be impossible to determine whether soya meal or rape oil has been derived from a GM or a conventional crop (or a mixture of the two).

An important concept that is used here is that of substantial equivalence, i.e. that with the exception of the specific trait modified, the GM plants and products are equivalent in composition to their non-GM counterparts. This means that if the analytical composition and toxicological properties of, for example, oil from herbicide-tolerant rape are within the range found for batches of oil from conventional rape, there is no reason why it should not be used in the same range of applications. Incidentally, the potential difficulty in determining whether a processed product is derived from a GM or a conventional crop is one reason why governments are reluctant to impose labelling requirements that may be meaningless and unenforceable. However, arguments about the labelling of GMOderived food are less to do with safety than with consumer choice, which is discussed below.

The use of a GM crop as animal feed may involve different considerations from its use as human food; for example, maize may be eaten unprocessed by cattle, but is cooked or processed for human consumption. The system for approval of animal feeds is similar to, but separate from, the human food regime.

Other forms of approval may be required for particular applications, such as herbicide-tolerant crops. The idea behind such crops, for example, glyphosate-tolerant oilseed rape, is that a wide-spectrum herbicide becomes entirely effective in that crop; glyphosate should kill all weeds but leave the rape unaffected. However, herbicides may only be used for approved purposes under approved conditions, and there is currently no approval for the use of glyphosate on oilseed rape, because conventional rape would be killed by it. To gain approval for the use of glyphosate on GM oil seed rape, a company must present the Pesticides Safety Directorate of MAFF with data on the efficacy of the herbicide and on its safety, including residues in soil and groundwater, effects on wildlife, the potential build-up of tolerance in weed species, etc. These data will of course have to be obtained in R&D release experiments that are themselves subject to consents. Concerns have been expressed about the effects of the widespread adoption of herbicide-tolerant crops on overall patterns of herbicide use and on crop rotations, and it is at this stage that such issues are properly addressed. Herbicide tolerance in a crop such as oilseed rape may well prove to be impractical in circumstances where the GM cultivar quickly becomes resident in the buried weed 'seedbank' of arable fields and waysides. Any factor, such as herbicide tolerance, which causes greater persistence of oilseed rape as a weed, will also increase the probability that a future crop of oilseed rape or turnip rape will be contaminated by the weeds left behind by a previous crop. The efficacy of the GMO as a weed will thereby act as its own regulator. If problems of this kind arise in practice and a worst-case scenario is realized, herbicide approvals can quickly be withdrawn.

Another issue that developers of GM crops have to face is how best to protect their property. In the US, there are two significantly different systems for the protection of plants: plant variety protection/plant breeders rights and the regular patent system. However, in Europe, even after the long-awaited EU Directive on the Legal Protection of Biotechnological Inventions, the position of patenting plants is still unclear. The Directive passed by the European Parliament on 12 May 1998 after some 10 years of debate, and a rejection by the European Parliament in 1996, clearly states under Article 4.1 that the following shall not be patentable:-

- (a) plant and animal varieties, and
- (b) essentially biological processes for the production of plants or animals

The term 'essentially biological' has not been judicially defined but is currently viewed as being applicable to traditional processes used to breed new plant varieties. Article 4.2 of the Directive states "Inventions which concern plants or animals shall be patentable if the technical feasibility of the invention is not confined to a particular plant or animal variety."

In Europe, the protection of plant varieties and plant breeders rights are covered by national law established in the 1960's and an international convention, the International Union for the Protection of New Varieties of Plant (UPOV, 1961). The protection is less robust than that of patents and includes exemptions for the use of protected varieties for further plant breeding and development, and for 'farm-saved seed', whereby a farmer can use seed saved from harvest for subsequent sowing on his own farm, subject to safeguarding the legitimate interests of the breeder. New varieties are granted protection if they meet Distinctness, Uniformity and Stability (DUS) criteria. The 1991 revision of UPOV has removed the prohibition on double protection by both patent and vari-

ety rights, and introduces rights to 'essentially derived varieties'.

It is currently held in professional patent circles that a plant patent should be protectable so long as it meets the criteria of patentability. In reality, because it is random and unpredictable, it would be hard to justify an 'inventive step' in traditional plant breeding of new varieties and, moreover, one could not define the genetic reason for or composition of most new traits emerging in a traditional breeding programme. Describing the invention is essential for the granting of a valid patent. Therefore, plant variety rights (PVR) are the preferred legal protection at the level of specific varieties.

**Public perception** Because the success of all GM crops used as food products will ultimately depend on

acceptance by the consumer, public confidence in their safety and desirability is very important. Currently, such public confidence seems to be at a low ebb throughout Europe, in contrast with the position in North America where acceptance of GM crops by the majority of the populace does not seem to be a problem.

Despite the strict EU/UK controls described above, and many years of experience of safety, the public does not seem to believe that GM crops are safe. Part of the difficulty arises from differences between the scientific and lay perceptions of risk. The sci-

entist will always try to quantify the risk, and because of this the scientist is never able to say that there is zero risk. He has to fall back on an expression such as 'negligible' or 'effectively zero', by which he means that he can detect no risk, but appreciates that there is a limit to the sensitivity of his methods of detection. Notice, however, that this refers to absolute risk; at no point in the regulatory regime can a risk, however small, be traded off against a potential benefit, however great.

The lay person's perception of risk is rather different and does involve an implicit risk/benefit analysis.

How else would anybody accept the risks of travelling by motor car, crossing a road, or bungee jumping? There has been no serious objection to the many vaccines that are now produced using GMOs, because the benefit is plain to see. In the case of GM crops, people are unwilling to accept even an infinitesimal risk unless they can see some benefit. It is notable that tomato paste made from GM tomatoes has sold well, despite being clearly labelled as derived from a GMO. Consumers can see the advantage because it is cheaper than paste made from conventional tomatoes and is of equal quality (Fig. 5). In contrast, products containing material from GM herbicide-tolerant soya beans have met with extreme consumer resistance. Here the consumer can see no personal benefit; the obvious benefits accrue to the grower, the seed producer and the herbicide manufacturer. The issue is not helped in this

SAINSBURYS
Californian
TOMATO
PUREE
WAR MITH GENTLE STATE
ROHLE CONCENTRATI

**Figure 5** Tomato puree derived from GM tomatoes, a product that has been accepted by consumers.

case by the fact that the beneficiaries are in North America and include multinational conglomerates. Furthermore, when EU governments insisted on scrutinizing the material under their own regulatory procedures before permitting its importation, threats were made to force the issue through the World Trade Organization, using trade sanctions. This only served to harden the antipathy of European public and political opinion, and it is to be hoped that the biotechnology companies involved now recognize that they scored a significant own-goal. It is unfortunate that the first

major GM-derived foodstuff to come to the market in Europe was a commodity crop, where segregation to provide consumer choice is impracticable and uneconomic; even for non-GM crops, up to seven different varieties of soya beans are mixed at co-operative farm silos and shipped in bulk to processing facilities.

Effects on science and technology Despite the fact that in 1998 GM crops are occupying more than 60 million acres throughout the rest of the farming world, the political climate dictates that this is clearly not the time to relax the controls that are applied in Europe to releases of GM plants. There is however

some scope for simplification, and this is being addressed in the EU at the moment. For example, repeat experiments can be dealt with relatively simply; if there have been no significant changes, the risk assessment next year is likely to be the same as it was last year. There are also many facts that can be accepted without having to present detailed arguments each time. Strawberries are never going to become a weed problem in Scotland, and it is now well established that potatoes do not form viable hybrids with any native British species. Simplifications such as these save time and paper for the applicants as well as allowing the regulators to concentrate on any really contentious issues, without impairing the rigour of the regulatory regime.

Meanwhile, however, the complexity of the regulations causes problems for research organizations such as SCRI. The preparation of an application for even a simple GMO field trial is a substantial undertaking, and the monitoring of the trial, which may have to continue for several seasons after the termination of the experiment itself, ties up resources in an unproductive way at a time when those resources are becoming increasingly limited. If this work is to be carried out at public research centres such as the SCRI, then it is crucial that the costs associated with applications for and monitoring of such trials are reduced accordingly. Indeed, there is a very strong argument that research for the public good should be carried out free of extraneous charges. Moreover, our overseas competitors can make more rapid progress where they are in a less rigid and restrictive regulatory climate.

There is a noticeable reluctance on the part of independent scientists, such as those at SCRI, to become involved with releases of GM plants, because the benefits are often not commensurate with the huge resource costs. This is likely to lead to an increasing concentration of research on GM crops in the hands of large commercial companies. Although companies have to take account of the welfare of their customers, and are aware that they operate in a litigious society, their ultimate motive is profit. There is therefore a need for the independent public sector to invest in a thorough understanding of GM crops and processes. Especially in the area of risk assessment research, it is vital that a vigorous independent capability is maintained, but the rewards from this kind of work are minimal. The big rewards, in terms of added value and wealth creation, come through ownership of intellectual property rights or of a commercially successful cultivar. However, such rewards flow only from very substantial investments, and there is a need for investment to secure public ownership of platform technologies. It should be noted that even the large multinationals have not yet seen a return on their research investment. In 1995 in the US, sales of agricultural biotechnology products amounted to \$100 million, whereas R&D expenditure was \$2000 million. Economic analysts predict that 1998 will be break-even year for some sectors.

What is the future of GM crops? The demand for high quality, inexpensive food and food products has intensified in recent years, with retailers and consumers also expecting environmentally-friendly agricultural practices to be applied in generating such food supplies. The reality is that consumers have little knowledge of the methods and technologies used to generate the food supplies they currently purchase. Relatively cheap supplies of quality produce cannot be produced in large quantities without the use of agrochemicals. The developed world has evolved a sophisticated system to ensure food supplies and cannot revert to the practices used 50 years ago. However, agriculture must continue to evolve and, as we all realise, must encompass the issues of sustainability, environmental protection and safety to humans and animals. Are our current practices satisfactory in all of these respects? Clearly not. Pesticides can kill not only pests, herbicides can kill not only weeds, agrochemical residues may be detectable in groundwater and food. The use of arsenic and Bordeaux mixture (which contains toxic heavy metals) is permitted in organic farming. However, many perceive our current farming practices as relatively benign by comparison with the use of transgenic crops. There are many arguments that can be developed to demonstrate that this should not be so. Valid discussions must take into account our current methods of food production and the fact that the use of biotechnology provides approaches to crop improvemnt which are as safe as, if not safer than, our established practices. We must also be fully aware that sustainabilty will not simply depend on the use of GM crops in the future. Integrated Crop Management systems will still need to be applied in agriculture and horticulture. There will still be a place for agrochemicals and new germplasm generated in the traditional way by plant breeders. We cannot, as a nation, afford to close the doors on biotechnology. History has shown that such major advances in scientific discovery are rarely if ever held back. This is how we have reached

our present level of civilization and more than doubled average life expectancy in the past 100 years. Like it or not, science and technology have delivered a safer, more secure life for millions. However, we must follow best practice to ensure safety. The current legislation is there to protect. If there were to be a moratorium on GM crops in the UK, how could we possibly progress our understanding of the issues that arise? If we cannot evaluate these issues in controlled field trials without the plants being destroyed by a self-appointed faction of eco-vandals, the sound scientific answers that the public require will never be obtained.

At the present moment, public opposition, fuelled by the media and activist groups, against GM crops and products derived from them, seems likely to damage the biotechnology industry in Britain and Europe. Some of this opposition may be sincere, although much of it is misinformed and misguided, and to try to dismiss it out of hand is unprofitable. It has to be admitted that not all developments in biotechnology are necessarily beneficial, and some possible applications may need to be curbed. Indeed, almost all of the case studies cited by anti-GM activist groups are actually exaggerated versions of small-scale experiments (many in containment) where the beneficial trait failed to live up to expectations or a risk was detected that was unacceptable. This is precisely why GM crop

technology has been subjected to over 15 years of extensive, unprecedented, precautionary risk analysis and testing. Rather than responding to risks or problems after the event, as with cattle feed contamination or food hygiene failures, GM crops have been, and continue to be, subjected to intensive risk analysis before being released.

Just as importantly, there are many GM crop applications that can be economically advantageous, environmentally beneficial and/or socially desirable. Perhaps the most important in all three respects will be applications that decrease the use of agrochemicals. Indeed, it has been suggested that the better public acceptance of GM crops in North America compared to Europe has been in part due to greater awareness of the benefits to the consumer and to the environment of diminishing agrochemical inputs.

Independent research organizations, like SCRI, must show the public what our GMO research is really about. We are not in the business of ramming transgenic crops and products down consumers' throats. We are exploring what is scientifically possible, whether what is possible is desirable, and whether risks are real or imaginary. This is not glamorous or short-term research, nor is it exactly wealth-creating, but in the present political climate it is probably wealth-preserving.

# Are diseased and blemished foodstuffs good for you? The need for plant pathology

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#### **Background**

Tankind is cropping the same 6 million square miles of land as in 1960 but feeding 80% more people. To keep pace with anticipated population increases, agricultural production from this area must triple by the year 20401. Failure to achieve this will result in the destruction of substantial amounts of the world's precious native flora and fauna. Stark predictions such as this emphasise the fundamental importance of efficient use of our limited land area. Increasing salinity and soil erosion continue to reduce the potential area for agricultural production. Crop yield is also limited by drought and pest and pathogen attack. Advances in the science of crop protection have lessened the impact of the latter, yet the threat from insects, nematodes, fungi, bacteria and viruses is perpetual and pre- and post-harvest losses on a world scale are huge.

In contrast to the Developing World, where production of sufficient staple crops remains the priority, abundance is taken for granted in more-developed nations and consumer demand is now for consistently higher-quality, diverse foodstuffs, available year-round and produced in ways considered ethically and environmentally sound. The great challenge is to realise these objectives without threatening food security or facing drastic increases in food prices.

There is a feeling among the media, the public, and some circles of government, that Developed-World agriculture has reached a point in which the battle against pests and diseases is in equilibrium and further scientific development redundant. Outright condemnation of further technological development in agricultural practices is gaining a favourable political hearing. Many of these fears are irrational, being fuelled by a largely ignorant media interested in 'sound bites' and sensationalism rather than in scientific accuracy or ethical integrity. A great deal of misplaced optimism exists in the belief that a 'greening' of agriculture can meet the future requirements of farmers, environmentalists and the public. Such a romantic

notion was recently described as 'a misleading and dangerous illusion'<sup>2</sup>. The demonisation of science, to create a popular perception that it is part of the problem rather than the solution, is dangerous and unfounded.

We should, of course, be concerned about the demands we are making on fragile ecosystems and do our best to harmonise agricultural production with environmental protection. But this will only come about through technological and scientific advances, with efficient training and advisory systems, to ensure advances are translated into tangible benefits for farmers. Ignorance is not a foundation for progress - future food security depends on scientific endeavour to overcome the daunting challenges.

Crop protection is central to the wider debate since its primary tools, pesticides and, increasingly, genetic engineering, are the cause of much consumer anxiety. With this in mind, this article aims to examine the need for further scientific research in crop protection, revealing the challenges, possible solutions and the threats of ill-conceived changes to the system.



**Figure 1** The benefits of host resistance are clearly seen in two potato breeding lines resistant (left) and susceptible (right) to potato late blight.

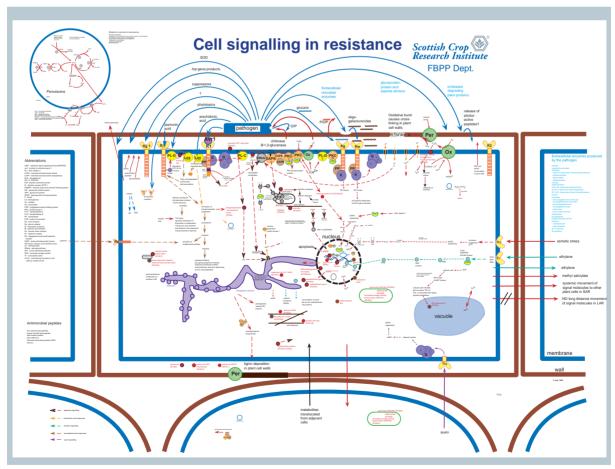
#### **Breeding for resistance**

Conventional methods Using natural plant defences is clearly the best strategy to reduce yield losses and maintain quality. The benefits of host resistance are clear; the control measure is always in place, the risks of environmental damage are negligible and the need for pesticides is reduced. In many cases, sufficient natural resistance is found in wild plant populations. The challenge is to incorporate the trait into a crop species while maintaining satisfactory yield and quality criteria. This is a lengthy and complex process in which 'crossing the best with the best and hoping for the best' often forms the basis of the breeding strategy.

However, in recent years our understanding of plant genetics has improved greatly as advances in biotechnology have allowed detailed genotypic examination (i.e. DNA-based) rather than reliance on phenotypic markers (e.g. morphological traits). Through the application of such molecular markers, genetic maps are now available for many of the major food crops and we are able to identify and monitor the inheri-

tance of genes or regions of chromosomes responsible for agronomically important traits. Such Marker Assisted Selection (MAS) is already having a great impact on conventional breeding through accelerating selection programmes.

Although MAS is yielding benefits for well-characterised traits or resistance based on single genes, the real leaps in understanding will only come from detailed investigation of the complex patterns of signalling, gene expression and biochemical pathways involved in host-plant resistance. Parallel studies on pests and pathogens during successful or unsuccessful colonisation of a host will also help pinpoint fundamental steps in the interaction. At SCRI, such targeted gene discovery programmes are already underway and yielding exciting data on gene expression in both host and pathogen. With time, such information will underpin plant breeding strategies, as trait specific markers for MAS or single genes incorporated through gene transfer (see below). Such work does not rely solely on molecular biology, as other specialist



**Figure 2** In this diagrammatic representation of host-plant cells, only a portion of the complex response to pest or pathogen attack is shown.

knowledge is fed in at every step. For example, resistant germplasm must be tested for stability, its utility in other climates, and careful deployment is needed to ensure it is effective and durable in the field. We are already involved in a world-wide programme to test the stability of quantitative resistance to potato blight in tropical, sub-tropical and temperate regions. Identification and exploitation of sources of resistance among wild relatives is vital and, at SCRI, the Commonwealth Potato Collection is proving an excellent genetic resource.

Gene transfer Traditional means of gene transfer within a plant species (crossing and repeated backcrossing) have been augmented by an ability to create wide interspecific crosses by tissue-culture techniques such as embryo rescue or protoplast fusion. In addition to this, through advances in biotechnology over the past 18 years, the ability to move individual genes, or groups of genes, across genus or even kingdom boundaries is now feasible. 'Genetic engineering' (genetic enhancement or manipulation) will certainly play a key rôle in the future of global food production, but still faces many scientific and socio-political challenges. Such a quantum leap in technological capabilities naturally evokes both positive and negative feelings. The technology offers the potential to provide secure and stable food supplies with minimal environmental damage, but doubts about the ethics, environmental impact, suitability for developing nations, and fears of dependency on enhanced crop varieties must be recognised and addressed before widespread adoption is possible. In terms of public acceptance, it is vital that consumer fear is allayed by communicating the results of thorough, independent scientific assessments of risk in a balanced way. This must include the extensive data from testing prior to release and monitoring over several years after release (see article on page 44).

Just as significant are the scientific questions to be answered as this new technology is taken forward. Identification of target genes is, of course, fundamental to the process. To date, the approaches have been relatively crude, few natural pest or pathogen genes have been identified and those advanced to commercial production primarily produce toxins or antifeedant molecules against insect pests. More subtle approaches using viral coat protein genes to mimic the widely applied phenomenon of cross-protection are being applied, but we need to learn more of the complexity of host/pathogen interactions to enable progress. Very sophisticated methods of isolating

genes involved in natural defence responses are currently being used at SCRI. A knowledge of the complex signalling pathways involved is emerging and will enhance our ability to manipulate host/pathogen interactions through transgenic approaches which may not involve gene transfer between species. Overexpressing or silencing of existing plant genes may, for example, provide a way forward.

The path to successful commercialisation involves gene discovery, confirmation of function, efficient transformation protocols, control of expression and assessment of the optimal deployment strategy to avoid breakdown of resistance and escape to the environment. All areas, incidentally, in which SCRI has proven expertise. It is only stable, well-funded and independent research teams, with a holistic view of the field, that have the necessary skills for the task. A moratorium on biotechnology research will only stifle progress, reducing the competitiveness of UK science, shifting the responsibility for refinement and development work from the public to the private sector, preventing inward investment to 'UK Crop Protection Research Ltd' and putting our growers at a disadvantage in international markets3.



**Figure 3** Without fungicides, raspberries rapidly become infected with grey mould.

#### The future of pesticides

Compared with natural plant resistance, the application of synthetic biocidal compounds to crops is very inefficient. It is costly, application times are restricted by weather conditions, much of the product (95%) misses both the target organism and even the crop, while some are highly toxic with risks to the user and the environment. In addition, efficacy frequently declines as pesticide resistance builds in the target organism. Moreover, there are few agrochemicals

active against bacterial pathogens and none against viruses per se, which cause considerable problems in the tropics. So why are biocides used? The answer is quite simply that they are an absolute necessity in the majority of cropping systems; the extent of their use is testament to that fact. Consider the control of potato late blight caused by the fungus Phytophthora infestans. At present, potato growers in the United Kingdom spend c. £150 per hectare on chemical control of blight and very conservative estimates suggest around \$1 billion is spent annually world-wide. Chemical sprays are not applied, as is sometimes perceived, out of a wanton disregard for the environment, but out of necessity to reduce yield losses. Failure to control blight in a commercial crop on the central lowlands of Scotland will result in severe financial loss, but in a vital staple crop in the S. American highlands, the price can be measured in human life.

The pressure for farmers to reduce inputs of 'synthetic' products for the perceived benefit of health, the environment and promote 'sustainability' is increasing, and some fail to understand why we cannot ban the use of all pesticides and become 'organic' tomorrow. Such simplistic solutions are rife amongst those that Norman Borlaug (Nobel Laureate and architect of the 'Green Revolution') described as "the extreme elitists and doom-sayers in the environmental movement in affluent countries who have never personally experienced poverty nor have produced a single ton of



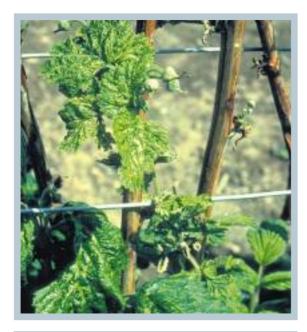
**Figure 4** Cabbage white caterpillars, the bane of every gardener, can rapidly devastate a crop.

food"4. Limited organic production is feasible, particularly in annual crops, because of the premium prices paid willingly by some consumers and through the benefit of being surrounded by pesticide-treated crops which are relatively free of pests and pathogens. This latter point is analogous to human immunisation against disease (e.g. whooping cough); the risk of disease in a few untreated children is low, so long as the majority are immunised. The epidemiological consequences of widespread organic growing need careful consideration as pest and pathogen populations would be certain to increase as a result, thus increasing the threat, both within organic production systems, and on surrounding farms. Even organic organisations have acknowledged the difficulty with some diseases such as late blight and permit the use of a calcium hydroxide and copper sulphate mix called Bordeaux mixture. This is a product of the late nineteenth century, deemed 'traditional', and therefore judged acceptable by organic organisations. However copper sulphate is neither organically 'natural' nor particularly safe.

The hard truth is that to feed 5.8 billion people, current intensive agricultural practices are reliant on pesticides. Pressure for drastic reductions, without sufficient research into alternative crop protection strategies (such as host resistance), will create more problems than they solve. Inevitably, we would see reductions in yield and quality, more stocks rejected by retailers, an impact on import and export markets, and increased food prices.

We must continue to refine the use of current compounds through improved modelling of epidemics to allow a move from prophylaxis to the much vaunted integrated crop management (ICM) programmes, where all methods of control are combined in an optimal manner<sup>5</sup>. Such ICM approaches are particularly relevant in the tropics where crops are threatened by a complex of many pests and diseases and control of any one without consideration of the others will likely fail. One only has to look at the difficulties in controlling rice plant-hoppers in Indonesia where, after initial success, insecticide-based control failed because the natural predators of the plant hoppers were also killed.

Although the trend has been towards the development of increasingly safe chemicals, there are still some highly toxic compounds in use. Soil sterilants are particularly toxic and compounds such as aldicarb are routinely applied to the soil prior to carrot production to prevent the nematode-induced 'fanging' of carrot



**Figure 5** Both virus and aphid vector must be understood to prevent viral damage such as this on raspberry.

roots. Methyl bromide is damaging to the ozone layer yet each year, 70,000 tons are applied to agricultural soils to fumigate against nematodes and other soilborne pests and pathogens. A reduction in the use of such compounds is clearly advisable and research is needed to optimise targeting and development of other means of control.

#### Monitoring of pests, pathogens and vectors

Effective breeding programmes and optimised pesticide applications may be rendered futile by the fact that pests, pathogens and their vectors are, genetically speaking, a 'moving target'. Only by knowing the distribution, diversity and ecology of these organisms, can we evaluate current problems, predict future threats and apply rational control strategies.

Despite the efforts of regulatory authorities, the changing world climate and increasing world trade in plants have resulted in pest and pathogen introductions in new biogeographic environments. There are all too many examples; the inadvertent introduction of *P. infestans* and Dutch Elm Disease into Europe; the 1995 outbreak of bacterial brown rot of potatoes which, in The Netherlands caused emergency EU legislation; the pine wilt nematode, indigenous to N. America, which devastated hundreds of thousands of trees in China and Japan, and the multi-billion dollar trade deal between China and the USA which was threatened by Karnal bunt of wheat.



**Figure 6** Early detection of the pathogen, *Phytophthora fragariae* var. *rubi*, in raspberry propagation material will help prevent devastation such as this.

The maintenance of a critical mass of trained staff is vital to prevent such introductions and minimise the impact of any outbreak. The development of new tools to aid diagnostics and monitoring is also essential. For example, at SCRI, new PCR-based diagnostics for *Phytophthora* diseases in soft fruit are allowing rapid detection and identification of the disease and, in the long term, will be used in international quarantine programmes to monitor propagation stocks and prevent disease outbreaks.

As well as preventing new problems, we must evaluate changes in current pest, pathogen and vector populations. Monitoring of the virulence of UK cereal pathogen populations has, for example, improved breeding strategies and allowed advisors to recommend cereal cultivars according to current pathogen populations. Careful studies on the build-up of resistance to pesticides has extended the life of many key products. The phenylamide fungicide, metalaxyl, for



**Figure 7** Blemish diseases, such as common scab, are increasingly important as tubers are sold pre-washed

example is a vital product in the control of potato late blight and was used extensively until resistance in the fungal population threatened its efficacy. Changes in patterns of usage by alternating products and mixing with other active ingredients have been successful in extending its use. Bt toxin (a 'natural' insecticide from the bacterium Bacillus thuringiensis) is either applied to the crop as a spray or introduced into crop plants through genetic engineering. In either form, its use must be carefully managed to prevent build-up of pest resistance. A refinement of such monitoring is the development of predictive models of epidemic development which incorporate information on pest/pathogen incidence, epidemiological data, plant resistance, weather conditions and pesticide efficacy to allow optimal integration of all control measures.

#### **Food quality**

There is no doubt that, in developed nations, the diversity, quality and freshness of food, especially fruit and vegetables, is continually increasing while the price falls in real terms as a proportion of income. The majority of food sales are led by a few large supermarket chains who set high standards. We increasingly regard agricultural produce in a similar way to manufactured goods, expecting a uniform size and shape and unerring freedom from disease, blemishes and pests. Misshapen or scabbed potato tubers, or a single aphid on a lettuce can result in rejection of an entire stock. This is driven by a combination of supermarket demands, EU bureaucrats and a fickle generation of consumers increasingly detached from the realities of agricultural production systems. While we would not advocate a lowering of such standards, perhaps the price of such perfection should be consid-



**Figure 8** Like many soil-borne pests, turnip root fly is very difficult to control and may require many pesticide applications.

ered? Removal of every carrot fly, cabbage aphid or apple scab requires exceptional husbandry and intensive pesticide use. There is a conflict of interests here - demands for perfect produce and zero pesticide use are presently incompatible. In fact, public fears of pesticides are often irrational. Comparisons have been made between the doses and risks associated with consumption of 'natural' and 'synthetic' pesticides. The finding that we are exposed to 10,000 times less 'synthetic' pesticides than 'natural' pesticides (i.e. plant defence products), of equivalent toxicity<sup>6,7</sup> should serve not to scare us from consuming such foodstuffs, but to increase awareness of the context in which these issues should be viewed. Despite claims that pesticides are unnatural, risky, toxic and unethical, average human lifespans have more than doubled over the past 100 years, in part through medical advances but also through safer, sufficient supplies of food.

A double irony is that organic produce, while free of synthetic agrochemical residues, may well be detrimental to health because of increased levels of natural plant defence products and, more importantly, toxins produced by the very agents of plant disease and food spoilage which are removed by pesticides. There have been few studies on the impact of natural plant 'pesticides', but considerable work on the damage caused by toxins. A range of such toxins has been detected in foodstuffs. The best known are the aflatoxins formed in grain and nuts after infection with the ubiquitous fungus, Aspergillus flavus. Aflatoxins are amongst the most carcinogenic and teratogenic (causing foetal defects) substances known, yet they are entirely 'natural'. Rigorous international safety standards and procedures for screening for aflatoxins have been implemented; yet, in the UK there are still sporadic contamination problems, possibly from unregulated imports of infected foodstuff8. Patulin, a toxin produced by the fungus Penicillium expansum, has to be monitored carefully, particularly in products such as apple juice. Ochratoxins, produced by some toxigenic species of Aspergillus and Penicillium, are nephrotoxic, hepatotoxic, teratogenic, carcinogenic and immunosuppressive. They are frequently reported as contaminants on food stuffs, particularly on cereals in temperate regions, and are implicated in an irreversible and fatal kidney disease referred to as Balkan Endemic Nephropathy. Most familiar are the toxins produced by the fungus Claviceps purpurea which infects the grains of cereal crops, particularly rye. Toxins in rye have often been found in concentrations sufficient to induce delirium and reduced fertility.

Such Ergotism or 'St Vitus's dance' was of historical importance in Europe and may once again be increasing since the sclerotia of *Claviceps purpurea* in modern rye cultivars are smaller than those found in traditional varieties and cannot be simply sieved from harvested rye grain. We should therefore be careful in donning our rose-tinted spectacles and assuming organic produce to be more 'nutritious, healthy and safe'9 when there is little supporting evidence<sup>10</sup>.

#### **Conclusions**

The above discussion highlights just a few of the areas of research necessary to meet current needs and future challenges. Methods developed by the UK plant pathology community present a significant benefits to our agricultural industry, attract inward investment, develop export markets, protect the environment, and transfer technology to developing nations to solve local problems and humanitarian needs. Undermining this skill base will have serious implications. Such a resource cannot be created overnight.

There is no *status quo*. False romantic notions of medieval, subsistence agriculture will not feed an extra 5 billion mouths. We have not, and probably will not, master pests and diseases; but we do need to keep one step ahead. This will not be achieved by reducing research investment but through the maintenance of a stably funded community of scientists able to face the challenges of reducing inputs, maximising production and protecting the environment. This must be coupled with increases in training of growers, the public and, of course, future generations of scientists.

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#### Wayne Powell and Howard V. Davies

Reorganisation of the Institute's research activities brings enhanced prominence to the thematic area of plant molecular and cellular biology. As the primary sequences of most plant genes become identified, new opportunities will arise for their exploitation in fundamental and applied research. In the future, greater emphasis will need to be placed on basic genetics and biochemistry to fill the knowledge gap between genome structure and function. This will demand that we have a better understanding of how living cells operate, function and most importantly interact and communicate. These scientifically challenging goals will require a focused commitment from us all.

RNA processing and gene expression This year has seen the culmination of 3-4 years of research on the novel organisation of small nucleolar RNA (snoRNA) genes in plants. These genes encode small RNAs required for the production of ribosomal RNAs (rRNAs). The molecular characterisation of the unique organisation, transcription and processing of these snoRNAs in plants has been complemented by cell biological localisations of various rRNA and snoRNA components carried out in collaboration with Dr Peter Shaw's group at the John Innes Institute. This exciting and productive area has had impact in the field of rRNA processing and ribosome biogenesis in general.

Research on pre-mRNA splicing continues to maintain a high profile and has concentrated on demonstrating the importance of exon scanning mechanisms in plant splicing. The establishment of experimental systems to show exon scanning and the molecular characterisation of particular *Arabidopsis* mutants, has provided the majority of evidence for such mechanisms in plants. In addition, progress is being made in understanding the regulation of alternatively spliced systems, and, in particular, how a mini-exon of only 9 nt becomes included in potato invertase genes. A greater knowledge of these post-transcriptional processes will allow better understanding of how plant genes and transgenes are expressed and how expression may be regulated.

Finally, the genes which encode the various components of the pre-mRNA splicing or rRNA processing machinery, provide a rich source of promoters for transgene expression in plant biotechnology. As most genes are organised in multigene families with great variability in expression levels and patterns, a novel approach has been developed to allow the identification and isolation of promoters with the required expression characteristics. This approach will prove valuable for future promoter isolation and exploitation.

The DNA Sequencing Facility has been run from June 1997 by Clare McQuade. The facility is well-organised and runs efficiently to meet the current sequencing needs of users across the breadth of the Institute.

The structural characterisation of all potato apoplastic invertase gene promoters has been completed. This included determination of the full nucleotide sequence of the single promoter for which a genomic clone was available and the nucleotide sequence for three further invertase gene promoters which were obtained by genomic walking. The acquisition of single copy sequence information from closely-related members of a gene family by genomic walking in the large tetraploid genome of potato, represents a significant achievement in the application of this technology to polyploid plant genomes. Transcription start points were determined for each promoter by the use of a sequence-specific RT-PCR approach which was also employed to obtain expression information at the organ level. This expression analysis was extended and refined by the construction of promoter-reporter gene fusions for each of the invertase genes. A set of confirmed transgenic potato plants was prepared for each of these fusions allowing accurate histological assessment of gene expression at the level of specific tissues.

Intellectual property rights have been obtained to protect the information generated from these analyses which offer a detailed insight into the function of these enzymes in plant sucrose metabolism. Through this insight, novel projects examining gene expression and targeted mutation in pollen have become possible. Potential commercial applications of the manipulation of sucrose metabolism in cold-stored potato tubers have also been advanced with the completion of a series of constructs designed to manipulate levels of invertase and other enzymes of potato carbohydrate metabolism. An extensive interdepartmental effort

has produced *c.* 10,000 transgenic potato plants which are undergoing field-trialling extending over several years. A further collaborative effort is under way to characterise the signals which regulate processing of the unique mini-exon found in plant invertase genes.

Our work on plant carbohydrate metabolism has recently been extended by the initiation of new projects which will yield essential knowledge and technology to underpin the manipulation of quality traits of the barley grain. The first of these involves the generation of a catalogue of gene expression in malting barley - the determination of an initial 1,000 expressed sequence tags (ESTs) is well under way. These, when extended into an expression profile and map, will provide both the raw material for genetic manipulation and an indication of the metabolic systems in which it might most usefully be employed. Such manipulation will require robust and efficient cereal transformation systems - work on both barley and wheat has commenced, with the engineering of virus-resistance in Chinese wheat, analogous to that engineered effectively into dicots, providing a real framework for the establishment of this key technology.

1997/98 saw the development of further plant RNA virus-based, foreign protein expression vector systems utilising the '2A-linker to coat protein' rationale behind the original Potato Virus X-OVERCOAT®. Specifically, Tobacco Mosaic Virus was found, counter intuitively, on X-ray diffraction derived structure data and from prior reports, to be able to accommodate large OVERCOAT® proteins up to the size of the green fluorescent jellyfish protein (27 kDa), fused to 5% approximately of the 2,400<sup>+</sup> coat protein sub-units required to encapsidate the recombinant genome. In addition, a vector was created which increased the efficiency and frequency of co-translational release of the upstream, 'foreign' OVER-COAT® moiety by virtue of arranging a double 2A linker peptide sequence between the foreign 'gene' and the TMV coat protein gene (work done by Christophe Lacomme).

A substantial Scottish Office/CHABOS co-ordinated programme initiative (£938 K) was compiled and funded during this period (SCR/824/97) with the objective of studying novel delivery routes for therapeutic/prophylactic or growth promoting peptides and/or proteins with veterinary applications (collaboration with the Hannah, Moredun and Rowett Research Institutes).

Work also began on a six partner, EU-INCO-funded programme between the UK, Germany and China to produce virus-resistant elite germplasm in Chinese cultivars. Several 'biolistic' transformation vectors for wheat embryos have been constructed and are being tested at present.

Genomics Marker technology development has progressed well in potatoes, barley and various tree species. For potato, more than 100 nuclear- and 20 chloroplast-derived simple sequence repeat basedmarkers have been assembled. Approximately 60% of the nuclear SSRs have now been genetically mapped. As with barley, the development of this class of markers for potato has provided tools which are highly appropriate for a range of applications. In diploid potato populations, the SSRs have been used to study the inheritance of quantitative resistance to late blight and other characters such as vigour and earliness, and SSRs in regions of the potato genome which harbour genes affecting these characters (particularly on potato chromosome V) have been identified. Establishing the map location of SSRs in experimental diploid populations has been particularly valuable in attempts to unravel some of the complexities of genetic inheritance in cultivated, tetraploid potatoes. This is because the number of distinguishable SSR alleles at a single locus allows a large portion of the parental genomes to be accurately followed in segregating populations. Thus, the SSRs have been used for the first time to examine the inheritance of quantitative resistance to late blight, S. tuberosum subsp. andigenaderived polygenic resistance to PCN and sensitivity to the important processing characteristic of low temperature sweetening in a tetraploid population. Multiple allelism allows allelic-bridges to be formed between (the 12 sets of 8) independently segregating genetic linkage groups and these linkage groups to be assigned specific chromosomal designations. Information derived from these analyses can then be directly compared to that available from diploid populations. Informative SSR-markers which explain a large proportion of the phenotypic variation in the population for late blight and PCN resistance, have been identified on potato chromosome IV. A particularly informative application of nuclear and chloroplast SSRs is in assessing the amount and distribution of genetic diversity in collections of germplasm. These marker types are complementary, allowing bi-parental and maternal inheritance respectively to be studied with a very high degree of resolution. Both have been applied to genotype the complete list of potato cultivars on the current UK National List and a genotypic database has been established and installed at SASA as a reference for comparison when applications are made to register new potential varieties. Eight nuclear SSRs reveal a substantial amount of genotypic diversity and can uniquely fingerprint all of the test varieties (except two pairs of 'sports'). In contrast, analysis with cpSSRs reveals a substantial cytoplasmic bottleneck in the cultivated genepool with more than 85% of the analysed lines having an identical chloroplast haplotype. In this study, 26 chloroplast haplotypes were revealed using cpSSRs. This compares favourably with the five haplotypes identified previously using cpRFLPs and demonstrates the power of resolution of this approach.

European Union-sponsored research on conifer genomics is focused on comparative gene mapping in maritime pine and Norway spruce. Six research groups are utilising the tools of contemporary genetics to examine gene synteny, genome organisation, gene discovery based on ESTs and the development of software to support comparative mapping and QTL analysis.

The development of new technologies for functional genomics is of major significance. In this context, a new BBSRC-GAIT funded project to develop radiation-hybrid panels for plants is underway in the Unit of Barley Genomics and Breeding. The goals of this project are to develop new approaches to map gene sequences which are not dependent on the detection of polymorphism and meiotic recombination.

# The barley genome: a source of genes for breeders and biotechnologists

W. Powell

Two of our main goals are to develop molecular markers for use in breeding programmes and to isolate genes for use in transgenic approaches to barley improvement. These approaches are complementary and are described below.

Molecular marker technology development and **deployment** Phenotypic differences between individual accessions have provided the basis for successful plant breeding. However, phenotypic appearance is not always a good indicator of genetic potential. Recently, the ability to detect polymorphism at the DNA level has profoundly changed plant genetic analysis and is poised to impact on barley breeding and plant biotechnology. An important element of this development is the technology for detecting DNA sequence variation. Microsatellites, or simple sequence repeats (SSRs), provide an important intermediate technology for barley breeders, since SSRs are PCR-based, exhibit co-dominant inheritance and are multi-allelic. The high information content of SSRs means that diagnostic markers will have a high probability of detecting polymorphism in germplasm of direct relevance to breeders. For this reason, we have invested considerable resources in the area of microsatellite discovery and mapping (SCRI Ann. Rep. 1996/97, 82-83). To date, 385 functional microsatellites have been identified, and 258 SSRs have been mapped, generating 299 loci (Table 1).

In parallel with the microsatellite marker development and mapping, we have also been active in deploying microsatellites for various projects:

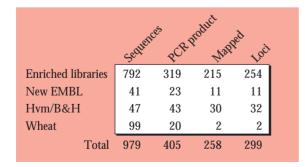
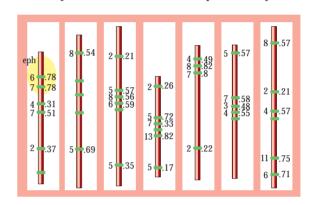


Table 1 Discovery and characterisation of SSRs.

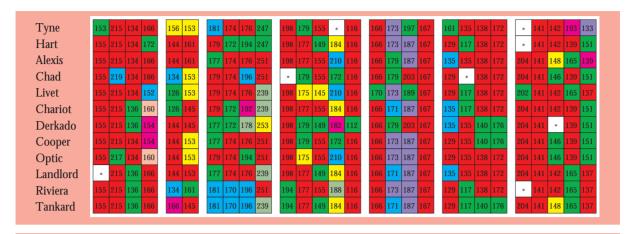
Genotypic diversity in the cultivated barley genepool A key factor in this programme is the utilisation of mapped microsatellites to create a genotypic database for barley. The microsatellites are represented by both



**Figure 1** Allele numbers and diversity indices at chosen SSRs - 67 genotypes.

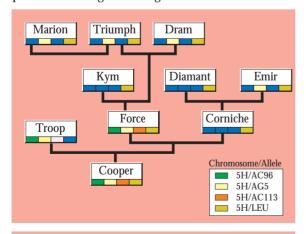
anonymous SSRs and SSRs that are associated with genes of known function. A sample of 100 cultivars, represented by genotypes produced in the last century, together with modern day cultivars, was genotyped with 30 SSRs spanning the seven barley linkage groups. A total of 168 alleles was observed with on average 5.6 alleles per SSR being detected. The number of alleles and diversity indices for each SSR are given in Figure 1, providing a two-dimensional representation of the pattern of genetic variability present in the barley genepool.

An alternative method of presenting the information on allelic diversity is shown in Figure 2. In this graphical display, the pattern of allelic variability is given for two of the seven linkage groups and 'pinpoints' specific allelic substitutions that have accompanied the introduction of new cultivars. This form of graphical genotyping, when coupled with data on pedigree relationships, provides a means of monitoring the flow of alleles through ancestral lineages and identifying regions of the genome that have been preferentially transferred through selective breeding. This is illustrated for the pedigree of Cooper in Figure 3 where the inheritance of alleles at four SSR loci is



**Figure 2** SSRs in selected modern spring barley cultivars arranged in chromosome order (1H-7H).

shown. Based on this pedigree, it would appear that chromosome 5H of Cooper has been 'inherited' directly from Force. The high discriminatory power of microsatellites allows closely related genotypes to be distinguished, enabling SSRs to be used in delineating complex pedigrees constructed from a narrow genetic base. The relationship between genetic and kinship estimates of relatedness is shown in Figure 4 for the cultivars Cooper and Force. Since Force is one of the 'grandparents' of Cooper, on average one would expect 25% of the genetic information from this cultivar to be inherited by Cooper. The radar plot given in Figure 4 shows the poor correspondence between the two estimates of relatedness. Furthermore, it shows that, based on this data, chromosomes 5, 6 and 7 have been inherited directly from Force rather than receiving contributions from Corniche and Troop. This retrospective analysis of cultivar production will provide new insights into significant 'historical recom-

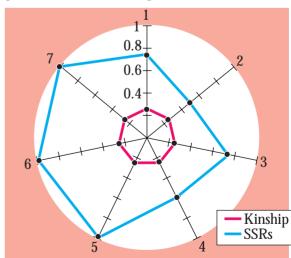


**Figure 3** SSRs in the pedigree of Cooper - origin of chromosome 5H alleles.

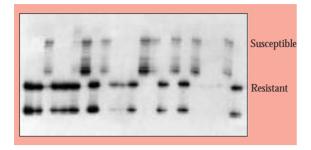
bination events' and provides an important tool for selection of parents based on genetic rather than phenotypic information.

Identifying a microsatellite marker linked to the Ym4 virus resistance locus in barley Barley yellow mosaic virus (BaYMV) and barley mild mosaic virus (BaMMV) are important diseases of winter barley in Europe. The fungal vector Polymyxa graminis is responsible for transmission of virus particles to roots of susceptible plants. The soil-borne transmission of the pathogen causes problems for both chemical control of the disease and for the testing of resistant lines for eventual deployment in plant breeding programmes.

Previous studies  $^{1}$  have localised the recessive resistance gene Ym4 to the distal region of chromosome  $^{3}$ H.



**Figure 4** Radar plots of genetic (SSRs) and pedigree similarity (kinship) for chromosomes 1H-7H.



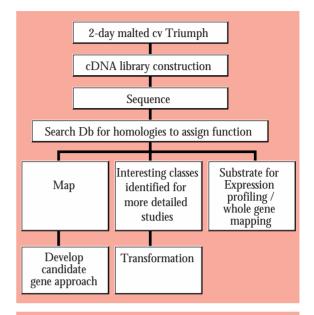
**Figure 5** A co-dominant, PCR-based assay for Barley Yellow Mosaic Virus (BaYMV) and Barley Mild Mosaic Virus (BaMMV).

The microsatellite MAC029 has been shown to be linked (0.9 cM) to Ym4 in a doubled haploid population (Igri x Franka) segregating for response to BaYMV. Germplasm surveys have confirmed the value of this microsatellite in discriminating between resistant and susceptible genotypes. Furthermore, the allelic differences between the two groups can be resolved on agarose gels (Fig. 5) providing an ideal codominant, diagnostic marker for use in barley breeding programmes.

#### Gene isolation in barley

The genome of barley is made up of 5.3 x 19<sup>9</sup> bp. If one assumes that barley possesses 50,000 genes, then on average one would expect one gene every 100 kb. Thus, gene isolation in species such as barley is not trivial. However, recent advances made initially in biomedical science provide fast routes to gene isolation. The approach is simply to sequence the ends of cDNA clones to produce expressed sequence tags (ESTs).

Advantages of this approach include direct access to coding sequences, information on tissue specificity and abundance of different mRNAs in various tissues sampled, and identification of putative function by homology to genes in databases (bacterial, yeast etc). Disadvantages include the cost, the difficulty of identifying low abundance messages and redundancy. In order to evaluate the potential of ESTs in barley, we have initiated a project to isolate 1,000 genes from 2-



**Figure 6** Strategy for development and exploitation of ESTs.

day malted barley. The strategy being pursued is shown in Figure 6 and, to date, about 70% of the sequences generated exhibit significant homology to known function genes in databases. Approximately 5% of the ESTs also contain short SSRs, providing one potential route to transcript mapping.

#### Conclusions and future directions

Gene discovery programmes will be less demanding in the future and greater emphasis will need to be placed on understanding the relationship between gene sequence and function. We will therefore need to develop (or have access to) barley transformation and create transcript-based linkage maps. For the latter, the potential of whole genome radiation-hybrid mapping will be explored. These biotechnological programmes will be complemented by large-scale genotyping efforts to expand our microsatellite databases for barley.

#### Reference

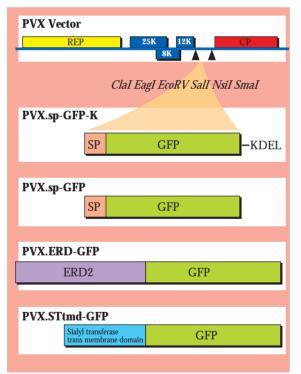
<sup>1</sup> Graner, A. & Bauer, E. (1993). *Theoretical & Applied Genetics* **86**, 689-693.

# New insights into the plant secretory pathway using virus delivered GFP

P. Boevink, C. Hawes<sup>1</sup>, D. Prior, S. Santa Cruz & K. Oparka

In addition to the study of basic virology undertaken at SCRI, we are developing virus-based vectors for fundamental cell biology research.

Potato virus X (PVX) has been engineered to express the green fluorescent protein (GFP) as a free protein and as a fusion to the viral capsid protein. These constructs are being used in a variety of ways to study the movement processes of PVX itself. Similar work is being carried out with cucumber mosaic virus, tobacco rattle virus and groundnut rosette virus. Recently, PVX also has been engineered to express GFP fused to proteins and peptides of interest in the secretory pathway. These modified GFPs act as powerful *in vivo* 



**Figure 1** Diagrammatic representation of the GFP fusion constructs. The PVX genome has five genes: the replicase (REP), three overlapping genes encoding movement functions, and the coat protein (CP). The PVX vector contains duplicated coat protein subgenomic promoters, represented by the black triangles, and a polycloning site (expanded). Each GFP fusion was independently inserted between the *Eag*I and *Ns*II sites.



markers for protein localisation and illuminate the structure and dynamics of the plant secretory pathway.

Published work on the plant secretory system generally assumes that most processes are homologous, if not identical, to those in animals and yeast. However, our recent work suggests this may not be the case.

Targeting GFP to the ER In the first step of the secretory pathway, proteins enter the endoplasmic reticulum (ER). The translocation of nascent polypeptides from the cytoplasm to the ER is dependent on a signal peptide sequence being present at the amino terminus of the polypeptide. To determine whether GFP could be targeted to the ER when expressed from the PVX vector, a signal peptide sequence was fused to the amino-terminus of GFP<sup>1</sup>. Two different signal peptide sequences were tested, derived from the vacuolar storage proteins patatin and sporamin. To

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**Figure 2** Fluorescent cortical ER in a PVX.sp-GFP-K infected *Nicotiana clevelandii* epidermal cell.

ensure high levels of GFP in the ER a four amino acid signal that specifies ER retention of proteins, KDEL, was fused to the carboxy-terminus of GFP (Fig. 1). Figure 2 shows an epidermal cell infected with the construct PVX.sp-GFP-K. The ER fluoresces brightly with the GFP and the polygonal network structure of the cortical ER is revealed. Since GFP allows imaging of live cells, we were able to observe that the cortical ER is a fairly stable structure, but on a smaller scale is constantly changing. The polygonal network constantly changes shape, new tubules

extend from the network and fuse to another part or contract, and the small patches of sheet-like ER appear and disappear.

The *in vivo* effect of the fungal toxin, Brefeldin A (BFA), which inhibits secretion, was also investigated. High concentrations of BFA caused a dramatic and reversible change in the morphology of the cortical ER (Fig. 3). After several hours in BFA, the ER had formed an almost complete sheet.

**Secreted GFP** Proteins which enter the secretory pathway but which have no further targeting signals, are secreted. Secretion is therefore considered to be the default pathway.

To target GFP for secretion, a GFP was made with the sporamin signal peptide at the amino terminus and no other signals (Fig. 1). In cells infected with this construct, no GFP fluorescence was observed in the ER. A very faint fluorescence was seen which appeared to be in the cytoplasm. This fluorescence may be from GFP which did not enter the ER due to overloading of the ER translocation pathway by the high level of expression from PVX. This cytoplasmic fluorescence was possibly also present in the cells infected with the KDEL-containing construct but could not be detected due to the saturating fluorescence from the GFP-labelled ER.

Evidence that the GFP was being produced in cells and then secreted was obtained when secretion was inhibited by cold shock or with BFA. Fluorescence built up to detectable levels in the ER when secretion

was inhibited. GFP was also detected in the apoplast by western blotting of fluid extracted from the extracellular space of PVX.sp-GFP infected leaves with anti-GFP antibody.

Targeting GFP to the Golgi apparatus The Golgi apparatus is a stack of membranous disks and associated budding vesicles and is considered to be the main processing and sorting organelle in the secretory pathway (Fig. 4). Proteins and other molecules enter the Golgi at the *cis* face in vesicles derived from the ER and

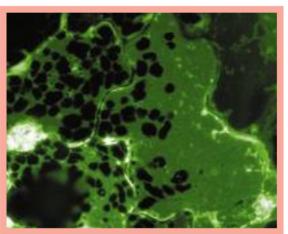
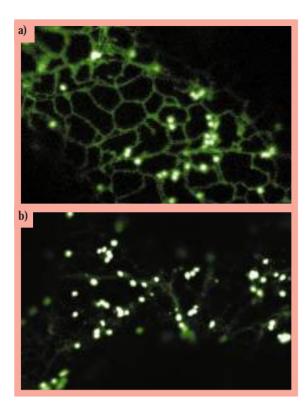


Figure 3 PVX.sp-GFP-K infected epidermal cells treated with  $100\mu g/ml$  BFA for 4 hours. The cortical ER was transformed from a network of tubules into large sheet-like structures.



**Figure 4** The *cis* face of the golgi stack, reconstructed from serial electron microscope sections.



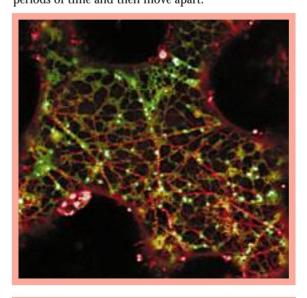
**Figure 5** a) Fluorescent Golgi and ER in a PVX.ERD-GFP infected epidermal cell. b) Fluorescent Golgi in a PVX.STtmd-GFP infected epidermal cell.

leave from the *trans* face in specifically targeted vesicles. From electron microscope and immunofluorescence studies, it is known that plant Golgi are arranged very differently to animal Golgi. They are dispersed throughout the cytoplasm and are presumed to be subject to cytoplasmic streaming. It is thought that this arrangement may reflect a predominant function of the plant Golgi in the synthesis of cell wall components, as opposed to protein secretion for the animal Golgi².

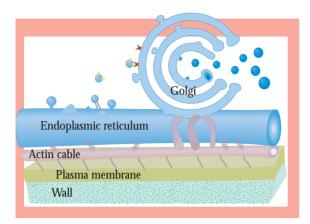
In the first construct, the entire *Arabidopsis* ERD2 homologue sequence (provided by N. Raikhel, East Lansing) was fused to the amino terminus of the GFP (Fig. 1). In addition to targeting the GFP to the Golgi, it was hoped that this construct would help clarify the localisation and rôle of ERD2 in plants. From yeast and animal studies, ERD2 is known to be the receptor for the K/HDEL peptide signal. It resides predominantly in the Golgi apparatus where it binds to KDEL-containing proteins that have escaped the ER and recycles them back to the ER. For the second construct, the amino terminal 52 amino acids of the rat sialyltransferase (ST; provided by S. Munro, Cambridge), a protein localised to the *trans* Golgi and

*trans* Golgi network of animal cells, was fused to the GFP (Fig. 1). The amino terminus of ST encodes the *trans* membrane domain (tmd) of ST and it has been shown that this region determines the localisation of the protein.

PVX.ERD-GFP infected cells displayed GFP fluorescence in the ER and the Golgi (Fig. 5a) while PVX.STtmd-GFP infected cells displayed GFP fluorescence almost exclusively in the Golgi (Fig. 5b). Evidence that the circular fluorescent bodies observed were in fact Golgi bodies was provided by immuno electron microscopy. Electron micrographs showed that the ERD-GFP was located throughout the Golgi, whereas the STtmd-GFP was located predominantly in the trans Golgi. This latter result demonstrated that there is sufficient homology between animal and plant systems for the animal Golgi targeting information to be functional. Observation of live cells with fluorescent Golgi revealed that the Golgi were in almost constant motion (videos of movement of GFP labelled Golgi can be seen at http://www.brookes.ac.uk/ schools/bms/research/molcell/hawes/gfp/gfp.html). They appeared to be closely associated with the ER and could be seen moving around the outline of the polygonal network of the cortical ER. Their motion was multidirectional and at various speeds. They moved very rapidly along cytoplasmic strands, at a lower speed along grouped cables of ER and slowest around the cortical ER network. Pairs, or small groups, of Golgi appeared to move together for short periods of time and then move apart.



**Figure 6** Double labelling of an epidermal cell to show labelled ER and Golgi (green) and actin filaments (red).



**Figure 7** Model of Golgi association with the ER and actin.

Colocalisation of ER, Golgi and actin Previous studies have indicated a rôle for the actin cytoskeleton in plant Golgi function. When the cells with GFP-labelled Golgi were treated with an inhibitor of actin motor protein function, N-ethyl maleimide, the Golgi ceased to move. Treatment of the cells with cytochalasin, which depolymerises actin filaments, arrested

Golgi movement and caused them to clump together at specific vertices on the polygonal ER network. The actin filaments in lightly fixed leaf cells were stained with rhodamine-conjugated phalloidin. Double imaging of the rhodamine and GFP in these cells revealed that the ER and the Golgi colocalised closely with the actin filaments (Fig. 6).

The close association of the ER, Golgi and actin has led us to propose a model in which the actin cytoskeleton underlying the ER network functions to transport the Golgi around the plant cell, using motor proteins that link the cytoskeleton to the Golgi stacks (Fig. 7). In this way, the Golgi remains closely associated with the ER and it is envisaged that in plants, transfer of proteins from the ER to Golgi occurs while the latter is in active transit.

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- <sup>2</sup> Stahelin, L.A. & Moore, I. (1995). *Annual Review of Plant Physiology and Plant Molecular Biology*

# Splicing regulation of a potato invertase mini-exon

C.G. Simpson, P.E. Hedley, C.M. McQuade, J.M. Lyon, G.P. Clark, G.C. Machray & J.W.S. Brown

The removal of non-coding intron sequences from precursor messenger (pre-mRNA) transcripts by

the process of splicing is an essential step in plant gene expression (SCRI Ann. Rep. 1991, 42-44). Selection of the cleavage (splice) sites must be accurate to retain the correct translational open reading frame. One mechanism aiding splice site selection is exon definition (Fig. 1a), which has been demonstrated

a) Exon definition

b) Intron splicing enhancers

**Figure 1** Model of exon definition and mini-exon/intron enhancers. (a) Splicing factors (circles) associate with splice sites at the ends of or on exons (boxes) and interact across the exon prior to intron (solid line) removal (dashed lines). (b) Factors associate with intron splicing enhancers (small boxes within intron) stabilising factor assembly on the mini-exon, permitting splicing.

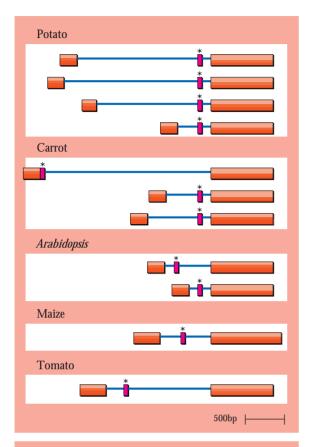
in vertebrate and, more recently, plant intron splicing (SCRI Ann. Rep. 1996, 102). In exon definition,

splicing components associate at the 3' splice site region and 5' splice site bordering an exon, and interact across the exon to stabilise assembly of splicing factor complexes at each of the sites. This process may also be mediated by factors bound to the exon itself. Following exon definition, interactions across introns occur to initiate spliceosome formation and removal of introns. However, when

exons are small (less than 50 nucleotides), efficient assembly of splicing factors at the splice sites may be adversely affected due to steric constraints, precluding the involvement of exon definition in splice site selection.

Different mechanisms have been proposed to explain inclusion of short, mini-exons (as small as three nucleotides) in vertebrates. Firstly, sequential splicing may occur whereby one intron is removed, splicing factors dissociate from the mRNA and the spliceosome reassembles on the second intron, which is then removed. Such a mechanism has been described for the seven nucleotide long, troponin I, mini-exon 3 from chicken. Secondly, mini-exons may be flanked by strong splice sites, improving their inclusion in mature mRNA transcripts. In some cases, however, this is not sufficient to promote inclusion, and other elements/factors are required. Thirdly, and most commonly, intron splicing enhancers (ISEs) have been described which promote the selection of splice sites (Fig. 1b). ISEs are usually found downstream of the mini-exon, are diverse in sequence and often consist of a series of direct repeats. Binding of specific protein factors to ISEs is thought to recruit splicing factors to the exon to initiate splicing (Fig. 1b).

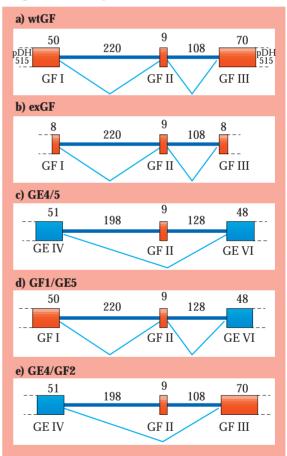
One of the smallest exons discovered in plants to date is a nine nucleotide mini-exon found within invertase



**Figure 2** Structure of plant invertase genes. Exons (boxes) and introns (solid lines) are shown for all characterised plant invertase genes. The mini-exon 2 is present in all but one of the carrot genes.

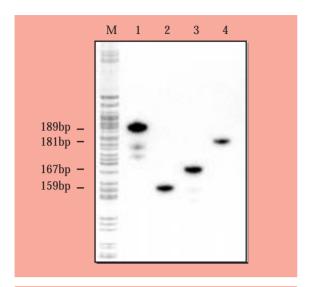
genes and flanked by introns of varying sizes (Fig. 2). Invertase is one of two key enzymes known to catalyse the breakdown of sucrose in plants. This central role in plant carbohydrate metabolism is executed by a variety of invertase isoenzymes specific to particular cell compartments, tissues and developmental stages, encoded by a family of invertase genes. With one exception, all characterised plant invertase genes include the mini-exon which encodes three amino acids of a highly conserved five amino acid motif found in all invertase enzymes and which is therefore likely to be critical to their function. In potato, we have characterised four invertase genes, each of which contains a mini-exon, and we have shown that under cold stress conditions, alternative splicing, which results in skipping of the mini-exon, can occur at low frequency (SCRI Ann. Rep. 1995, 52). The physiological consequences of exon skipping to the function of the resulting enzyme are being examined alongside the mechanism which ensures the accurate inclusion of the mini-exon under normal conditions.

It is likely that inclusion of this mini-exon into mature invertase mRNA will require splicing signals in addition to the flanking splice sites. To identify such signals, we have made a series of intron/mini-exon constructs based on the potato invertase genes, invGE and invGF (SCRI Ann. Rep. 1996, 100-101). All of the constructs were inserted into the expression and splicing analysis vector, pDH515, and their splicing behaviour analysed. Each construct was introduced into tobacco protoplasts and, following expression of the transcripts, RNA was isolated and examined by reverse transcriptase-PCR (RT-PCR) using a labelled primer in the PCR reaction. Primers were designed to amplify from the sequences in pDH515 flanking the intron constructs and mini-exon inclusion or skipping was determined from the sizes of the generated PCR products.



**Figure 3** Intron replacement constructs. Schematic diagram showing the region from the invGF gene (red) containing the mini-exon and the modifications where introns from invGE (blue) replace those of invGF. (a) WtGF - wild type. (b) exGF - As a but with reduced sizes of exons 1 and 3. (c) GE4/5 - double intron replacement. (d) and (e) Single intron replacements.

## Plant molecular & cell biology



**Figure 4** RT-PCR analysis of splicing of intron replacement constructs. Electrophoretic separation of labelled RT-PCR products from wtGF (lane 1), GE4/5 (lane 2). GF1/GE5 (lane 3) and GE4/GF2 (lane 4).

To demonstrate that correct mini-exon splicing (inclusion) occurs in the tobacco protoplast experimental system, a region of invGF containing 50 bp of exon 1, intron 1 (220 bp), exon 2 (the 9 bp miniexon), intron 2 (108 bp) and 70 bp of exon 3 was isolated by PCR amplification (Fig. 3a; wtGF). In this construct, the potato invGF mini-exon is flanked by its authentic introns: introns 1 and 2, both of which are UA-rich (72% and 84% UA respectively), an important signal in plant intron splicing. RT-PCR analysis of the wtGF transcript (Fig. 3a) revealed a fully spliced product of 189 bp which shows that the mini-exon was included constitutively in the mature mRNA transcript when flanked by its authentic introns and exons (Fig. 4, lane 1). Although it was likely that the signals for mini-exon inclusion lay in the flanking introns, to exclude the possibility that exons 1 and 3 were needed for correct splicing, a construct was made where the exons in wtGF were reduced to 8 bp each (Fig. 3b; exGF). Splicing analysis of this construct showed that the mini-exon was present in the mature transcript, demonstrating that these flanking exon sequences do not influence miniexon inclusion (results not shown).

The first construct designed to show that at least one of the introns flanking the mini-exon was important for its correct splicing, was a double intron replacement construct. Introns 1 and 2 in wtGF were replaced by introns 4 and 5 (and their flanking exons) of the second invertase gene (invGE). These introns were selected as they were similar in size and UA content to the introns which normally flank the miniexon. This construct therefore contained the invGF mini-exon flanked by two introns derived from a completely different region of invGE (Fig. 3c; GE4/5). In this case, the mini-exon was no longer included (i.e. it was skipped), as reflected by the spliced product of 159 bp (Fig. 4; lane 2). Therefore, inclusion of the mini-exon in the mature mRNA required its authentic flanking intron(s).

Finally, to determine whether one or both introns were necessary for normal splicing, single intron replacement constructs were made. Firstly, the intron downstream of the mini-exon (invGF intron 2) was replaced by invGE intron 5 (Fig. 3d; GF1/GE5). Secondly, the upstream intron (invGF intron 1) was replaced by invGE intron 4 (Fig. 3E; GE4/GF2). Splicing of GF1/GE5 in tobacco protoplasts resulted in the inclusion of the mini-exon in the final transcript as shown by a 167 bp RT-PCR product (Fig. 4, lane 3). However, splicing of GE4/GF2, always resulted in skipping of the mini-exon as shown by a 181 bp RT-PCR product (Fig. 4; lane 4). Thus, the presence of the authentic upstream intron 1 was essential for the inclusion of the mini-exon and the downstream intron 2 was not required for correct splicing.

Clearly intron 1, upstream of the mini-exon, regulated inclusion of the mini-exon into invertase mRNA. However, intron 1 does not contain sequences similar to known intron splicing enhancers from vertebrate introns nor are there obvious direct repeat sequences, apart from U-rich regions. Therefore, a systematic deletion analysis of this intron is currently underway to identify the signals responsible for mini-exon inclusion. To date, a polyuridine tract and the branchpoint have been shown to be required for exon inclusion. Ultimately, it may be feasible to develop biotechnological applications in targeted regulation of gene expression, by controlling the inclusion or exclusion of mini-exon containing genes, resulting in the alteration in the functional capacity of alternatively spliced proteins.

## Breeding and genetics

#### George R. Mackay & Ronnie J. McNicol

The ultimate objective of the research into breeding and genetics at SCRI is to facilitate the application of new technologies to end-user industries. The achievements which will mark the success of this goal are new, improved crop cultivars, produced in association with industrial partners, and improved scientific knowledge and practical technologies that will increase the efficiency and efficacy of crop improvement processes. In collaboration with various commercial sponsors, Kentish Garden Marketing Ltd and Scottish Soft Fruit Growers Ltd and the Hannah Research Institute, we have been profiling the flavour and mouthfeel of a wide range of raspberry and blackcurrant genotypes in fresh and various processed products. This will permit the more accurate targeting of the breeding programmes to produce new cultivars specifically suited to the needs and desires of the eventual end-user, the consumer.

The main target crops for improvement through breeding are potatoes, raspberry, blackberry, blackcurrant, strawberry and spring barley. In addition, underpinning research, funded by external contracts (e.g. EU), continues on legumes, and a small swede breeding (brassicas) programme is funded by an industrial partner. SCRI breeders have been highly successful historically, and currently the UK area share of SCRI-bred cultivars is significant: potatoes (17%), soft fruit (90%), and spring barley (10%).

Numerous new, improved cultivars have been released in recent years, as reported in previous SCRI Annual Reports, and others are currently undergoing statutory, National List Trials. Many of these, including the new so-called Glen MARS (Magna, Ample, Rosa, Shee) series of raspberry, and Symphony, our most recent strawberry, continue to perform well. Demand for Symphony and Glen Ample has continued to outstrip supplies. Estimates now indicate that Symphony will be the second most widely grown strawberry cultivar in the UK in 1999. It is largely the stability of cropping that has attracted growers to Symphony. Its tough skin gives it a distinct advantage in terms of reduced grading losses, especially under difficult harvesting conditions. This toughness, fortunately, is combined with a juicy flesh that has a good acid/sweetness balance. In addition, the cultivar has good tolerance of soil-borne pests and diseases.

New cultivar releases from SCRI are achieved by the collaborative efforts of breeders, geneticists, pathologists, nematologists, virologists, chemists and biochemists, and increasingly involves the fundamental researches of molecular biologists and tissue culture experts. This multidisciplinary activity allows the rapid adoption and development of novel technologies into the crop improvement process and, equally important but often neglected, permits research into the efficacy and efficiency of these technologies compared to classical methodology. It is one thing to propose a new experimental technique that will 'help the breeder', another to validate those proposals in a practical breeding programme

which may be constrained by the real demands of the market-place and economics. The approach has con-



ferred many advantages to the breeders and is at least partially responsible for past successes.

The research is also aided by access to SCRI's considerable germplasm resources, maintained in a high health state and immediately available to SCRI researchers. The Commonwealth Potato Collection, one of the smaller international genebanks of potatoes, has contributed disproportionately to its size in the improvement of the European Potato. Practically all modern cultivars, recently released, now possess the H1 gene originally discovered in the CPC S. tuberosum subsp. andigena accession CPC 1673 for example. The Ribes and Rubus collections are the

world's largest, and unique to SCRI.



## Applied potato genetics and breeding: the way ahead for potato breeding

J.E. Bradshaw, H. Barker, M.F.B. Dale, G.R. Mackay, S. Millam, R.M. Solomon-Blackburn & H.E. Stewart

**Introduction** The principal cultivated potato (*Solanum tuberosum*) is a tetraploid species (2n = 4x = 48) which was introduced into Europe, and thence the rest of the world, from the Andes of South America in the late 16th century. By the end of the 18th century, it had been adapted to long-day conditions through selection by its early cultivators for earlier-tubering, higher-yielding clones. These were

derived from seedlings from naturally occurring berries, the consequence of uncontrolled, largely self-pollination.

Potato breeding in the modern sense began in 1807 in England, when Knight made deliberate hybridisations between different varieties by artificial pollination, and flourished during the second half of the

19th century when many new cultivars were produced by farmers and hobby breeders.

However, it was the rediscovery, in 1900, of Mendel's 1865 paper on Experiments in Plant Hybridization that marked the birth of modern genetics, and opened the way to crop improvement by scientific breeding methods based on a sound knowledge of the inheritance of economically important traits. The development of such methods for potatoes was one of the challenges which faced scientists at the Scottish Plant Breeding Station (SPBS) on its foundation in 1921, and one which now faces the members of the newlyformed Applied Potato Genetics and Breeding Research Unit at SCRI.

This article reviews the way ahead for potato improvement at SCRI, in terms of the needs of the British Potato Industry, the germplasm available to breeders, the possibilities for modifying existing cultivars by genetic transformation and the technologies available for enabling faster, more efficient, and novel breeding strategies based on genotypic selection. It is concerned with producing improved cultivars for clonal propagation by tubers, and not with ones for propagation by True Potato Seed (TPS). This is because SCRI breeders are sceptical about the place of TPS in the highly developed markets of Europe and North America, whilst acknowledging that there is much

interest throughout the tropics in TPS as a means of avoiding some of the disease problems associated with the maintenance of vegetative stocks.

Priorities for potato improvement In Britain, 150,000 hectares of potatoes are grown for ware each year, primarily from seed-tubers produced in Scotland (15,000 hectares). Approximately 30 per cent of the crop is for processing (French fries and crisps) and 70 per cent for table use, mainly through supermarkets.

Seasonal fluctuations in supply require storage of tubers for long periods in order to ensure continuity of supply.

Today, as never before, the commercial success of new cultivars is heavily dependent on meeting the quality requirements of processors and supermarkets, and this is a trend that is likely to continue. Hence, the priorities shown in Table 1 reflect our recent discussions with these important end-users, an assessment of disease priorities in northern Europe, and the potential for increased seed exports to southern Europe and N. Africa. The majority of today's most popular cultivars are susceptible to a range of pests and diseases which have to be controlled by the widespread use of chemicals, such as fungicides for late blight, nematicides for cyst nematodes and insecticides for aphid-transmitted virus diseases. This is particularly true of cultivars for processing, where old ones such as Russet Burbank and new disease-susceptible ones such as Shepody are increasing in area. However, chemical control is



#### 1 Cultivars with the processing quality demanded by the manufacturers of crisps (chips) and French fries.

Whilst many traits are important, particularly adequate dry matter content and fry colour, major thrust is still:

 resistance to low temperature sweetening (and hence dark, bitter-tasting, fry products) so that tubers can be stored at 2°C to 4°C to control the development of diseases, weight loss and sprouting in store, with reduced reliance on sprout inhibiting chemicals.

#### 2 Cultivars with the table quality demanded by supermarkets.

Again, many traits are important: tubers must be resistant to after-cooking-blackening, then:

- · attractive skin finish paramount
- flavour and texture as judged by taste panels
- low levels of glycoalkaloids
- special purpose cultivars e.g. salad and punnet types

#### 3 Combining quality with durable resistance to pests and diseases

Priorities for resistance:

- potato cyst nematodes, particularly *G. pallida* the most serious pest problem in UK
- late blight (in foliage and tubers)

   world-wide
- blackleg and powdery scab common criticisms of Scottish seed
- ullet blemish diseases<sup>(a)</sup> supermarkets want good skin finish
- storage diseases(b) less important now, but cannot ignore
- viruses:

 $\begin{array}{c} PLRV \\ PVY \end{array} \hspace{0.5cm} \bigg\} \hspace{0.5cm} \text{- most serious worldwide} \\ TRV \\ PMTV \end{array} \bigg\} \hspace{0.5cm} \text{- cause of spraing in tubers, particularly those} \\ \text{of some important processing varieties} \\ PVX \hspace{0.5cm} \text{- less important now} \\ \end{array}$ 

#### 4 Cultivars with potential for seed export to southern Europe and N. Africa

- resistance to warm temperature diseases and pests e.g. early blight, Fusarium dry rots, Verticillium wilt and potato tuber moth
- resistance to abiotic stresses e.g. heat, cold, drought, salinity

(a) silver scurf, black dot, black scurf, skinspot

(b)gangrene, dry rot

#### Table 1 Priorities for potato improvement.

expensive, not always effective, and raises environmental and food safety concerns, particularly over large-scale insecticide use and pesticide residues in tubers for human consumption. Hence, cultivars with higher levels of disease and pest resistance are highly desirable, but they must retain the marketable yield and quality required for a modern cultivar to be successful.

Although Britain does not have a potato starch industry, it is worth mentioning that the potential variation in the chemical and physical structure of starch is immense and, hence, there is tremendous scope to produce novel starch for use both in the food and non-food market sectors. Plant breeding and biotechnology have a major rôle to play in generating new starches, as already seen in The Netherlands with the development of amylose-free potatoes by genetic modification of the starch variety, Karnico<sup>1</sup>. Amylose production was completely suppressed by antisense, RNA-mediated inhibition of granule-bound starch synthase, an approach made possible by the identification of an amylose-free mutant produced by techniques associated with conventional breeding.

It may also be possible to engineer the tuber synthesis of commercial quantities of fructans from sucrose for use in the food industry, or modify potatoes to produce and store novel compounds such as pharmaceuticals<sup>2</sup>. Finally, it might be worth considering improvements in the nutritional value of what is already a highly nutritious food, by correcting its methionine and cysteine deficiency with genes encoding proteins rich in these amino acids.

Germplasm for potato improvement At the beginning of the 20th century, progress in potato breeding was being impeded by a narrow genetic base tracing back to the few original introductions of *S. tuberosum* subsp. *andigena* from South America to Europe in the latter part of the 16th century, limited further casual introductions in the 17th and 18th centuries, and a single cross with a Chilean Tuberosum (*S. tuberosum* subsp. *tuberosum*) in the 19th century (Fig. 1).

Furthermore, it is believed that relatively few of the 228 wild tuber-bearing taxonomic species of the genus *Solanum* were involved in the early domestication process in the Andes - probably just several closely related and inter-fertile members of series Tuberosa. Compared with the wild species, cultivated potatoes also evolved under a very limited range of environmental conditions in cool temperate regions. As a

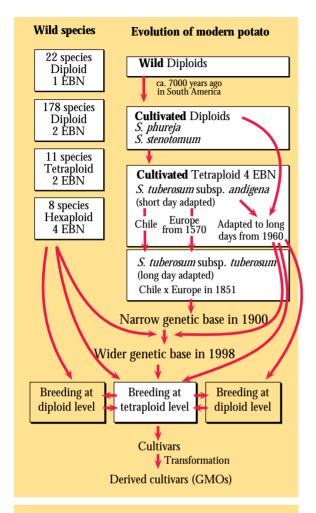


Figure 1 Germplasm for potato improvement.

consequence, they were often unable to resist the attacks of pests and diseases occurring over the much wider range of conditions in which they eventually became cultivated.

During the 20th century, the genetic base of the European potato (S. tuberosum subsp. tuberosum) has been widened in a number of ways, as shown in Figure 1. At SPBS/SCRI, high levels of resistance to viruses PVX and PVY, to cyst nematodes and to late blight have been introgressed from wild and cultivated species, and have proved sufficiently durable to remain useful today, with the exception of R gene resistance to late blight. However, it is hoped that the high levels of field resistance achieved in the SCRI-bred cultivars, Torridon and Stirling, will prove more durable, despite also being derived from relatively few accessions of S. demissum. Encouragingly, recent unpublished results from an experiment in CIP's Global Initiative on Late Blight<sup>3</sup> have confirmed that Torridon and Stirling are highly resistant in a wide range of environments. Nevertheless, as insurance against lack of durability in the longer term, new sources of resistance to late blight are being evaluated from *S. papita* and *S. verrucosum*, and to cyst nematodes from *S. boliviense*, *S. kurtzianum* and *S. sparsipilum*.

SCRI also has long-day adapted populations of S. tuberosum subsp. andigena (Neotuberosum) and S. phureja/S. stenotomum derived from accessions of the Commonwealth Potato Collection (CPC) through the work of Simmonds, Glendinning and Carroll in the 1960s (see Bradshaw & Mackay<sup>4</sup> for brief review). Experience has shown, however, that it is relatively easy to introgress desirable genes from short-day adapted S. tuberosum subsp. andigena into clones capable of becoming commercially successful cultivars, and hence the long-day adapted *S. phureja/S. stenotomum* (also referred to as *S. tuberosum* Group Phureja/Stenotomum) material looks set to have a greater impact than Neotuberosum. It appears to be a good source of resistance to Erwinia soft rot and blackleg, powdery scab and skin spot, as well as having interesting cooking qualities. Indeed, although this adapted diploid population has only 60 to 70 per cent of the yield of tetraploid cultivars, there is interest in it as a speciality vegetable and MRS Ltd entered a commercially-promising clone into National List Trials in 1998.

Finally, clones resistant to low temperature sweetening, including one which became cv. Brodick, and others with simply inherited resistance to PLRV multiplication, have been identified in SCRI's tetraploid breeding material.

In planning future breeding work, a balance needs to be kept between making use of the genetical variation already available in *S. tuberosum* subsp. *tuberosum*, utilising long-day adapted S. tuberosum subsp. andigena and S. phureja/S. stenotomum, and locating and introgressing new genes from wild and cultivated species. During the 20th century, samples of many of these species have been collected and held in international germplasm collections such as the CPC, which is kept at SCRI in true seed form and contains representatives of 81 species. The use of molecular marker technology to study the genetic differences and similarities among this germplasm, should assist breeders to make more rational decisions on its utilisation, and should also help to establish core collections of germplasm for detailed evaluation. Although there is scope for further understanding of species relationships, it would appear that breeders can introgress genes from virtually any potato species into S. tubero-

sum [by manipulating effective ploidy as determined by endosperm balance number (EBN) or by embryo rescue] and somatic fusion may widen opportunities still further. As many wild species are diploids which readily cross with dihaploids (2 sets of chromosomes) of *S. tuberosum* (both 2EBN), there is scope for breeding at the diploid level before returning to the tetraploid level, but the optimum amount is still a matter for debate and further genetical research.

Genetically modified potatoes Today, the genetic improvement of existing cultivars with specific genes is a reality, made possible because the potato has proved amenable to *Agrobacterium tumefaciens* Ti plasmid-mediated genetic transformation, although a major research effort is always required to isolate, clone and incorporate a desired gene into the Ti plasmid. The first such derived commercial cultivar was Monsanto's NewLeaf<sup>TM</sup> Russet Burbank with Bt (*Bacillus thuringiensis*) resistance to Colorado beetle, which was granted registration in the USA in 1995. NewLeaf Plus<sup>TM</sup>, with the addition of replicase-mediated resistance to PLRV, is set to follow this year. In

Europe, the first transgenic potato in agriculture is almost certain to be a Dutch amylose-free starch cultivar, as mentioned earlier. However, whether or not SCRI becomes actively involved in releasing genetically modified cultivars, as opposed to doing transgenic research, will probably be determined more by who owns the relevant patents and intellectual property than by what is biologically feasible and desirable.

In the meantime, three main thrusts to work on genetically modified potatoes can be discerned. 1) The molecular cloning of natural resistance genes and their transfer into well-adapted but susceptible cultivars is being pursued in a number of laboratories world-wide. 2) The search for novel forms of genetically-engineered resistance to pests and diseases constitutes a major effort at SCRI and elsewhere. SCRI virologists have demonstrated the effectiveness of coat protein-mediated resistance to PLRV and PMTV, and others have done the same for PVX and PVY. Genes coding for lytic enzymes from bacteria and insects are being evaluated at a number of laboratories world-wide as a way to achieve transgenic resistance to a

#### Further research and development required:

#### Anther (microspore) culture

 may prove better than inducer pollinations for dihaploid and monohaploid production of any genotype in large numbers for breeding and genetics at diploid and monoploid level

#### Disease and quality tests

 need ones that breeders can use on the few tubers available early in breeding programme but which truly reflect field and processing performance

#### Genetic (molecular) markers

• for marker-assisted-selection, tracking introgression, identifying sexual and somatic hybrids, fingerprinting new cultivars for identification, prerequisite of map-based cloning, assessing biodiversity in germplasm collections, choosing parents that complement one another genotypically

#### Micropropagation

 to increase rate of multiplication and hence availability of new cultivars, and controls for experiments - fairly routine but some recalcitrant genotypes

#### **Progeny testing**

• to identify best parents and crosses - very useful, but not yet available for all economically important traits e.g. blackleg and powdery scab

#### **Protoplast fusion**

• still has a place as a way to overcome barriers to sexual hybridisation with wild species and for limited chromosome transfer, but genotype-dependent, and barriers can be overcome by manipulating ploidy levels etc.

#### Transformation (Agrobacterium) and regeneration

• for production of transgenic potatoes - still some difficult cultivars - and there are limits to size of DNA in vector

#### **Table 2** Enabling technologies for potato improvement.

number of bacteria and fungi, and a whole range of novel strategies can be anticipated, along with increased understanding of the biochemical basis of host-pathogen interactions. However, it is too early to say which will be successful in providing high levels of durable resistance. 3) Transgenic approaches at SCRI and elsewhere have also provided new ways of understanding and manipulating carbohydrate metabolism aimed at developing genetically in-built resistance to low temperature sweetening caused by an accumulation of glucose and fructose. There are a number of encouraging examples where about a 50 per cent reduction in sugar content can be achieved through modulating single genes, but it appears that the more extensive reductions which are needed will require the concerted regulation of more than one gene. This in turn will require multiple transformation or appropriate new vectors (Howard Davies, personal communication).

It would appear that the genetic modification of existing and future cultivars will have a major place in agriculture, provided that the general public is satisfied by adequate scientific evidence that it is safe to release transgenic potatoes into the environment, and that it will be safe to eat their tubers. This will require the conventional skills of plant breeders to test and trial such material, as well as ensuring its agronomic suitability.

**Enabling technologies for potato improvement** The strategies and methods for achieving the breeding objectives outlined earlier, depend on the enabling technologies available, as well as the germplasm; and so will the rate of progress. The key technologies now being used, or under development, are listed in Table 2 and deserve further comment.

Many modern aspects of potato improvement have developed from the amenability of the potato to tissue culture techniques. These include the ability to infect many types of potato tissue with *Agrobacterium tume-faciens* for transformation, regeneration of plants from culture, rapid methods of micro-propagation, anther culture and protoplast fusion. However, some of these techniques are still genotype-dependent, and there is certainly scope for further increases in efficiency.

Rapid progress in potato breeding requires the correct choice of parents and crosses, and efficient selection procedures. At SCRI, the development, validation and use of seedling and tuber progeny tests for disease resistance and quality traits have proved of immense value in selecting the most promising progenies for further breeding at the earliest opportunity, as well as for using biometrical methods to study the inheritance of quantitative traits and to identify the best parents for future breeding. Such research has already led to the submission of three clones for National Listing within 6 years of crossing (Ann. Rep. 1996/97, 40-45). Likewise, the development of reliable tests on clones has enabled the most promising ones to be identified in the selected progenies, and multiplied for evaluation as potential new cultivars. The two most important traits for which we still require reliable progeny tests are blackleg and powdery scab resistance. This is because it has proved difficult so far to get symptom expression of these diseases under glasshouse conditions, and success in field trials is weather-dependent.

It can be seen in Table 2 that many applications are envisaged for molecular marker technology, including molecular-marker-assisted selection strategies for the introgression of desirable genes from wild species and for breeding at the diploid and tetraploid level. These strategies should avoid the problems associated with selection for the many economically important traits which are substantially modified by environmental factors or which can only be detected in special tests, for example, virus resistance. The proviso is that there has to be tight linkage between the markers and the desired (or undesired) genes, the ideal being markers within the desired genes. In the longer term, locating the genes underlying quantitative traits through their linkage to molecular markers, should have a big impact on determining breeding strategies and methods, because one will know the number of genes involved, their chromosomal locations, and the magnitudes and natures of their actions.

Concluding remarks Although this article has looked at the way ahead at SCRI, many of the objectives, strategies and methods are relevant to potato breeding world-wide. Hence, one further way ahead is likely to be increased participation in international collaboration on what is, after all, the fourth most important food crop in the world after wheat, maize and rice.

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## Genome bioinformatics at SCRI: engineering the datastream

D.F. Marshall & L. Cardle

One of the main tasks of the new Bioinformatics & Information Technology Research Unit (BITR) at SCRI is to provide the computational and data-handling infrastructure that is required to underpin plant genomics research. This essentially breaks down into three main areas.

- the development of efficient procedures for handling and validating data, in particular the large data volumes that modern genomics research can generate.
- the development of novel software tools for data analysis and visualisation.
- the development of an appropriate framework for storage and retrieval of data.

The data resources that are being generated at SCRI must also be continually reviewed, not only in a local context, but also in a national and international context to maximise the value of our data.

Modern PCR-based techniques for molecular marker genotyping and recent developments in sequencing technology have dramatically increased both the rate at which genotype and sequence data can be generated and the volumes of data that must be efficiently handled. The critical rate-limiting step in genotype and sequencing projects is now no longer the data generation phase but rather the rate at which the data can be captured, validated, fully analysed and finally placed in context. This means that we now need to develop new data handling and storage procedures.

One of the major factors we have to deal with is the fact that, though there are a wide range of software tools, many of them public domain, for molecular biology, often they are only available on certain hardware platforms or operating systems. For example, much of the software that is available for directly handling automated sequencing or genotyping is specific to the Apple Macintosh, whereas the majority of major analysis tools are available on Unix systems. Finally, the most important factor is that, no matter what software tools and hardware platforms we use along the way, the final results must be available for the research scientist to browse from his or her desktop computer.

Ideally, we would like to write all the software components used from scratch to provide a uniform software

platform. In many cases this may well be our ultimate goal. However, we need to take a more pragmatic approach in the shorter term. An ideal computer language to help implement this approach is Perl, which was originally developed on Unix systems, but is now available on Apple Macintoshes and PCs running Windows 95 or Windows NT. Larry Wall originally conceived Perl as a 'glue' language. It has superb text processing capabilities and can operate as a command language running external commands and programs. These features make it ideal for our purposes. We can automate our data handling by the use of Perl programs or 'scripts' to run one or more software tools and process the output from each for input into the next step in the process. It is also possible to write new modules entirely in Perl, which, despite the fact that it is an interpreted language, is remarkably fast and efficient for many sequence and genotype data handling tasks.

The need for such new approaches to data handling can best be illustrated by a couple of simple examples.

#### SSR development and genotyping

In the last few years at SCRI, we have developed over 300 barley simple sequence repeat (SSR) genetic markers and are continually adding to this total. The first stage in the process is the development of genomic libraries enriched at the pre-cloning stage for an appropriate microsatellite repeat, usually either (CA)<sub>n</sub> or (GA)<sub>n</sub>. This enrichment process maximises the rate at which SSR-containing clones can be extracted from the library. The next stage is to sequence SSR-containing clones in order to design suitable PCR primers that amplify a particular SSR sequence. It is at this stage that bioinformatics can begin to make an impact. If we are dealing with a single sequence, or even 10 sequences, it is a relatively straight forward matter to take each sequence in turn, identify the SSR sequence and, if possible, design suitable PCR primers for subsequent testing. However, if rather than 10 we have several hundred sequences to analyse, the situation is more complex. If we break the problem down into its component stages, we can identify steps at which we can improve the efficiency of the entire process by either implementing existing software tools or developing our own software.

Sequencing Currently, we sequence putative SSR-containing clones using an ABI 377 with supporting software running on an Apple Macintosh. The major task at the initial stage is to check that the sequence is of sufficient quality and to remove any sequence that has come from the cloning vector. We then need to transfer the sequence data, via our local area network, to a Sun Unix Workstation for further analysis.

SSR location The next step is to confirm that the clones containing an SSR sequence have sufficient flanking sequence on either side to design suitable PCR primers, to precisely locate the SSR repeat and then mask it to avoid spurious database matches.

Database searches We then need to take each SSR-containing sequence and compare it against our local database of existing sequences to ensure that it is unique. It is also sensible at this stage to compare the sequence against all publicly available sequences by BLAST searching against GenBank or other appropriate databases. This gives us the opportunity to identify any possible sequence matches and identify corresponding predicted function(s) associated with sequences flanking the SSR.

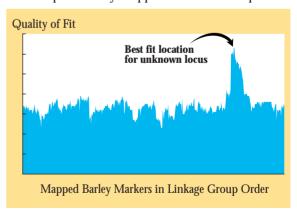
**Primer design** If the SSR-containing sequence is unique, we need to design appropriate PCR primers using the program Primer. This enables us to design primers with appropriate position and amplification conditions. Indeed, it is possible at this stage to automatically order the synthesis of the best primers by E-mail.

We have designed a series of Perl scripts that enable us to automate most of the analytical steps in this process. These scripts run each sequence through a series of analytical tools, pre-processing the input or postprocessing the output from each analysis. The remote database searching is also automated, with the results of each BLAST analysis being returned as formatted HTML files for subsequent analysis. We have also adapted this approach for the analysis of sequences from a number of Expressed Sequence Tag (EST) programs currently underway at SCRI. In this case, the primary aim of the analysis is to assign a function to each unknown cDNA sequence through database homology to characterised sequences. Since the number of characterised sequences in the major international databases is rapidly expanding, we need to repeat this process at regular intervals to find matches for still unknown sequences or to improve the quality of the matches and thereby our confidence in the assigned function. We are now processing several thousand EST sequences in this way from a range of crops, their pests and pathogens. We are also examining ways to handle the large body of information that is returned from these searches so that we can efficiently mine it for relevant information and build suitably structured indexes. We hope to be able to provide automatic notification of significant changes to the BLAST scores of individual sequences that result from each new pass of database searching.

#### Quick and dirty (QAD) mapping

As there are now good quality genetic maps in of all of the major crop species, we are frequently faced, not with the problem of how to generate a new genetic map from scratch, but rather how to efficiently add more loci onto existing saturated maps. Conventional linkage analyses, using *standard* programs such as JoinMap or Mapmaker, are relatively slow and inefficient for such purposes.

We have developed an alternative approach, Quick and Dirty (QAD) Mapping, which is based on a Perl module that uses simple pattern-matching to map each new SSR or other locus by comparison with the genotypes of existing mapped markers. For a saturated map the best matching locus gives us a good location for our unknown locus. This process is illustrated in Figure 1 which shows a pattern-matching scan of an unknown locus across the entire barley genome with markers in linkage group order. A clear peak in the 'Quality of fit' shows the location of the unknown locus. We are currently generalising this QAD mapping approach to a range of barley, potato, *Arabidopsis* and *Brassica* populations. The QAD tool can also be used to place newly mapped markers in map order



**Figure 1** A QAD mapping pattern matching scan of an unknown locus against the entire barley genome. The most likely map position of the unknown locus is at the 'quality of fit' peak.

within the existing raw data files. QAD mapping is particularly suitable for adaptation as a WWW-based mapping service linked to HTML forms controlled through a web browser. We are developing this aspect for use on our own intranet, but, in future, we hope to be able to offer this via our extranet Web-server as a service to rapidly map new loci in our target crops using public domain mapping populations and datasets.

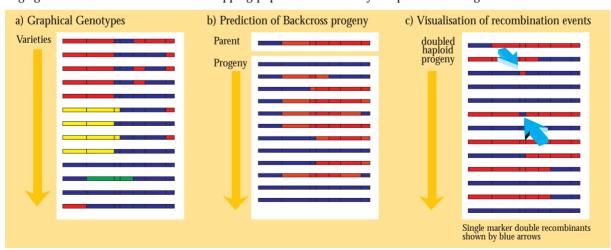
#### **Data validation**

One of the major concerns with respect to molecular linkage map data, is that given the extremely large number of individual data items, there is a significant chance of introducing errors. Such errors can arise at several steps in the data generation, data entry and data analysis process and may significantly distort or inflate the resulting genetic maps. A major challenge, therefore, is first of all to audit these processes to minimise the risk of data errors, and, secondly, to try and develop data validation processes which can serve to indicate data items that are, to some extent, inconsistent with the main volume of data. In the former case, we can take some simple steps to ensure that our procedures for naming lines and DNA samples are robust. We also must make sure that the location of samples at every stage of the PCR, gel and auto-radiography processes is robust and auditable. Even once we have established such procedures, we have to realise that there is an appreciable probability of data errors occurring. In the case of map data, it is possible to identify a number of factors which are at least indicative of poor quality data or data inconsistencies. Segregation distortion often occurs in mapping popu-

lations, especially those generated from wide crosses or involving doubled haploid populations in diploids. However, individual loci with high levels of segregation distortion, especially those that show significantly different patterns of distortion from adjacent loci, should be treated with suspicion. A second element, often associated with data errors, is the occurrence of what are known as single marker double recombinants. A mistaken genotype at a single locus can often generate two spurious recombination events in a very small genetic interval. However, since chiasma interference prevents two or more cross-over events occurring close together on the same chromosome, a pair of such recombination events is likely to occur with an extremely low probability in saturated genetic maps with high marker density. Therefore, we can treat such a data item with care and closely re-examine the original raw data. We are currently evaluating a range of algorithms to objectively gauge the quality of marker data sets and identify loci, or individuals, which are in tension with the bulk of the data, for subsequent reappraisal.

#### Graphical genotypes and data visualisation

Single-marker double-recombinants are one of a series of problems of handing genomic data where visualisation of the data, based on a *Graphical Genotype* of either a single chromosome or the entire chromosome complement of an individual, can play a considerable rôle in evaluating what is a complex array of data. We are developing a general Graphical Genotyping tool in the platform-independent language Java. This will enable us to use a visualisation approach to investigate not only the presence of single-marker double-recom-



**Figure 2** Three applications of the Graphical Genotypes interface in a) diversity analysis; b) simulation of the outcome of backcross conversion experiments; and c) the visualisation of errors in mapping data.

binants but also to evaluate diversity data (generated, for example, by the SSR analysis of a wide range of barley germplasm). We will also be able to monitor and predict the size of introgressed chromosomal tracts in directed backcrossing programmes to introduce disease resistance genes from exotic germplasm into advanced breeding lines. These graphical genotyping scenarios are illustrated in Figure 2. The combination of such a visual approach with the estimation of appropriate genetic parameters and/or the simulation of the outcomes of projected experiments, will provide a valuable 'Visual Genetics Workbench' to support a broad range of marker-enhanced breeding activities at SCRI.

#### Plant genomic databases

SCRI forms one node of the UK CropNet consortium of Plant Genome databases. Other members of UK CropNet include the John Innes Centre, Nottingham University and IGER, Aberystwyth. With funding from the BBSRC Plant and Animal Genome Analysis Initiative, CropNet has established a series of genome

databases for plant species relevant to UK agriculture. At SCRI, we have responsibility for establishing and curating databases for barley and potatoes. The first of these, BarleyDB, is now publicly available through the UKCropNet web site at http://synteny.nott.ac.uk/ (see Fig. 3). At the end of 1998 a much more extensive version of this database will be released. Development work is now also underway on the potato database, SPUDB, and we plan to have an initial version of this database available on the CropNet Webserver in early 1999. These database projects serve not only as a focus for handling our genome data locally but, increasingly, will provide a national resource for the storage, maintenance of and access to data from publicly funded work in these species. We are currently in discussion with groups in Europe and North America to build a coordinated international framework for plant genomic databases. Genomic databases are becoming an increasingly significant resource. It is crucially important that funding agencies provide the appropriate levels and continuity of funding for stable database development and data curation.

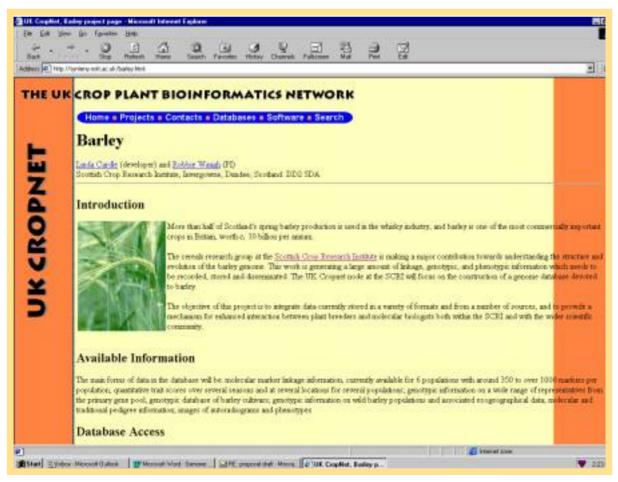
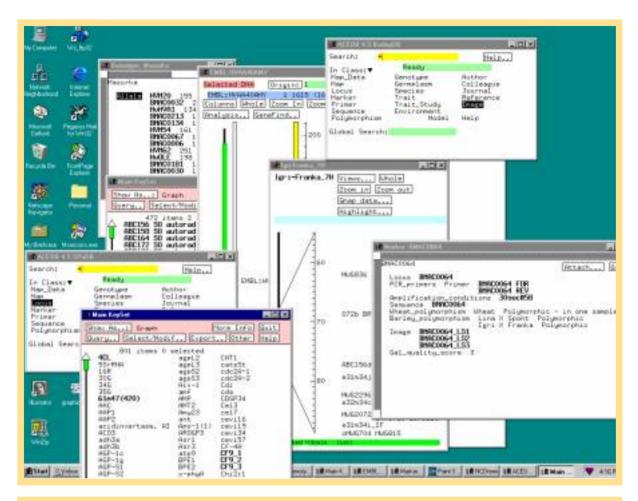


Figure 3 The SCRI Barley page on the UK CropNet Webserver.



**Figure 4** A Windows NT 4 Desktop showing a range of X-Windows open to both BarleyDB and SPUDB running on a SUN Unix Workstation.

A common theme which underpins most of our current software tool development is the need to provide a suitable common graphical-user interface (GUI) for laboratory and field scientists. The development of standard WorldWideWeb (WWW)-browser client software (e.g Netscape Navigator or Microsoft's Internet Explorer) for a range of hardware and operating system platforms provides us with a common environment with which most of our local users are familiar. By designing our software tools so that they can be readily accessed though WWW pages on our local intranet, we can fully exploit this familiarity. This can be achieved by a combination of HTML forms-based web pages with both client-side analytical and visualisation tools written as Java applets and server-side CGI programs on our Intranet web-server. Again, we can exploit the 'glue' language capabilities of Perl to link together otherwise disparate software components.

Overall, our aim is to provide scientists at SCRI with efficient computational tools to underpin their laboratory and field science. However, our design and implementation philosophy is a pragmatic one which gives a priority to ensuring that we can provide a series of integrated but unsophisticated tools which can then be developed in response to both user requirements and software developments. In the rapidly changing world of plant genomics, the development cycle of software tools needs to be fully integrated into that of the molecular biology.

## Development of markers for potato genetics and breeding

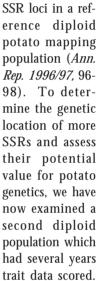
R. Waugh, A. Collins, D. Milbourne, L. Ramsay, R. Meyer, C.A. Hackett, J.E. Bradshaw, C. Gebhardt<sup>1</sup>, C. Chatot-Balandras<sup>2</sup>, E. Bonnel<sup>2</sup> & N. Bonar

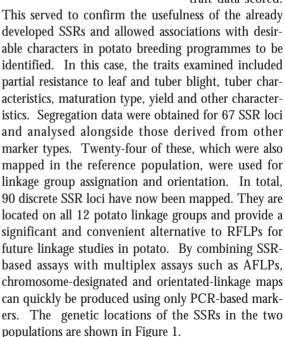
In practical terms, the adoption and more efficient application of DNA-based molecular markers in marker assisted selection (MAS) schemes, particularly by non-molecular biologists, would be increased if the markers exhibited a combination of features which

made them more user-friendly. Ideally, they should identify the same defined locus (or loci) every time they are used and they should be PCRbased, highly informative and evenly distributed throughout the genome. There presently two basic ways of

obtaining markers of this type. Either previously identified markers (RFLP probes, RAPDs, AFLPs etc.) are converted into such an assay (usually called CAPs), or a new set of markers, with all of these attributes, is developed *de novo*. If polymorphic, these could then be used in any population to monitor the inheritance of a specific chromosomal segment in crossing programmes or to evaluate the variation available in the genepool at that particular locus. If that chromosomal segment was associated with a trait, then the marker could be used to indirectly follow the inheritance of the trait. As the level of polymorphism associated with CAPs markers is usually low and the assay involves a post-PCR enzymatic cleavage step, we chose the de novo option by developing a class of markers from the potato genome which are known as microsatellites or simple sequence repeats (SSRs). SSRs are a tried and tested marker assay. They are the principal assay used in human and animal genetics, largely because they are abundant, and have a high information content. As such they have a high subsequent value for genetical analyses. Their adoption in plant genetics has been slow because, until recently, they have been difficult to characterise from plant genomes in sufficient numbers to make them really useful for the majority of potential applications.

Previously, we reported the genetic mapping of 47





Having constructed a linkage map, a quantitative trait locus (QTL) analysis was performed using the map

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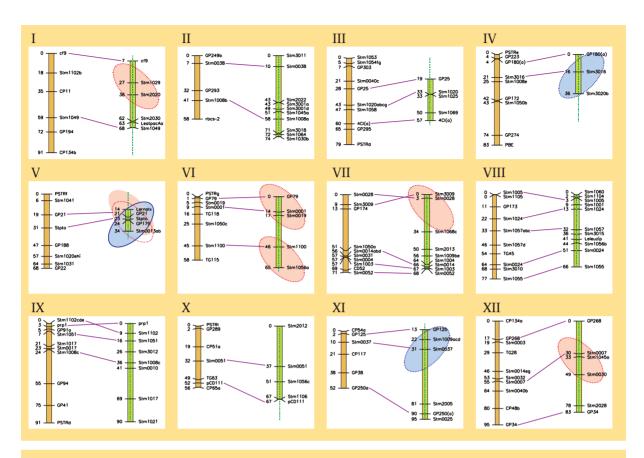


Figure 1 Location of SSRs on a genetic linkage map of potato. LG's I - XII are indicated. The maps on the left of each pair are the 'reference' population. Only a few of the >400 loci mapped on this population are shown. The markers designated PSTR are sub-telomeric repeats indicating the ends of linkage groups. The maps on the right show the position of the SSRs on the QTL mapping population. Only the SSRs (from >360 markers in total) are shown. Dotted lines show the extent of the maps of this population when all markers are included. Arrows indicate the position of the common loci mapped in both populations.

The regions of the genome associated with resistance to late blight in the QTL population are highlighted. Solid ellipses are QTL-detected in all seasons, dotted ellipses in a single season. Those originating from the male parent (susceptible) are in blue, and the female (resistant) parent in red.

and the assembled trait scores. Loci affecting resistance levels to leaf and tuber blight, earliness, plant vigour and other characters were detected on a number of linkage groups. The most significant QTLs for leaf blight were detected on linkage groups V and VI from both the male and female parents. The QTL from the male parent was significant over all three seasons in which data were collected. Interestingly, major QTLs for plant vigour and earliness were coincident with the major QTL (from the susceptible parent) for late blight on linkage group V. Informative SSR markers spanning these regions have been identified.

The major QTLs were at approximately the same position as those detected in previous studies on diploid populations<sup>1</sup>, suggesting a fairly robust association of these regions of the genome with quantitative resistance to late blight. The most significant QTL

for LB resistance is in the same region of the genome as the R1 resistance gene which is effective against pathotypes of *P. infestans* expressing avr1. The coincidence of QTLs for LB resistance with those for earliness and plant vigour may suggest that the mechanism of resistance in this population at the locus on LG V is a consequence of genes controlling physiological factors which affect the ability of the pathogen to develop a successful lesion. This conclusion is consistent with the well-known correlation between earliness and susceptibility to natural infection by late blight in the field. While this is potentially contributing to the overall quantitative resistance level of any given line, the data are also consistent with the presence of additional resistance factors (e.g. on the top of LG's IV, V and VI). Our studies on partial resistance to late blight at the tetraploid level (the ploidy level at

which the majority of potato breeding is practised) have not, to date, identified a QTL associated with resistance on chromosome V - even though the population exhibits a spectrum of maturity types. Rather, the major component of resistance is located on linkage group IV, in the same location as an environmentally sensitive QTL found here. In the tetraploid studies, the SSR markers have been particularly useful in identifying specific chromosomal segments and providing multi-allelic bridges between linkage groups assembled using mono-allelic single dose markers (AFLPs). This has allowed us to identify the location of components of H3<sub>(adg)</sub> derived horizontal resistance to *G. pallida* (Pa2/3) at the tetraploid level and

has provided a platform for developing strategies ultimately to clone the component genes.

Thus, SSR markers are a particularly attractive tool for examining allelic variation. They have a high probability of directly detecting polymorphism in any potato cross and, as such, can be used simply and effectively to evaluate allelic composition or haplotypes at mapped SSR loci. Their potential for use in MAS in diploid and tetraploid potatoes is currently being evaluated. The results so far look encouraging.

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## Blackcurrant breeding and genetics

R.M. Brennan, S.L. Gordon & P.G. Lanham

**Introduction** Blackcurrant (*Ribes nigrum* L.) is the most widely grown bush fruit in Europe, and is comparatively recent in its domestication from wild accessions.

The blackcurrant breeding programme at SCRI was established in the 1950s, with a remit to provide cultivars suitable for cultivation in northern parts of the UK. Initially, the work covered only the establishment in 1952 of variety trials for both black- and redcurrants, under the aegis of Malcolm Anderson, but results from the trials showed scope for considerable improvement within the existing genetic base, and breeding of blackcurrants was inaugurated in 1956.

The initial objectives of the programme were to produce improved germplasm with cold tolerance, especially in the spring, even and earlier ripening and fungal disease resistance. Initial hybridisations were made between established UK cultivars and cultivars from Canada, Scandinavia and northern Europe, together with a small interspecific programme, and the first commercialised product of the continuing breeding effort, 'Ben Lomond', was released in 1972.

The Industry The blackcurrant crop in the UK currently occupies ca. 1.4 kha. Most is grown on contract to SmithKline Beecham plc (SB) for the production of Ribena<sup>TM</sup>. In the lifetime of the breeding programme at SCRI, the scale of production has changed with an overall increase in farm size and complete mechanisation of all processes involved, including harvesting.



Figure 1 Blackcurrant hybridisations.

Since 1990, the breeding programme has been entirely funded by the SB Growers' R&D Fund, to produce cultivars meeting their processing and agronomic specifications.

Germplasm The *Ribes* genus consists of ca. 150 species, usually classified into 6 subgenera, distributed mainly in temperate areas of Europe and North America, although species are also found in South America and North Africa. All species are diploid, and an extensive species collection is held at the Institute, with new accessions added on a regular basis. Several species have been used to develop breeding strategies, such as the use of *Ribes dikuschka* and its derivatives for introgression of resistance to Blackcurrant Reversion-Associated Virus (BRAV)<sup>1</sup>.

Breeding of new commercially useful cultivars is based on recurrent selection from seedling populations at SCRI, with initial selections in the first (non-fruiting) year based on vegetative characters, followed by selection on fruiting characters and longer term agronomic traits. Trialling of the most promising seedlings is carried out at sites in Norfolk and Gloucestershire (Fig. 2) where commercial large-scale fruit assessments are made. Recommendations for release are made after 3 years' trialling.

**Breeding objectives** FROST TOLERANCE The objectives of the programme were initially to improve the frost tolerance of the available cultivars and thereby to produce consistency of cropping. This has been done

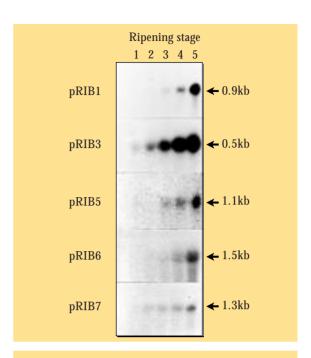


**Figure 2** Commercial blackcurrant trials in the West Midlands.

by introducing a late-flowering character into many of the SCRI cultivars, reaching its highest expression in Ben Tirran, released in 1990. Beyond this point, however, further delays in flowering are likely to compromise the eventual yield, and therefore genuine physiological tolerance of freezing temperatures is sought. Further introgression of germplasm from northern latitudes is in progress within the breeding programme to provide the required tolerance. The physiological basis of genotypic differences in frost tolerance, relating to rates of ice movement through tissues, is under investigation in collaboration with J. Carter of the University of Minnesota, and work to examine genotypic differences in the differential expression of cold-induced genes during acclimation in the autumn is in progress, using cDNA-AFLPs.

FRUIT QUALITY Of the various fruit quality components in *Ribes*, sensory attributes have hitherto been neglected in breeding terms, due to the complex nature of their inheritance and the uncertain origin of variation between genotypes. Recent collaborative work with the Hannah Research Institute and BioSS has demonstrated the range of genotypic variation in sensory characters, notably appearance, aroma, flavour, aftertaste and mouthfeel, in juices made from



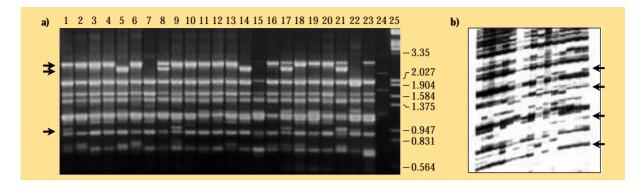


**Figure 3** RNA blot analysis of pRIB genes from black-currant fruit at five ripening stages (1= green, 2 = green/red, 3 = red/green, 4 = red, 5 = black), showing increasing expression levels as ripening progresses.

individual genotypes<sup>2</sup>. Flavour appeared to be the most important sensory attribute, and initial studies have suggested that some flavour characteristics are dominant in their inheritance. Overall, however, sensory attributes show highly complex patterns of inheritance and further work is in progress to examine this so that specific breeding strategies can be used.

Work with M. Woodhead and M. Taylor (CEP Dept.) showed that blackcurrant is a non-climacteric fruit in its ripening. Qualitative and quantitative changes in mRNA populations were found as ripening progressed, leading to the isolation of cDNA clones of five genes showing enhanced steady-state transcription levels in fully ripe fruit<sup>3</sup> (Fig. 3). Genomic clones of some of these genes have been isolated, and two of the genes (RIB1 and RIB7) showed highly fruit-specific expression. The promotors driving the expression of these two genes may therefore be of considerable value in the future manipulation of ripening processes in transgenic fruit.

Ascorbic acid levels represent a major part of the appeal of blackcurrant to consumers and the processing industry. Levels of ascorbate production in blackcurrant fruit are highly variable: most commercial UK cultivars such as Ben Alder contain typically 120 mg/100 ml juice, whereas most Scandinavian cultivars



**Figure 4** Molecular markers detected in *Ribes* germplasm
a) RAPD markers in *R. nigrum* germplasm
b) Anchored microsatellite markers in *Ribes* subgen. *Ribesia* germplasm, DNA polymorphisms are indicated by arrows.

are barely half that value. However, breeding lines within the SCRI programme containing up to 400 mg/100 ml have been identified, and are being used in combination with appropriate parents. Additionally, work to manipulate the biosynthetic pathways for ascorbate within fruit is in progress.

Recently, interest has been expressed by researchers and commerce in the role of antioxidants in a nutritional context. Blackcurrant is high in ascorbic acid, as already mentioned, but it also has a high concentration of other antioxidants, such as anthocyanins. Whilst breeding for high juice colour has long been an objective of the *Ribes* breeding programme, more recent information suggests that (i) the anthocyanins present in blackcurrant (mainly delphinidin- and cyanidin-3-glucosides and -rutinosides) have antioxidant capacities in their own right<sup>4</sup> and (ii) the anthocyanin content of blackcurrant is exceeded only by some genotypes of blueberry (*Vaccinium* spp.).

It remains a matter of concern that little information on the inheritance of fruit quality components or on the genotype-environment interactions affecting fruit quality is currently available for any of the berry fruits.

PEST RESISTANCE The development of new cultivars with resistance to gall mite (*Cecidophyopsis ribis*), and to BRAV which is transmitted by the mite, remains a high priority within the programme. Uncertainty regarding the future use of acaricides on toxicological and environmental grounds could potentially leave the industry without effective means of controlling this damaging pest. Resistance breeding for *C. ribis* is still based mainly on the dominant *Ce* gene from *Ribes grossularia*, and commercially acceptable types are now in commercial trials. Further screening of *Ribes* species to identify other sources of resistance is in

progress, although collaborative work with the Agricultural Research Centre, Jokioinen, Finland has demonstrated that *Ce* is effective against other *Cecidophyopsis* mites, as well as Finnish strains of *C. ribis*.

The identification of BRAV as the causal agent of blackcurrant reversion has made the development of a rapid detection assay possible<sup>5</sup>; this will then enable the mode of resistance to be accurately assessed by screening of graft-inoculated progenies segregating for resistance. Initial work produced unclear results, and the use of rapid assays will enable further work to be done and the range of BRAV-resistant cultivars available to the industry to be increased.

Marker-assisted selection The development of molecular markers affords considerable opportunities for breeders, particularly those working with highly heterozygous perennial crops, to both characterise the available germplasm at the DNA level, and to indirectly select for agronomic traits faster and more accurately than otherwise possible. In the specific context of the Ribes breeding programme, we have so far characterised the germplasm using RAPDs in the Eucoreosma subgenus<sup>6</sup> (Fig. 4a). AFLPs and ISSRs (anchored microsatellites) were used for the Grossularia and Ribesia subgenera<sup>7</sup> (Fig 4b). Work to develop AFLP markers for the gall mite resistance gene Ce is also well-advanced, with test progenies in field infestation plots providing the required susceptible and resistant bulks from which the analysis can be made. The use of a molecular marker for mite resistance will obviate the present 3-year screening of mature plants to identify resistant genotypes, and enable screening of seedlings to be carried out prior to planting.

Further use of markers to map important multigenic traits, such as fruit quality characteristics and freezing tolerance, as well as resistance genes including those controlling resistance to BRAV, is also under investigation using mapping populations established in the field.

**Transgenic blackcurrants** Protocols for the *Agrobacterium*-mediated transformation of blackcurrant have been optimised in collaboration with S. Millam (Crop Genetics Dept.), and plants transformed with a range of genes controlling various aspects of fruit quality are currently being assessed.

Cultivars The first cultivars from the SB-funded breeding programme are now in commerce; Ben Hope and Ben Gairn, both released in 1997, provide alternative strategies for pest and disease management, since Ben Hope demonstrates a high degree of resistance to gall mite, while Ben Gairn is resistant to BRAV. Another three, presently un-named, seedlings have been approved for propagation and release by SB, and a further 20 seedlings are currently in commercial trials with SB, including several gall miteresistant genotypes.

**Future advances** Blackcurrant breeding at SCRI is strongly and uniquely placed to make large advances in the future, as the conventional breeding is assisted by a range of enabling technologies.

It is envisaged that there will be further releases of gall mite-resistant cultivars, in order to provide the industry with a firm basis for successful integrated pest management in the future. Also, further BRAV-resistant seedlings will be developed as the means of identification of resistant segregants becomes easier.

Marker-assisted selection is being developed to identify desirable genotypes within the programme at a much earlier stage, particularly for characters that have hitherto required long selection periods. Also, there is likely to be increased use in the future of interspecific hybridisation to provide genes controlling desirable resistance and quality characters.

The use of molecular studies relating to fruit ripening and increased quality makes large improvements in fruit quality components, and hence commercial desirability, possible, especially through the use of transgenic methods.

#### Acknowledements

Work on fruit-specific promotors and ripening-related clones was carried out in collaboration with Mark Taylor, Mary Woodhead and Howard Davies of the Cellular and Environmental Physiology Department. Financial support from the SmithKline Beecham Blackcurrant Growers' R & D Fund (breeding programme), SB Consumer Healthcare and the Scottish Office Agriculture, Environment and Fisheries Department, including the Flexible Fund (sensory work), is gratefully acknowledged.

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## New swede cultivar

Virtue (SS17): Virtue was placed on the UK National List and granted Plant Breeders' Rights on 19 March 1997. It is an F6 family which was produced by single seed descent from a cross made in 1985 between a line from cv. Criffel and one from cv. Marian. Virtue was selected primarily as a shopping swede, but can be fed to livestock. It has an attractive purple skin, circular shape and yellow flesh, at a low to medium dry matter content (similar to cv. Marian). Its powdery mildew resistance is also similar to that of cv. Marian, but it has a higher dry matter yield. Virtue is being marketed by Sharpes International Seeds Ltd.



#### Howard V. Davies & William W. Christie

Biochemistry and phytochemistry infiltrate the SCRI's core and externally funded programmes, complementing and enriching the skill bases in breeding, pathology and molecular biology. One of the Institute's key objectives is to harness its multidisciplinary skills in focused programmes which evolve and adapt to meet the needs of the agricultural, horticultural, and industrial communities. Programmes on environmental monitoring and protection proceed in parallel to provide an holistic approach in our science strategy. Below, we present an overview of the programmes and achievements for the past year, which embrace the skills of the Institute's phytochemists, biochemists and molecular physiologists that work alongside them.

Genotype-environment interactions and potato glycoalkaloid accumulation High-performance liquid chromatographic (HPLC) methods have been utilised to study the effects of storage temperature on the glycoalkaloid content of potato (*Solanum tuberosum* L.) tubers. The results have indicated that low temperature storage (4°C) of tubers immediately post-harvest, results not only in a cultivar-dependant increase in tuber total glycoalkaloid content but also increases the rate of glycoalkaloid accumulation upon their subsequent exposure to light. Storage at a higher temperature (10°C) prior to their movement to low temperature stores, significantly reduced glycoalkaloid accumulation in the tubers stored continuously in the

dark. However, tubers stored at  $10^{\circ}\text{C}$  for a period of eight weeks followed by six weeks at  $4^{\circ}\text{C}$ , when exposed to light, accumulated significantly more glycoalkaloids than tubers of the same cultivars stored continually for 14 weeks at  $10^{\circ}\text{C}$ . The use of HPLC combined with mass spectrometry, has also been evaluated for the identification of individual glycoalkaloids. Conditions have been optimised for the production of molecular ions from both  $\alpha$ -solanine and  $\alpha$ -chaconine and these techniques have been applied to check the identity of glycoalkaloids detected in tubers of a number of *Solanum* species, closely related to the domesticated potato.

Novel approaches to plant lipid analysis Gas chromatography-mass spectrometry has been used to identify a number of interesting fatty acids in conifer seed oils. These include a branched-chain component, 14-methylhexadecanoic acid, which otherwise is found only in animal and microbial lipids.

The nature of the phospholipids and glycolipids in plant membranes have an impact on the physiological condition of the plant. Procedures have been developed to determine the detailed molecular species compositions of complex lipids. The polar head groups of phospholipids and glycolipids are removed by enzymatic and chemical procedures, respectively, to yield diacylglycerols. After conversion to nicotinate derivatives, the molecular species are separated according to the chain-length and degree of unsaturation of the acyl moieties by reversed-phase HPLC linked to mass spectrometry. The nicotinates have excellent mass spectrometric properties when examined both by particle-beam and atmospheric-pressure chemical ionisation (ACPI) interfaces, but the latter technique has proven more robust. The nature of the fatty acids on the glycerol backbone can be readily determined and reverse isomers (i.e. diacylglycerols with the same acyl groups but on different glycerol carbons) differentiated. Progress has also been made in analysing a variety of lipid classes (e.g. sterol esters, plant glycolipids) by normal-phase LC-MS (APCI, electrospray).

Leaf waxes and insect resistance As part of the Unit of Phytochemistry's studies of cuticular wax chemistry, techniques are being developed for determination of the spatial distribution of cuticular waxes over different parts of the plant. Differential extraction of

wax from upper (adaxial) and lower (abaxial) leaf surfaces and from stems of broccoli has revealed considerable differences in the composition of wax in these regions. Waxes are usually isolated by solvent extraction, but use of a water spray to fracture and detach individual wax crystals from leaf surfaces has been investigated. This appears to extract components of the upper crystalline regions of the wax differentially, leaving the

lower ones substantially intact. There are significant compositional differences between these extracts and this may be correlated with visible wax structures.

Work continued on the investigation of the chemistry of the leaf surface of kale, broccoli, potato, raspberry and blackcurrant. In a multiple-instrument approach, both volatile and non-volatile components have been investigated, and this necessitated the development of mass spectral data translation techniques to compare results from different instruments.

Free radicals, senescence and differentiation A component of the free radical research at SCRI is concentrating on plant senescence processes. Initial experiments have been devoted to the study of cereals, partly because of their great global importance, but also because of their architectural suitability for studies in vivo (which are part of the ultimate aim of this work) using the electron paramagnetic resonance (EPR) technique. Emphasis has been placed on senescence processes induced by biotic or abiotic stresses, and results show that changes in the chemical forms of iron and manganese, as well as the amounts and chemical natures of free radicals, are influenced by these stresses. This work provides a background for a more comprehensive study of the roles of free radicals and transition metal ions in different ageing processes. Over the next 3 years, this will be supplemented by an EU-funded project in which the roles of active oxygen species in plant pathogenesis will be investigated.

Also relevant to the free radical research programme is a collaboration project with researchers at the University of Abertay, Dundee. In this project, the aldehydic products of lipid peroxidation, malondi-

aldehyde and 4-hydroxynonenal, have been profiled
in callus cultures of *Daucus*carota. This is the first occasion that the latter has been
detected in plant tissues.
Clonal lines differing in
embryogenic potential have
shown different contents of
the two compounds, and
indeed different ratios. Both
the absolute contents and
the ratio have been shown to
be good indicators of
embryogenesis.

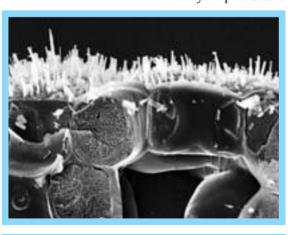


Figure 1 Leaf wax.

Stable isotopes technologies and applications

Continuous flow analysis of sulphur stable isotopes is now a routine analytical procedure within the stable isotope facility in the Chemistry Department. The methods described for sulphur-rich minerals (SCRI Ann. Rep. 1994, 97-100) have been extended to include a range of biological samples with sulphur contents between 0.1 and 1%. This is achieved by ensuring rapid and complete sample conversion by the addition of vanadium pentoxide to the samples, and allowing only pure sulphur dioxide to enter the mass spectrometer. Samples analysed successfully include invertebrates and plants from studies of both terrestrial and marine food webs. The timing of sulphur uptake and reallocation in wheat plants throughout the growing season has been studied using this analytical approach and sulphur sources with naturally different <sup>34</sup>S content. Experiments covering the growing season are best done with stable isotopes due to the short halflife and radiation hazard of <sup>35</sup>S. Rapid, automated stable isotope analysis makes such studies a practical proposition. An accompanying, fuller article outlines developments in the interpretation for  $\delta^{15}N$  in plants.

Plant fibres, plant cell walls and crops for industrial use Within the Unit of Plant Biochemistry, the activity of enzymes capable of oxidising and polymerising monolignols has been shown to be present in cell wallassociated proteins from the developing xylem of a range of taxonomically-diverse trees. This ubiquity of monolignol-specific oxidases indicates that they are required for lignin formation. Their rôle is not fully understood but the initial products of monolignol oxidation formed by these oxidases are not significantly different from those obtained by oxidation with peroxidase/hydrogen peroxide. This suggests that they do not make lignin with different sub-unit linkages. The coniferyl alcohol oxidase from Sitka spruce has been purified and amino-terminal sequence obtained. Electron paramagnetic resonance spectra of these oxidase-enriched extracts contain signals indicative of a free-radical and type II copper atoms. The free radical signal appears to be involved in the catalytic mechanism of the oxidase, as its intensity is diminished by addition of coniferyl alcohol and is re-established by reaction with oxygen. The free radical intermediate has properties that are different from those of other known free radical intermediates of plant enzymes. The involvement of a bound free radical intermediate may explain the usually broad substrate specificity of this oxidase.

Work has been initiated on the identification of xylotransferases involved in the biosynthesis of the major secondary cell wall polysaccharide, xylan, and their purification using protocols previously established at SCRI. Developing flax xylem has been identified as a good source of this enzyme, as it is present at much higher specific activity with lower contaminating glycosyl transferase activities than previously used model systems such as pea.

Many transgenic lines of tobacco with genetically modified lignins have been analysed to assess the effect of modification of gene expression on cell wall composition and structure. Transgenic plants studied have modified activities of cinnamyl Co-A reductase and feruloyl-5 hydroxylase (in addition to previously reported work with cinnamyl alcohol dehydrogenase). Modified expression of these enzymes has a distinct effect on the constitutive lignin formed. For example, levels of lignin cross-linking have been reduced which will facilitate easier pulping.

The studies on Reed Canary grass as a potential source of pulp for paper and biofuel, continue. The varieties



Figure 2 Reed Canary grass.

grown have all become well established, giving potential yields well in line with those of other EU partners. The analyses of the quality parameters being determined at SCRI for material for the 1996/97 harvest have been completed but the interpretation and prediction of the data has still to be carried out.

The Crops for Industrial Use project (FIBSTORE), designed to determine the effects of storage regimes on baled straws, has been completed. Straw from different regions of a large stack, with a range of moisture contents, has been analysed chemically, spectroscopically and for microbial spoilage. In summation, it has revealed that storage outdoors causes bales on the outside of stacks to rot. Biodegradation is due to the action of fungi and their removal of hemicellulose (also causing some damage to cellulose microfibres). Treatment with biocide affords some protection initially, but this effect is lost with time.

The use of novel bleaching agents for use in the pulp and paper industry has continued with the work on oxone now completed. This successfully bleached/delignified all of the sources of annual fibres examined with the exception of oilseed rape straw. Promising results have been obtained using the metal ion chelator tetraacetylethylenediamine (TAED) in combination with hydrogen peroxide.

Starch: genetic variability and genetic engineering Continuing the theme of crops for industrial use, a SOAEFD Flexibly Funded project has been aimed at identifying the processing potential of starches from barley, wheat, oat and potato varieties/cultivars grown under Scottish conditions. This has required detailed comparative assessments of starch structure and rheology. Part of the programme has investigated barley starches with different genetic backgrounds, especially waxy and high-amylose types. With potatoes, the variation in composition and properties has been shown to be high. Variation between variety/cultivar appears to be greater than between growing sites. For example, a two-fold difference in phosphate ester content between varieties/cultivars is possible while even larger differences in viscosity parameters occur.

Complementing the above, a detailed analysis of transgenic potatoes expressing, ectopically, a range of genes with the potential for modifying starch structure, is well underway. One gene previously isolated as an  $\alpha$ -glucosidase by yeast complementation, has been identified as a plant equivalent of glucosidase II, an enzyme essential for glycoprotein processing in

eukaryotes. This is the first time an enzyme of this class has been identified in higher plants. As in mammals, the enzyme is enriched in the microsomal fraction of non-transgenic plants. Transgenic plants with the gene down-regulated show an extremely stunted phenotype. More recently, an additional  $\alpha$ -glucosidase gene has been cloned which is more similar to those believed to be involved in primary carbohydrate metabolism.

Also relevant to the future manipulation of starch synthesis and structure is the elucidation of the pathway of (floridian) starch biosynthesis in the red alga *Gracilaria tenuistipitata*. Unlike in higher plants and green algae, the elongation of polymeric  $\alpha$ -1,4 glucan chains occurs via a unique glucosyltransferase specific for UDPglucose. This novel UDPglucose-starch synthase has been partially purified and characterised and a putative N-terminal amino acid sequence obtained. A novel HPLC-based method for the assay of starch synthase has also been developed during the course of this work. The method is highly sensitive and does not require the use of radiolabelled substrates.

Developmental processes: biochemical, molecular and NMR imaging approaches Other aspects of carbohydrate research are relevant to developmental processes such as dormancy break and sprout growth in potato tubers (EU-funded programme). It has been established that initial bud growth is sustained by the mobilisation of soluble compounds from the tuber. This phase is followed by the onset of active starch degradation in the tuber which corresponds with the expression of a new  $\beta$ -amylase isoform and a large increase of soluble sugars. Sprout removal induces very rapid changes (within hours) in carbohydrate metabolism in the tuber, causing an induction of gly-



Figure 3 NMR of a blackcurrant.

colytic metabolism and starch biosynthesis. These changes occur prior to any quantifiable change in tuber sugar content and indicate the existence of a highly co-ordinated sink-source relationship between tuber and sprouts.

Within the Unit of Plant Biochemistry, several soft fruit biotechnology programmes have been initiated this year and funding has been secured from a widerange of sources including growers associations, industry, SOAEFD and the EU. Genes involved in fruit ripening have been isolated from strawberry and raspberry and already novel targets for the transgenic phases of these programmes have been identified. Efforts to understand the changes in carbohydrate biochemistry that occur during strawberry fruit ripening are also underway and a major programme designed to improve ascorbate levels in blackcurrant fruit has been established. An overview of progress on the chemistry, biochemistry and molecular biology of raspberry fruit ripening is provided in an accompanying review article.

An NMR-imaging, developmental study of blackcurrant fruits from flower to mature fruit has been completed. NMR images were corroborated by low temperature SEM and resin section light microscopy and included a 3-D time course of a fruit still attached to the plant. Gradient echo images revealed the 3-dimensional structure of gelatinous sheaths around the seeds and their vascular traces in over-ripe fruits. The seemingly paradoxical change with maturity in image contrast of the vascular bundles, was ascribed to changes in cell sizes and intercellular gas spaces around the vascular bundles which affected the magnetic susceptibility homogeneity.

In collaboration with the Germplasm Conservation Department at RBG Kew, the 3-D distribution of mobile protons and 2-D water and lipid distributions in the West African seed *Vitellaria paradoxa* have been monitored during dehydration and subsequent rehydration. The mobile water content never regains its initial level and its distribution is more amorphous. The gross water and lipid NMR signal intensities appear to correlate with differential scanning calorimetry measurements of water mobility.

NMR imaging has also been used to investigate water imbibition by different cultivars of malting and malted barley. This work was carried out in collaboration with the International Centre for Brewing and Distilling at Heriot-Watt University and preliminary studies have indicated that there are differences in the rate of water uptake and the distribution of water in the endosperm between samples of 'good' and 'poor' malting barley and malt grains. Also using seeds, a comparison has been undertaken of germinating Vicia faba seed at two different magnetic field strengths (0.47T at Wageningen NMR Centre and 7.1T at SCRI). At high field, the best contrast, which revealed internal structure, was obtained using an unweighted spin echo sequence; little discrimination was obtained at low field under the same conditions. Good visualisation of the same features was obtained from low field longitudinal relaxation rate (1/T<sub>1</sub>) images, although not as dramatic as in the high field images. The greater contrast in the spin echo images at high field is probably due to decrease in signal from the endosperm as a consequence of chemical exchange of protons between water and starch. This signal attenuation will be much less prominent at low fields, giving rise to a higher overall signal and less discrimination between the tissue types.

# Multidisciplinary approaches and the improvement of fruit quality in red raspberry (*Rubus idaeus* L.)

P.P.M. Iannetta, C. Jones, D. Stewart, M.A. Taylor, R.J. McNicol & H.V. Davies

Introduction The UK is a net importer of raspberries, mainly due to a restricted growing season and limited fruit shelf-life caused by natural softening and decay processes. These are often exacerbated by the ingress of diseases such as grey mould (*Botrytis cinerea*). Despite these limitations, consumers continue to demand high quality, long shelf-life fruit which is competitively priced. The soft fruit processing sector also encounters problems due to the high levels of soluble cell-wall polysaccharides and phenolics in juice and concentrate. The clarification and filtration processes required to resolve these issues are particularly expensive for products derived from raspberry fruit. The issues of improved fruit quality and storage characteristics are therefore central to the continued success of the raspberry industry.

Tomato has been a 'model' species for studies on fruit ripening related processes for many years. This reflects both its economic importance, its readily dissectible inheritance characteristics and, latterly, the ease with which it can be genetically manipulated to target the improvement of key traits using modern

biotechnological approaches. Research andripening quality of soft fruit, such as strawberry, is gaining momentum but with other, relatively minor crops, such as raspberry, our understanding of mechanisms is rather poor. Given SCRI's successful history of rasp-

berry breeding and the potential for adding value to fruit using modern scientific approaches and technologies, renewed emphasis on this crop is justified.

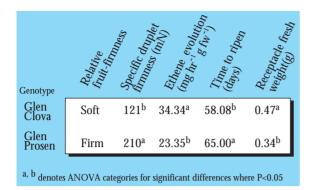
Within the Unit of Plant Biochemistry, the research effort is geared toward an understanding of the physicochemical, biochemical and molecular mechanisms underpinning the natural ripening of raspberry fruit. This will facilitate the identification of targets for fruit improvement, utilising biotechnology and traditional breeding-based approaches in a synergistic manner. The research approach encompasses the expertise of biochemists, cell wall chemists and molecular biologists, delivering high quality science which is of direct relevance to the raspberry industry.

Biochemical and molecular analysis of soft fruits such as raspberries, strawberries and blackcurrants, is intrinsically problematic. This is due to the naturally high levels of polyphenols and soluble polysaccharides and low protein levels. Despite these problems, we have developed an armoury of techniques which have been used effectively to identify numerous targets for raspberry breeders and genetic engineers. Furthermore, and in conjunction with the more fundamental aspects of raspberry ripening processes, the

retardation of post-harvest spoilage and decay has been studied using modern packaging technology, specifically modified gas atmospheres and packaging films with modified permeability characteristics. Expansion of work in this area will provide



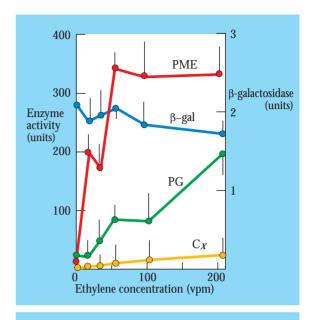
benefits in the short-term, while the longer-term approaches involving gene transfer technology are established.



**Table 1** Relationships between genotype, rate of ripening, ethylene evolution, fruit firmness and receptacle fresh weight. Values relate to fruit parameters quantified from red-ripe fruit. Increased rates of ethylene evolution are found in softer fruit which ripen faster and have heavier receptacles than firmer fruit.

Physiology and biochemistry of raspberry fruit ripening The raspberry fruit undergoes dramatic changes in firmness during ripening, particularly during the later stages (Table 1). The rate of ripening and degree of firmness maintained in ripened fruit is genotype-dependant. For experimental, comparative purposes, two SCRI raspberry varieties, Glen Prosen and Glen Clova, have been examined in detail. Fruit of these varieties have been classified, subjectively, as firm and soft, respectively, when ripe.

Changes in fruit firmness were found to be directly related to the rate of ripening and the rate of production of the gaseous plant growth regulator, ethylene, which exerts a strong influence on ripening processes in many fruit (e.g. melon, tomato). A correlation between the rate of ethylene evolution and the size of the raspberry receptacle (plug) has been established. Furthermore, exposure of green fruit to exogenous ethylene stimulates colour (anthocyanin) development, accelerates fruit softening and enhances the activities of cell-wall modifying enzymes (Fig. 1).



**Figure 1** Effect of ethylene on the activities of cell wall hydrolases. Ethylene applied to green fruit, enzymes measured after 48h exposure. Bars indicate S.E.M.s.

Therefore, ethylene plays an important role in the ripening of raspberry fruit.

The natural ripening of raspberry fruit is also associated with changes in the activities of cell wall modifying enzymes. The activities of polygalacturonase (PG), pectin methylesterase (PME), cellulase (Cx; endo- $\beta$ -1,4-glucanase) and  $\beta$ -galactosidase ( $\beta$ -gal) increase during ripening and appear to contribute to the soft fruit character of ripe raspberries (Fig. 2). The data suggests that PME may be important in determining the onset of softening whilst in the later, and post-harvest stages, PG and Cx activities appear to play more major roles in regulating firmness and texture. As described later in this article, molecular approaches are confirming hypotheses generated from the comparative biochemistry.

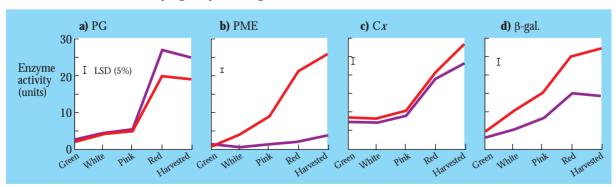


Figure 2 Activities of PG (a), PME (b), C<sub>X</sub>(c) and β-gal (d) in ripening druplets of Glen Prosen (red) and Glen Clova (purple).

Glen Prosen	Ara	Xyl	Man	Gal	Glc	UA	%Me
Green	2.9 (0.1)	24.1 (0.3)	0.7 (0.1)	2.7 (0.1)	1.3 (0.1)	28.0 (1.3)	41 (6)
White	3.0 (0.1)	24.0 (0.2)	0.6 (0.1)	2.7 (0.1)	1.4 (0.2)	22.0 (1.1)	35 (4)
Red	2.1 (0.2)	24.6 (0.3)	0.6 (0.1)	2.1 (0.2)	1.4 (0.1)	9.7 (0.8)	14 (3)
Glen Clova							
Green	2.2 (0.1)	23.2 (1.0)	0.5 (0.1)	1.7 (0.3)	0.6 (0.2)	26.8 (1.4)	39 (5)
White	2.2 (0.1)	23.5 (0.8)	0.5 (0.1)	1.2 (0.2)	0.7 (0.2)	21.6 (1.5)	31 (6)
Red	2.1 (0.1)	26.6 (0.4)	0.4 (0.1)	1.1 (0.1)	0.5 (0.1)	5.5 (1.1)	7 (4)

Figures in parenthesis are the standard errors. Non-cellulosic neutral monosaccharide and uronic acid contents are expressed as mg/100mg cell wall.

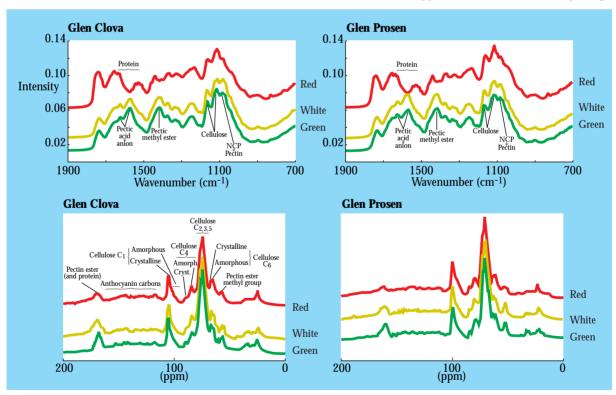
The non-cellulosic neutral monosaccharide contents are the mean of triplicates whilst the uronic acid contents and % methyl esterification are the mean of five replicates.

**Table 2** The neutral sugar, uronic acid and methyl esterification content of Glen Prosen and Glen Clova raspberries at the green, white and red stages.

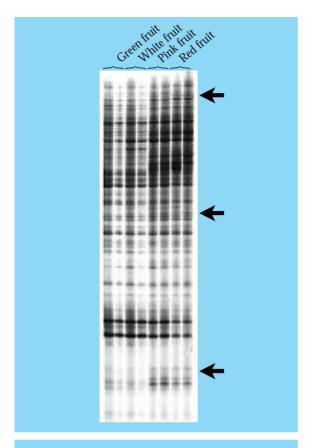
The cell wall chemistry of ripening fruit Physicochemical analysis of isolated fruit cell walls has also produced a broad agreement with results of biochemical studies. The most significant changes accompany the progression of fruit from yellow to red ripening stages. A large reduction in the cell wall constituents, uronic acid and associated methyl ester, account for the majority of these changes. There are also small but significant reductions in the levels of residual arabinose and galactose (Table 2). These are derived from pectic arabinogalactans. This agrees

with the extensive pectin solubilisation and demethylation reported for other fruit and concurs with the elevated activities of enzymes such as PG and PME during raspberry ripening.

This change in pectin structure is reflected in both the FT-IR and NMR spectra (Fig. 3). Both genotypes, Glen Clova and Glen Prosen, show similar reductions in the FT-IR absorbance at ~1660-1600 and 1440 cm<sup>-1</sup>, the regions associated with pectic acid/anion and ester, respectively. In the NMR spectra, the ester resonance at 172 ppm is reduced in the red-ripe stage



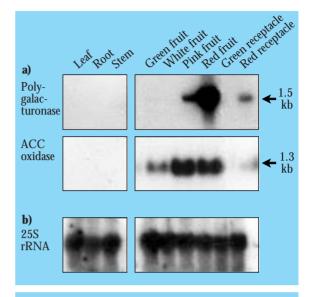
**Figure 3** The DRIFT (upper) and solid-state NMR (lower) spectra of Glen Clova and Glen Prosen cell walls showing reductions in pectin methyl ester and cellulose crystallinity and increased protein accretion accompanying ripening.



**Figure 4** Differential display gel. Arrows indicate genes up-regulated in the ripening raspberry fruit.

but not as much as the measured level of methyl ester content would suggest. This is due to the presence of increased level of protein in the red-ripe stages. The protein (amide) carbonyl resonance (175 ppm) is close to that of the methyl ester (172 ppm), producing an apparently inconsistent reduction in the latter.

Definitive evidence for increased protein levels is seen in the FT-IR spectra. At the red-ripe stage, both genotypes have significant absorbances at 1650 and 1550 cm<sup>-1</sup>, corresponding to the protein C=O and N-H absorbances respectively. These may be due to interstitial proteins, such as expansins, or to adsorbed cell wall hydrolases, such as cellulase. Cellulase levels increase enormously during the latter stages of ripening and the spectra show definitive evidence of cellulose breakdown. In the NMR spectra, the principal cellulose C<sub>2,3,5</sub> resonances, at ~73 ppm, have collapsed to a single peak in the red-ripe stage, especially in Glen Clova. Also, there are net increases in the amorphous cellulose resonances. Significantly, the cellulose absorbances in the FT-IR spectra are greater and sharper in the spectrum of Glen Prosen. This suggests that macromolecular cellulose breakdown has



**Figure 5** a) RNA blot analysis of two differentially expressed genes from raspberry fruit - polygalacturonase and ACC oxidase. Expression is shown to be upregulated in the ripening fruit and receptacle (or plug from red fruit. b) To ensure even loading and transfer of RNA, the membrane was re-hybridised with a 25S ribosomal probe.

been more extensive in Glen Clova, the genotype classified as producing softer fruit.

Molecular approaches to raspberry ripening Studies of the molecular aspects of raspberry ripening have been initiated, with the ultimate aim of improving raspberry fruit quality via a transgenic approach. Initially, technical problems in the isolation of pure, intact messenger RNA (mRNA) from raspberry fruit had to be overcome. Raspberry fruit extracts are strongly acidic and contain high levels of RNases and polysaccharides. Nevertheless, new methods were developed to overcome these obstacles, enabling the construction of good quality cDNA libraries. Our approach is to isolate genes, up-regulated in ripening fruit, as these are good candidates for having important rôles in this complex.

Three techniques for isolating differentially-expressed genes have been applied to raspberry fruit. As well as conventional plus-minus screening, two RNA finger-printing techniques have been employed. These PCR-based methodologies enable the rapid analysis of many transcribed genes (Fig. 4). Using these techniques, we have isolated clones representing over 30 genes that are differentially expressed during raspberry fruit ripening. Expression of two of these genes is shown in Figure 5. In agreement with the parallel

biochemical and physicochemical studies, some of these encode for enzymes that are involved in cell wall degradation. Genes isolated include those encoding polygalacturonase (PG; Fig. 5) and pectin methylesterase (PME). In the fruit of other species, most notably tomato, these activities have been down-

regulated using antisense and cosuppression technology in transgenic plants. This has led to the production of fruit with improved shelf-life and processing characteristics. Another gene that has been isolated from raspberry is that encoding a putative ethylene-forming enzyme, 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase (Fig. 5). As has been shown biochemically, ethylene is an important trigger for ripening processes in raspberry. With fruit of several species (for example melon and tomato), down-regulation of ACC oxidase can delay ripening significantly, resulting in a longer shelf-life. Genes we have isolated that encode for PG. PME and ACC oxidase are clearly now high priority targets for genetic manipulation in raspberry fruit.

The functions of some of the other genes isolated from ripening fruit are less obvious at present, but their study may provide an insight into the complex matrix of processes that constitute ripening. Genes that fall into this category include those encoding a latex-like protein, similar to the latex of opium poppy, which seals wound sites and stores secondary metabolites. Similar sequences are expressed at high levels in the ripening fruit of melon and pepper, suggesting a common ripening-related function, which may protect the ripe fruit against attack by pathogens.

Another example is a gene encoding a metallothionein-like protein. Similar genes are expressed in the fruit of many species, including kiwi, papaya, apple, blackcurrant and banana. Again, a common ripening-related function is implied. Metallothioneins are heavy-metal binding proteins, responsible for metal ion homeostasis and implicated in protection against oxidative stress. We are currently characterising the raspberry-fruit metallothionein at the biochemical level.

Our gene isolation programme has also provided us with the tools to isolate raspberry fruitspecific promoters which will be necessary to regulate the expression of genes in a tissue- and temporal-specific manner. The production of transgenic fruit

with improved quality, storage and processing characteristics is now the immediate goal.

#### Acknowledgements

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## Determination of the stuctures of fatty acids

W.W. Christie, G. Dobson & G.W. Robertson

atty acids are the basic building blocks of all lipids. The text books state that the common fatty acids of animal and plant origin consist of even-numbered linear chains of 16 to 22 carbon atoms, with zero to six double bonds of the cis configuration; polyunsaturated fatty acids have methylene-interrupted double

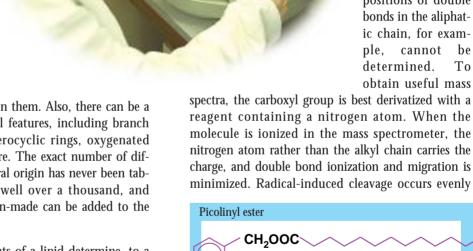
bond systems in general. However, there are countless exceptions, especially in the plant kingdom. Fatty acids can be both oddand even-numbered, with two to almost a hundred carbon atoms. Double bonds can have either cis or trans geometry, and acetylenic allenic bonds occur; these can be part of a conjugated system of unsaturation or there can be several

methylene groups between them. Also, there can be a host of further structural features, including branch points, alicyclic or heterocyclic rings, oxygenated functions, and many more. The exact number of different fatty acids of natural origin has never been tabulated, but it must be well over a thousand, and innumerable that are man-made can be added to the list.

The fatty acid components of a lipid determine, to a large extent, its physical and often its biological properties. It must be assumed that nature does not behave randomly and must synthesise each of the distinctive fatty acids that may occur in organisms for good biological reasons. In addition, fatty acids formed as byproducts in industrial or related processes may have biological effects on consumers. Therefore, it is important that we have rapid unequivocal methods for determination of fatty acid structure. When pure components can be isolated for study, a host of chemical degradative and spectroscopic methods are available. The real challenge is to identify minor components unequivocally among complex mixtures. Then, gas chromatography-mass spectrometry comes to the fore, and this, together with suitable derivatization methods and ancillary chromatographic techniques, has greatly simplified a complex task. The topic has

been reviewed comprehensively elsewhere by authors. 1,2

Fatty acids are usually analysed by gas chromatography as methyl esters, but the mass spectra of such derivatives rarely contain ions indicative of structural features; the positions of double bonds in the aliphatic chain, for examcannot ple, determined. obtain useful mass



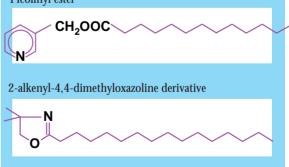
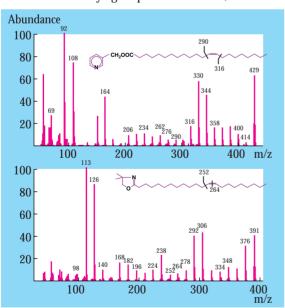


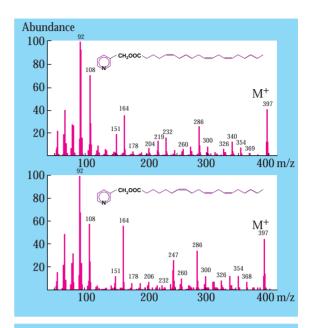
Figure 1 Chemical structures of picolinyl esters and 4,4dimethyloxazoline (DMOX) derivatives.

along the chain and gives a series of relatively abundant ions of high mass from the cleavage of each carbon-carbon bond. When a double bond or other functional group is reached, diagnostic ions tend to occur. Most analysts now prefer either picolinyl (3-hydroxymethylpyridinyl) ester or 4,4-dimethyloxazoline (DMOX) derivatives (Fig. 1). Both have their merits in mass spectrometry terms, and each has advantages for particular types of fatty acid; they are best considered as complementary. In our experience, picolinyl esters are by far the best for branched-chain and cyclopropane fatty acids, while DMOX derivatives have advantages for conjugated double bonds and other cyclic fatty acids. In most other applications, they are similar.

As an example, the mass spectra of the picolinyl ester and DMOX derivatives of erucic (13-docosenoic) acid, a major component of rapeseed and other brassica seed oils, is illustrated in Figure 2. That of the picolinyl ester is typical in that it has prominent ions at m/z = 92, 108, 151 (the McLafferty ion) and 164, which are all fragments about the pyridine ring. The molecular ion (m/z = 429) is easily distinguished and it is always odd-numbered, because of the presence of the nitrogen atom, but most other ions are even numbered. In interpreting such spectra, the simplest approach is to start with the molecular ion and progress downwards, as if one were unzipping the molecule one methylene group at a time. Thus, there is loss of a methyl group to m/z = 414, a further



**Figure 2** Mass spectra of picolinyl ester (upper) and dimethyloxazoline derivative (lower) of erucic acid.

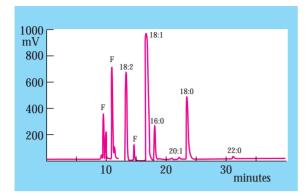


**Figure 3** Mass spectra of picolinyl ester of 5,11,14-eicosatrienoic acid (upper) and of 7,11,14-eicosatrienoic acid (lower).

methylene to m/z = 400, and so forth. When the double bond is reached, there is a gap of 26 amu between ions at m/z = 290 and 316, that, amongst other features, serves to locate the double bond.

The mass spectrum of the DMOX derivative of erucic acid and other fatty acids invariably has prominent ions at m/z = 113 and 126, the former representing cleavage between carbons 2 and 3 (the McLafferty ion). In this instance, the molecular ion is at m/z = 391. There are many similar features to the spectrum of the picolinyl esters, but in this instance the double bond is located by a gap of 12 amu between ions at m/z = 252 and 264 (carbons 12 and 13).

The efficacy of this methodology with closely related isomers is illustrated by Figure 3, which contains mass spectra of picolinyl ester derivatives of 5,11,14- and 7,11,14-eicosatrienoic acids, common components of Gymnosperm seed lipids (*Pinus contortus* in this instance). Both have the molecular ion at m/z = 397), and the spectra are virtually identical in the region from m/z = 260 to 397, where there are diagnostic features for the double bonds in positions 11 and 14. However, the ions at m/z = 219 and 232 in the mass spectrum of the 5,11,14-isomer help to locate the double bond in position 5, while in that of the 7,11,14-isomer, the distinctive ion at m/z = 247 points to a double bond in position 7 (odd-numbered ions are unusual).



**Figure 4** Separation of picolinyl ester derivatives prepared from the seed oil of *Dichapetalum toxicarium* by reversed-phase high-performance liquid chromatography. F = a fluorinated fatty acid.

When there is dubiety in the interpretation of a mass spectrum, the problem can usually be resolved by deuteration with Wilkinson's catalyst. With this method, deuterium adds to the double bonds in a simple way and is easily located by mass spectrometry. However, it is rarely possible to carry out this reaction with complex natural mixtures of fatty acids. Either the component of interest must be isolated in a pure state or in a simpler fraction in which other components do not interfere. We have developed two procedures for this purpose, silver-ion and reversed-phase high-performance liquid chromatography. The former is usually used with methyl ester derivatives and gives fractions which can differ in the position, geometry and number of double bonds in the molecules. Such methodology has proved extremely useful for the characterization of cyclic fatty acids formed when vegetable oils are heated to high temperatures, as in frying foods, for example.<sup>3</sup>

Reversed-phase HPLC is a mild method in that it is carried out at ambient temperature and involves only liquid-liquid interactions. With fatty acid derivatives, separation is based both on the chain-length and degree of unsaturation of components, each double bond reducing the retention time by the equivalent of about two methylene groups. Initially, it proved difficult to adapt the technique to picolinyl esters and DMOX derivatives, because of the basic nature of the molecules which resulted in tailing and poor resolution. However, new deactivated stationary phases of the ODS type are now available that permit elution of basic compounds as sharp peaks without addition of ionic species to the mobile phase. Figure 4 illustrates a separation of picolinyl ester derivatives prepared from the seed oil of *Dichapetalum toxicarium*, which is unusual in that it contains some fatty acids with fluorine atoms in the terminal methyl group. A column of Hichrom RPB<sup>TM</sup> was utilized with acetonitrile as the mobile phase and a flow gradient of 0.5 to 1.5 ml/min. It was then a relatively simple matter to collect the fractions of interest for further characterization, including gas chromatography-mass spectrometry.

We now have a battery of techniques available to us that permits determination of most fatty acid structures with relative ease.

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## Host pathogen interactions and crop protection

#### James M. Duncan, Peter F. Palukaitis & David L. Trudgill

The resistance of certain plants to bacterial and fungal diseases can be pre-formed, as anti-microbial compounds stored in healthy tissue, or as impenetrable barriers of dead or modified cells. However, much more important is resistance in which the host reacts to infection and metabolic changes create an environment within the plant that is unsuitable for the pathogen. Such active resistance is initiated by host recognition of attack by the pathogen. Central to host-pathogen recognition, especially with biotrophs, is the gene-for-gene concept which remains largely intact despite many years of argument between proponents of vertical and horizontal resistance. In any case, the distinction between the two has become slightly blurred as horizontal resistance in a number of plants has been mapped to major quantitative trait loci (QTLs) and may be at most oligogenic rather than polygenic. Moreover, both forms of resistance often involve activation of many of the same metabolic pathways within the plant, e.g. the synthesis of pathogenesis-related proteins and phytoalexins.

The increasing characterisation of sequences of resistance genes from the host, and virulence genes from the pathogen and their products, will result, hopefully, in an improved understanding of how the recognition mechanism initiates the cascade of events which eventually is manifest as a resistant reaction. However, although many genes and metabolites from the subsequent cascade have been characterised, many others remain undiscovered. A new research project (RO 494) designed to isolate such genes from potato infected by *Phytophthora infestans* (late blight) and *Erwinia carotovora atroseptica* (blackleg), was initiated in 1997. Already, using cDNA-AFLPs and new PCR-

based cDNA subtraction techniques, a number of genes not previously reported from plants have been isolated. Some of these, *e.g.* serine palmitoyltransferase, which is implicated in signalling the onset of programmed cell death or apoptosis in mammalian cells, are only up-regulated in the earliest stages of an hypersensitive response to infection with *P. infestans*. In parallel work, the same techniques are being used to isolate genes specifically activated within a pathogen *e.g.* genes produced by *P. fragariae* var. *fragariae* but not by the closely related var. *rubi*.

Activation of genes in diseased potatoes results in the production of a whole battery of proteins and other

## Host pathogen interactions & crop protection

compounds not found in healthy plants or present in much smaller amounts. Among them are phytoalexins, low molecular weight anti-microbial compounds implicated in slowing or halting the progress of pathogens through host tissue. Dr Gary Lyon presents his unparalleled knowledge of this area of potato metabolism in an article elsewhere in this report (see p.118). Uniquely, he has distilled everything into a comprehensive metabolic map or poster which is freely available on the World Wide Web.

Elsewhere within FBPP, the effort on molecular diagnostics continues. The lessons learned from the highly successful HDC-supported programme on Phytophthora diagnostics are now being applied practically in an SCRI-led programme under the European Union's 'Standards, Measurements and Testing' programme. The aim is to develop to ISO standards, PCR-based tests for the presence of P. fragariae (red core) in commercial strawberry propagation stocks throughout Europe. Similar diagnostics for Spongospora on potato tubers, funded by BPC, have proven very sensitive and early results suggest that they could be applied to soil as well as plants. Recently, the work was extended to other blemish pathogens, in particular, to black dot (Colletotrichum coccodes), silver scurf (Helminthosporium solani) and common scab (Streptomyces spp.), as part of an open contract with MAFF. Similar progress has been made with bacterial diseases, with PCR-based tests for detecting Erwinia on potatoes and Xanthomonas spp. attacking beans.

All of this work, which is funded by contract, is integrated with ongoing basic core research. For example, the development of *Phytophthora* diagnostics has led to molecular characterisation of a very comprehensive range of *Phytophthora* species in joint work with Professor Brasier of the Forestry Commission. Nearly every available *Phytophthora* species has been included. Likewise, PCR detection of *Erwinia* is closely linked to core work on molecular diversity within that genus. New studies on the molecular diversity present in cereal rusts hopefully will yield similar tangible results.

Research on various resistance strategies, as well as mechanisms of resistance, continues to be a major focus within the Virology Department, where both the mechanisms of natural resistance to plant viruses as well as pathogen-derived resistance to plant viruses are being examined.

A resistance strategy against groundnut rosette virus (GRV) was demonstrated in *Nicotiana benthamiana*,

utilising a hypovirulent satellite RNA as a transgene to suppress the replication of GRV. In fact, two types of resistance were observed. In one, GRV, as well as a pathogenic satellite RNA, were suppressed by the transgenic hypovirulent satellite RNA. In the other, the pathogenic satellite RNA was suppressed, but GRV was not, leading to a reduction in symptom expression i.e. tolerance. With improvements in transformation technology, it is hoped that such resistance could be introduced into groundnut (*Arachis hypogaea*) in sub-Saharan Africa, where groundnut rosette disease is a severe agricultural problem.

Pathogen-derived resistance to potato mop-top virus (PMTV) is being tested in potato, where there is no natural resistance to this virus. PMTV is an important potato pathogen in Scandinavia, and is becoming increasingly important in China. The mechanism of this resistance has been examined in transgenic *N. benthamiana* and involves the presence of the virus coat protein, expressed from the transgene, rather than transgene-mediated silencing mechanisms mediated by RNA. The resistance was maintained against a number of Scandinavian strains of PMTV.

Natural resistance to potato virus Y (PVY) and potato virus A (PVA) genes has been analysed. The  $Ry_{sto}$  gene from *Solanum stoloniferum* confers resistance to PVY, PVA and PVV, while the Ra gene, also from S. *stoloniferum*, confers resistance to PVA alone. The  $Ry_{sto}$  gene has been mapped and is being isolated elsewhere. The Ra gene is being mapped to facilitate its isolation, and potato protoplasts are being used to compare the mechanisms of resistance to PVY and PVA.

The mechanism of replicase-mediated resistance to cucumber mosaic virus (CMV) was analysed further. A hypothesis stating that the movement protein (MP) was inhibited from either binding to or moving through plasmodesmata, was analysed using two approaches: (1) The CMV MP, fused to the green fluorescent protein (GFP), was expressed from the potato virus X vector and was shown to associate with plasmodesmata; (2) the same MP-GFP fusion was expressed from CMV RNAs with limited cell movement, and the MP-GFP was shown to move through plasmodesmata. These observations refute the hypothesis that replicase-mediated resistance inhibits virus movement by inhibiting the MP from either associating with or passing through plasmodesmata.

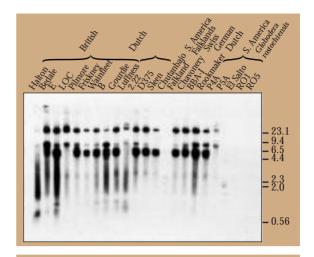
A novel form of resistance to CMV was observed in transgenic tobacco expressing CMV RNA 1. While

some regenerated plants could complement the replication of CMV, when only RNAs 2 and 3 were added, the selfed progeny of some of these plants showed resistance to systemic infection of inoculated CMV, even though they were still able to complement replication in the inoculated leaves. The mechanism of resistance involves a sequence-specific inhibition of RNA 1 accumulation, but does not involve either constitutive or inducible gene silencing. Further work on this mechanism and the other mechanisms of virus resistance described above, will increase our ability to apply different resistance strategies and prevent infection of agriculturally important plants by viruses.

The widespread and variable virulence of the white potato cyst nematode (PCN, Globodera pallida) is hampering progress in breeding for resistance. Consequently, attaining a better understanding of virulence differences in PCN has been a priority. Molecular approaches have been applied to explore the proposition that, as PCN is an introduced pest, virulence differences between populations derive from differences in the gene pools introduced. Exceptionally virulent populations, such as Luffness from Scotland, were of particular interest, and RAPD analysis supported the view that it represents a distinct introduction. Avirulent pathotype Pa1 populations were also confirmed as being genetically distinct and therefore probably deriving from a different introduction to the majority of populations which are of intermediate to low virulence.

As *G. pallida* is now widely distributed in the UK and Europe, it seems likely that post-founder events will also have influenced current patterns of virulence. This was supported by a molecular genetic analysis. Pathotypes Pa2 and Pa3 could not be distinguished and were shown to represent the extremes of a continuum of biological and genetic variation. Analysis with inbred lines demonstrated that this range of variation could be produced by genetic drift. It was also demonstrated that *G. pallida* is extremely heterogeneous, and that it readily responds to selection, even by apparently susceptible cultivars of potato. Resistant cultivars produced even greater selection.

Analysis of the internal transcribed spaces (ITS) region of ribosomal DNA (rDNA) provided evidence for substantial gene flow within UK populations. Restriction enzyme digestion of the amplification product from the ITS region with RsaI showed that Pathotype Pa2/3 populations contained three distinct ribo-types (Fig.1). One of these was found in Pa1 and

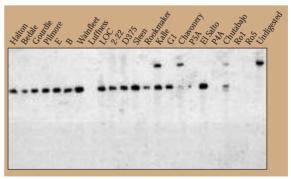


**Figure 1** RsaI digestion products of ribosomal PCR products from 17 *G. pallida* populations. Sizes of digestion products based on sequence information are indicated.

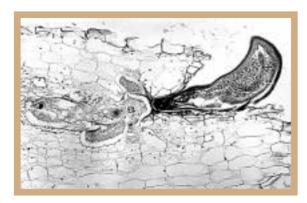
another in a population from South America (P5A), each of which contained a single ribo-type. The third ribo-type was present in a population (Pa375) from the Netherlands which also contained the ribo-type found in Pa1.

Analyses of mtDNA have revealed even greater divergence in *G. pallida*. Ten mtDNA probes, derived from a Pa2/3 population library were used to probe the DNA of populations of *G. pallida* from the UK, Europe and South America. Most, or all of the probes bound to total DNA from most of the UK and European populations, and to one population from South America (El Salto). However, less hybridisation was observed with the Luffness and Pa1 populations and several probes failed to hybridise to the P5A population (Fig.2).

The unique structure of the mtDNA genome of *G. pallida* was the greatest surprise. It was shown to be



**Figure 2** RsaI digestion products of ribosomal PCR products from 17 *G. pallida* populations. Sizes of digestion products based on sequence information are indicated.



**Figure 3** Young female cyst nematode (right) with enlarged cells forming the syncytium on which it depends for its food (left).

comprised of a population of small, circular DNAs that individually are too small (<13kb) to contain all the genes required for a fully functional mitochondrial genome. Various types of multi-partite mtDNA genomes are found in plants, fungi and some protozoa, but have never before been reported from a metazoan. Such an arrangement poses many questions, including how and why it has arisen. This may relate to the unusual biology of PCN, which includes a capacity to change sex, depending on the environment, and to persist as unhatched eggs for >20 years between potato crops. Current research is focused on sequencing *G. pallida* mtDNA.

Research continues to isolate the *Hero* gene from tomato, which we showed conferred c. 80% resistance to *G. pallida* Pa2/3. Cosmids spanning the *Hero* locus have been supplied by colleagues in Germany. Complementation studies seeking to transform potato with such cosmids are in progress. We are also involved in the early stages of the map-based cloning of genes from potato with quantitative resistance but, as avirulent nematodes are the probes, such research requires access to populations of *G. pallida* with specific avirulence characteristics.

Cyst nematodes are endoparasitic, sedentary and biotrophic root pathogens. To become adult, they have to induce the root cells at their permanent feeding sites to become enlarged and multi-nucleate, thereby providing the developing juvenile with a rich supply of food. As these enlarged "syncytia" (Fig.3) have to remain alive and metabolically active for the whole of the nematode's life cycle, it is imperative for the nematode that it induces a susceptible rather than a resistant response. Whether an incompatible or compatible response results, depends on the reaction of the host to the secretions produced/injected by the

nematode. We have developed techniques of collecting nematode secretions in sufficient quantities to analyse and to use for antibody production. We are also screening cDNA libraries made from PCN invasive stage juveniles in an attempt to isolate genes encoding secreted molecules.

Direct biochemical analysis of secretions has revealed the presence of a range of biologically-active molecules including antioxidant enzymes, plant cell wall-degrading enzymes and metalloproteases. Such biochemical studies are still extremely challenging due to the enormous numbers of nematodes required to produce sufficient secretions for even the most basic of biochemical studies. More progress has been made in characterising genes isolated from cDNA library screening. These genes can be sub-cloned into expression vectors which allow the production of almost unlimited quantities of protein for biochemical analysis, thus enabling details of the functional roles of each protein to be investigated. Genes investigated in this way include GPSEC-2, a secreted fatty acid binding protein from *G. pallida*, and a thioredoxin peroxidase from G. rostochiensis.

GPSEC-2 was isolated from a G. pallida expression library using an antiserum which bound to the surface of invasive stage juveniles of this nematode. Sequence analysis showed it to be extremely similar to proteins produced by a range of free-living and animal parasitic nematodes. Functional studies undertaken on protein produced from this gene showed that, like its counterparts in animal parasites, GPSEC-2 was capable of binding a wide range of fatty acid ligands including linolenic and linoleic acids. These fatty acids are used as substrates by plant lipoxygenases in order to generate free radicals; this reaction forms one of the first lines of defence used by plants against attack by pathogens. Subsequent work has shown that GPSEC-2 is capable of inhibiting the activity of this enzyme in vitro suggesting that it may have a secondary role in protecting the pathogen from host defence responses.

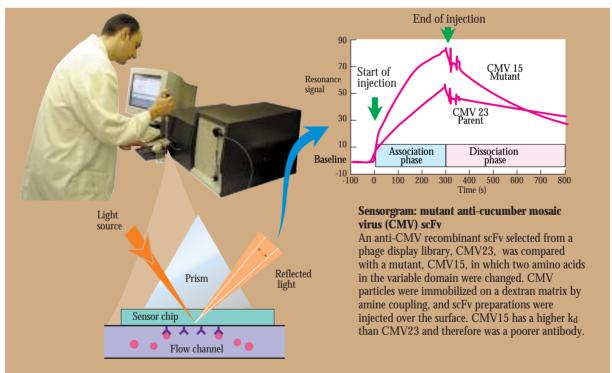
Work on the second species of PCN, *G. rostochiensis*, led to the isolation of a gene encoding a thioredoxin peroxidase. This gene was isolated from a cDNA library using an antiserum raised against secretions collected from this nematode. Biochemical studies, in collaboration with the University of Dundee, on the protein produced from this gene showed that, like its counterparts from animal parasitic nematodes, it operates in a different pathway to thioredoxin peroxidases from other organisms, making it an excellent candidate for targeting with novel control methods.

# Engineered antibodies: readily adaptable molecular tools for basic and applied research

L. Torrance, A. Ziegler, K. Harper & M. A. Mayo

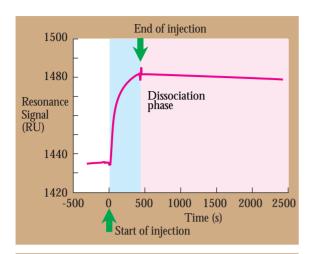
**Introduction** Antibodies are glycoproteins which have a modular structure with different domains having different functions. The variable domains that are responsible for specific binding to an antigen are in two chains, V<sub>H</sub> in the heavy chain, and V<sub>L</sub> in the light chain. We have cloned the genes encoding V<sub>H</sub> and V<sub>L</sub> and expressed them as a single polypeptide (scFv: single chain variable fragment) in Escherichia coli either on the surface of a bacteriophage (phage display), or as a soluble protein within the bacterial cells. Recent developments in antibody engineering have included the cloning of other domains from antibody molecules as well as other proteins, including enzymes, to express genetic scFv fusion proteins. These developments mean that it is possible to engineer different properties into the basic scFv molecule, such as multivalency to increase binding strength, bispecificity to broaden binding recognition, fusion to enzymes (or other reporter molecules) to facilitate detection of binding, and signal sequences to target expression to different sub-cellular compartments in plants.

Cloning and expression of antibody genes in heterologous hosts has opened up new areas for research, and new opportunities to devise novel diagnostics. For example, we have recently been successful in obtaining funding from the European Commission to explore the effects of expressing scFv in plants that bind to non-structural virus proteins, and from the DTI and industrial partners in the UK to develop novel diagnostics. The recent acquisition of a BIAcore  $X^{\rm TM}$  instrument will allow accurate estimation of binding strength, and measure of the specificity of interactions of the novel antibodies (see box). This article



The BIAcore  $X^{\rm TM}$  instrument (Biacore AB) measures interactions between molecules by surface plasmon resonance (SPR). The SPR response measures changes in mass at the detector surface, and thus the association or dissociation of the molecules. Test molecules can be immobilized to different surface matrices either directly by covalent bonds using amine, aldehyde or thiol groups, or indirectly through e.g. biotin/streptavidin linkages, or by binding to specific antibodies. The rates of association or dissociation are measured, and kinetic and equilibrium affinity constants are calculated from these.

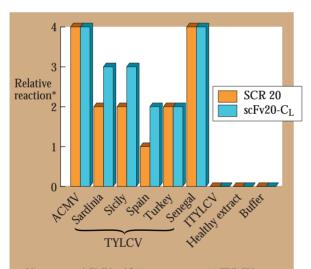
The BIAcore  $X^{\scriptscriptstyle{\mathrm{TM}}}$  instrument, and principle of detection method.



**Figure 1** BIAcore  $X^{TM}$  sensorgram of the interaction of MAb SCR20 with TYLCV.

describes some achievements of our current programme on novel diagnostics, and gives an indication of future research areas.

Novel diagnostics Whitefly-transmitted geminiviruses cause economically important yellow leaf curl dis-

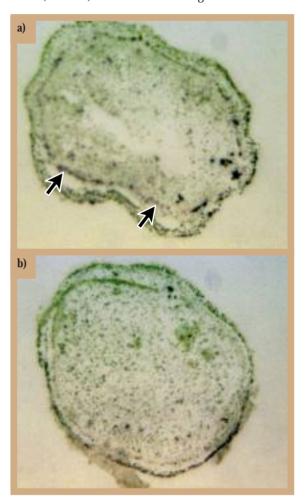


Viruses were ACMV = African cassava mosaic; TYLCV = tomato yellow leaf curl isolates from five different countries; ITYLCV = Indian tomato yellow leaf curl virus. Tests were done by triple antibody sandwich ELISA in which virus was trapped from sap extracts by anti-ACMV antibodies and detected by either SCR 20 followed by anti-mouse-alkaline phosphatase conjugate or scFv- $C_{\rm L}$  followed by anti-CL-alkaline phosphatase conjugate.

\*Absorbance values were ranked as follows: 4 = 1.21 - 1.80; 3 = 0.61 - 1.20; 2 = 0.31 - 0.60; 1 = 0.15 - 0.30;  $0 = <2 \times 10^{-2}$  x control.

Figure 2 Comparison of reactions in ELISA of geminiviruses with monoclonal antibody SCR 20 and recombinant scFv20- $C_L$ .

eases of tomato in many parts of the world. Different whitefly-transmitted geminiviruses have been shown to be serologically related and some monoclonal antibodies (MAbs) prepared against African cassava mosaic geminivirus were found to cross-react with viruses causing yellow leaf curl in tomato. In particular, MAb SCR 20 cross-reacts with tomato yellow leaf curl virus (TYLCV). When the binding of SCR 20 to a



**Figure 3** Detection of TYLCV in tomato stems by tissue print immunoblotting using scFv 20-C<sub>L</sub> and a) infected b) healthy.

Sardinian isolate of TYLCV was investigated by surface plasmon resonance, we found that the dissociation of antibody molecules from the antigen was extremely slow, and the dissociation rate constant  $k_d$  was lower than  $10^{-5}~\text{s}^{-1}$  (Fig 1). The half-life of such an antibody-antigen complex is c. 19 hours.

The V genes of MAb SCR 20 were cloned and expressed as scFv fragments and as fusions to another part of the antibody molecule, the constant domain of

the light chain,  $C_L$ , forming scFv- $C_L$  fusion proteins. The antibody fragments were readily expressed in E. coli and we found that they retained the same binding reactivity as the parent MAb (Fig 2). Tissue print immunoblotting tests using the scFv- $C_L$  fusions showed that the virus was located in the vascular cells of tomato stem sections (Fig 3).

Phage display libraries In addition to cloning and expression of antibody genes from existing sources, it is also possible to obtain antibody genes from large phage display libraries of V genes. The phage antibodies are selected by binding to the target antigen immobilised on polystyrene tubes. The advantages of this approach are many but the most important of them is that we can be sure of a continuing supply of antibodies to virtually any antigen from a phage dis-



play library stored in the refrigerator. We obtained scFv fragments that bind potato leafroll virus (PLRV) from a phage display library after four rounds of selection on virus preparations immobilised on tubes. The scFv were sub-cloned such that the transformed *E. coli* secreted a scFv-alkaline phosphatase fusion protein. This antibody readily detected PLRV in extracts of infected potato plants with a sensitivity comparable to detection using conventional antibodies.

In addition, we have obtained scFv from phage libraries that bind to other plant viruses. These include blackcurrant reversion associated virus, a virus against which we were unable to obtain useful antisera by conventional methods. Also, scFv have been obtained to plant enzymes involved in lipid biosynthesis (enoyl acyl reductase) and starch synthesis (granule bound starch synthase; GBSS) from a synthetic phage display library.

Future prospects The properties of recombinant antibodies, such as fast selection of many different binders from a single stock, and the relative ease of their subsequent genetic manipulation, mean that they can be useful tools in several areas of basic research. An example is the incorporation of the genes into specially designed vectors for targeted plant expression. We are currently pursuing some aspects of scFv expression in plants, in particular the effects on virus replication and plant metabolism of expressing scFv that bind to virus encoded non-structural proteins, and to the enzyme GBSS. Another potentially productive research topic for future study may be to investigate the cellular location of novel genes. ScFv can be obtained that bind to short C-terminal peptide sequences translated from expressed sequence tags. These antibodies can be used in immunocytochemical studies to locate the putative protein, thus providing a clue to biological function of the gene.

#### Acknowledgements

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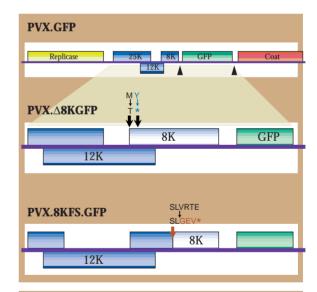
## Probing the virus long-distance transport pathway

S. Santa Cruz, P. Boevink, G. Duncan, A. Roberts, D. Prior & K. Oparka.

espite much progress in unravelling the mechanisms of intracellular and intercellular virus movement, the processes involved in systemic movement, most significantly phloem entry and exit, remain very poorly understood. For mechanically transmitted viruses, such as potato virus X (PVX), systemic infection results from the combination of both cell-to-cell and phloem-dependent movement. In the initial phase of infection, intercellular movement from epidermal cells, via mesophyll and bundle sheath cells, leads to infection of the minor vein phloem. Subsequent loading of virus into sieve elements leads to rapid dispersal of virus, through the phloem transport pathway, followed by phloem unloading of virus and cell-to-cell movement away from the vascular tissue. The pattern of virus movement mimics the translocation of photoassimilate in that both virus and solutes are exported from photosynthetic source tissues to sink tissues. Furthermore, this common pathway for phloem translocation of virus and solutes extends to the specific vein classes involved in phloem loading, which occurs in the minor veins, and unloading, which occurs exclusively from the major veins<sup>1</sup>.

We are investigating the requirements and pathways for both local- and long-distance movement of PVX through a combination of molecular and cell biological approaches. Precise, real time, analysis of viral movement processes is greatly facilitated by the use of the green fluorescent protein (GFP), as a reporter of virus-infected cells, and we have utilised a GFP-tagged PVX in order to investigate the movement phenotypes of a series of viral mutants<sup>2,3</sup>. PVX is the type member of the potexviruses and falls into a larger grouping of viruses that require the products of three overlapping open reading frames, the triple gene block (TGB), for cell-to-cell movement. Despite their central role in cell-to-cell movement, no clear function for the TGB proteins in either intracellular or intercellular transport has been established. In addition to the TGB proteins, PVX also has an absolute requirement for coat protein (CP) for intercellular movement<sup>2</sup>. In fact, the only potexvirus protein to date that has been shown to localize to plasmodesmata is the viral CP<sup>4</sup>, and available evidence suggests that PVX moves between cells as encapsidated virus particles<sup>5</sup>.

To gain a better understanding of the PVX movement process, a series of frame-shift mutations were introduced to disrupt each of the three TGB protein open reading frames. All mutations were introduced into a PVX genome tagged with the green fluorescent protein as a reporter for virus infection. Analysis of these mutants on Nicotiana clevelandii plants showed that whereas mutations in either the 25 kDa or 12 kDa TGB completely abolished cell-to-cell movement, the mutant 8 kDa protein still supported some local movement. Because the mutation introduced in the 8 kDa protein in PVX-8KFS.GFP permits the expression of the amino-terminal half of the protein, a second mutant was engineered in which translation of the entire 8 kDa protein is prevented (Fig. 1). This mutant, PVX-Δ8K.GFP, like the mutants carrying disrupted 25 kDa and 12 kDa genes, was incapable of



**Figure 1** Viral genomes of 'wild-type' and mutant forms of PVX.GFP.

Schematic representation of the 'wild-type' PVX.GFP genome and the mutant genomes used in the analysis of the 8 kDa protein. The triple gene block is shown in blue with the individual open reading frames identified by the predicted sizes of their translation products (K = kDa). In the mutant PVX- $\Delta$ 8K.GFP the initiating methionine codon of the 8 kDa protein has been mutagenized to prevent translation of the protein. In PVX-8KFS.GFP a truncated protein is translated due to the introduction of a +1 frame shift mutation that introduces a stop codon after 29 amino acids.

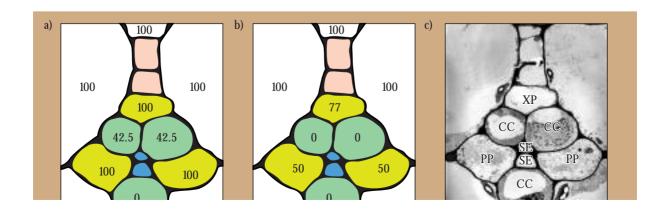


Figure 2 GFP fluorescence in *Nicotiana clevelandii* leaves 7 days after inoculation. Infections with either 'wild-type' PVX.GFP (a) or PVX-8KFS.GFP (b) show multicellular infection foci. Infection with PVX- $\Delta$ 8K.GFP (c) gives rise to infections that are restricted to single epidermal cells.

cell-to-cell movement and infections remained restricted to single inoculated cells (Fig. 2).

Further analysis of the attenuated movement mutant, PVX-8KFS.GFP, demonstrated that this virus was completely blocked in phloem-dependent movement. This failure to invade host plants systemically could be caused by blocks at a number of steps in the pathway either into or out of the phloem conducting tissue. In order to investigate the specific block to phloem-mediated transport exhibited by PVX-8KFS.GFP, inoculated leaf tissue was examined by electron microscopy. The results, summarised in Figure 3, showed that the inability of the mutant PVX-8KFS.GFP to move via the phloem reflected a barrier preventing infection of the minor vein phloem companion cells. The distribution of both wild-type PVX.GFP and PVX-8KFS.GFP in infected vascular tissue is illustrated in Figure 3. Thus, whereas both the wild-type and mutant viruses were equally efficient in infecting the bundle sheath cells, their ability to invade the phloem and phloem-associated cells differed. Most significant, with respect to access to the phloem transport pathway, was the absence of PVX-8KFS.GFP from the companion cells.

Figure 3 clearly shows that PVX-8KFS.GFP is able to exit the bundle sheath cells and infect both xylem and phloem parenchyma. However, the mutant was unable to invade the paired companion cells that for the wild-type virus appear to be the main route for phloem loading. Infection of phloem companion cells could result from either direct entry of virus from adjacent bundle sheath cells or, via a two-step process, in which virus first enters the phloem parenchyma and subsequently infects the companion cells. Previous studies of wild-type PVX infection have demonstrated that plasmodesmata between bundle



sheath and companion cells are heavily labelled with antibody raised against the viral coat protein whereas labelling of plasmodesmata between parenchyma and companion cells is rare<sup>5</sup>. This evidence suggests that direct invasion of companion cells from adjacent bundle sheath cells is the normal route by which PVX reaches the phloem. A significant feature of the findings presented is that, although PVX-8KFS.GFP is clearly able to move beyond the bundle sheath cells and invade the phloem parenchyma, the normal route for PVX invasion of the phloem is completely blocked. The results strongly suggest that, as well as a general requirement in facilitating the cell-to-cell movement process, the PVX 8 kDa protein plays an essential rôle in allowing invasion of the companion cells to occur.

The barrier to phloem entry exhibited by PVX-8KFS.GFP, between bundle sheath and companion cells, is of particular importance in the transport pathway of Nicotiana species as it marks the boundary between symplasmic and apoplasmic transport. In many species, including members of the family Solanaceae, phloem loading occurs apoplasmically, whereas virus movement is known to be symplasmic. Apoplasmic phloem loading involves the retrieval of solutes from the apoplast into companion cells and sieve elements by specific transporter proteins. The minor vein configuration in species of the genus Nicotiana is typical of apoplasmic phloem loaders and two lines of evidence point to a functional symplasmic barrier, sufficient to prevent solute transfer, between the companion cell-sieve element complex and surrounding cells. First, phloem loading of sucrose in tobacco is completely blocked by the sucrose symport inhibitor PCMBS<sup>6</sup>. Second, experiments performed on tobacco have shown that treatment of tissues with high concentrations of solute causes plasmolysis of bundle sheath and phloem parenchyma cells but not of companion cells<sup>7</sup>. Both these lines of evidence argue against the presence of an operational symplasmic pathway into the phloem of mature (i.e. source) leaves. However, the immunolocalization of PVX in companion cells of the minor vein phloem<sup>1,5</sup> (Fig. 3) clearly shows that PVX, and undoubtably other viruses, can exploit a symplasmic pathway that is not functional for solute transport in uninfected tissue. This in turn raises the question of whether PVX is directing the de novo production of intercellular channels or is opening pre-existing plasmodesmata that under normal circumstances are nonfunctional for solute transport.

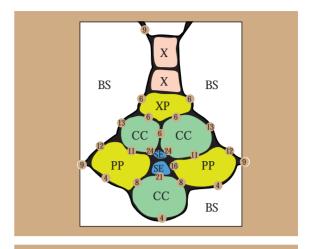


Figure 4 Plasmodesmogram of cells within and surrounding a Nicotiana clevelandii class V vein. Plasmodesmatal frequencies at the different interfaces between cells of a class V vein were counted from serial sections of veins imaged by electron microscopy. Numbers refer to the average number of plasmodesmata per 10  $\mu m$  of cell wall.

In order to establish the distribution of plasmodesmata in the minor veins of uninfected N. clevelandii. a detailed analysis of plasmodesmatal frequency was carried out using electron microscopy. The results, shown in Figure 4, demonstrate that in non-infected tissues the companion cells are highly connected to neighbouring bundle sheath and parenchyma cells. This apparent symplasmic continuity between bundle sheath and companion cells is clearly non-functional in the mature leaf, based on the evidence discussed above. Whether the observed high frequency of plasmodesmata connecting the bundle sheath to the companion cells reflects a legacy of development or whether some symplasmic continuity is maintained for transport of small molecules and ions (e.g. for signalling purposes) cannot be resolved from this study. However, the evidence obtained does clearly demonstrate that PVX is able to exploit a symplasmic movement pathway that under normal conditions does not permit the passage of solutes. Furthermore, the failure of PVX-8KFS.GFP to invade companion cells strongly suggests that a normal role of the 8 kDa TGB protein is to unlock this gateway to phloem entry.

Phloem loading of virus, like every other aspect of the viral life cycle, requires productive interactions between host and viral proteins. Mutants, such as PVX-8KFS.GFP, that are capable of cell-to-cell but not phloem dependent movement, suggest that the specific interactions required for these two phases of the movement process are different. The demonstra-

tion that, for PVX-8KFS.GFP, the barrier to phloem loading occurs at the same cell interface, between bundle sheath and companion cells, as the symplasmic barrier to solute transport in *Nicotiana* species, emphasises the key importance of this boundary in



regulating access to the long distance transport pathway of the phloem. Strategies designed to exploit this natural barrier to long distance transport, reinforcing the block to symplasmic movement, could provide an effective barrier to long distance virus movement without affecting the capacity of the plant to transport essential macromolecules via the phloem.

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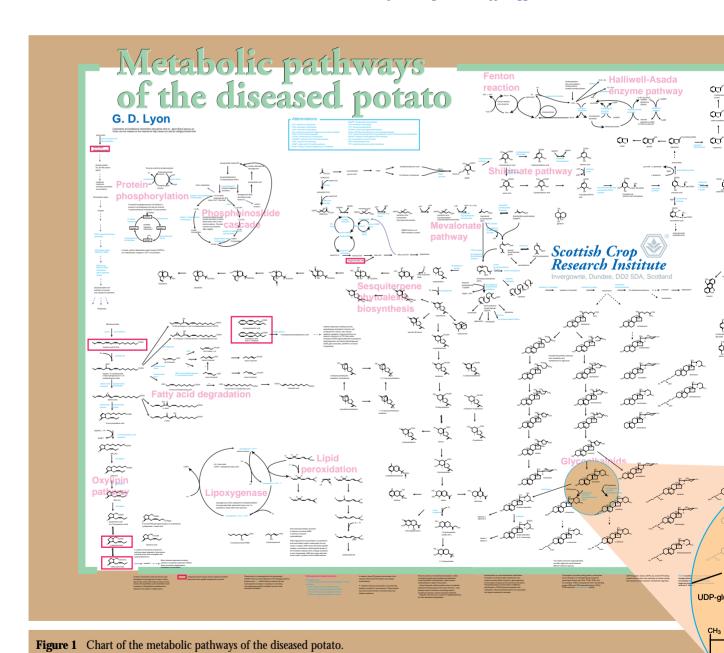
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## Metabolic pathways of the diseased potato

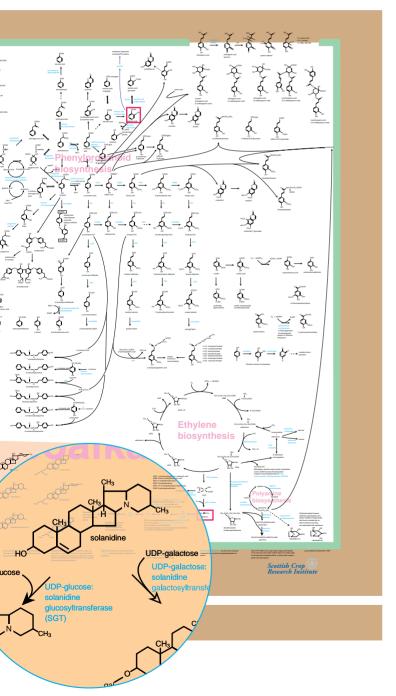
G. D. Lyon

One of the greatest constraints on food production is the potential for crop losses through pests and diseases. Understanding molecular interactions in diseased cells has therefore provided opportunities not only for academic, curiosity driven research, but also new opportunities for manipulating disease resistance.

Most biologists are familiar with wall charts of metabolic pathways of healthy cells and, indeed, this information can now be accessed on the internet at a number of different locations (for example, http://expasy.hcuge.ch/cgi-bin/search-biochem-index and http://www.genome.ad.jp/kegg/metabolism.html).



The molecular complexity of the plant's response to infection has long been known by pathologists working on resistance. The problem for plant pathologists is that the widely published charts on metabolic pathways only refer to healthy cells and do not include secondary metabolic events associated with resistance responses. There is a further complication: whilst some processes of resistance are common between plants belonging to different families, the chemistry of those induced responses varies and therefore it is not possible to provide a single unifying metabolic chart.





However, it is possible to present information on secondary metabolism for a single plant species. Information on secondary metabolism in the diseased potato has been published over many years, most of it associated with infection by Phytophthora infestans or Erwinia carotovora, or the application of resistance elicitors. Combining information in the format of a metabolic pathways chart<sup>1</sup> has a number of advantages. Not only does it draw attention to the complexity of the plant's response to infection, but it shows some of the responses which may not be so obvious when reading primary publications. It can act as a focus for designing new experiments and clearly highlights those areas where information is lacking. For example, enzymes involved in the biosynthesis of the sesquiterpene phytoalexins have not yet been purified or characterised. This contrasts with the greater knowledge about phenylpropanoid metabolism, although even this is still incomplete. Importantly, there is little firm data on signal transduction pathways in plants in general, even in healthy cells, and hence the chart includes some generalisations about potential signal induction cascades. For example, the MAP kinases and phosphoinositide signalling pathways have been poorly described in plants but are much better described in animal systems.

The chart also emphasises the importance of posttranslational modification of proteins through addition of phosphates, methyl groups, carbohydrates or lipids. For example, isoprenylation is a post-translational modification of proteins involving covalent attachment of an isoprenyl moiety (either farnesyl or geranylgeranyl) to the cysteine residue at the C-terminus of proteins. Prenylated proteins can be further

modified by palmitoylation, COOH terminal proteolysis and methylation. Most prenylated proteins are associated with signal transduction cascades. To understand 'resistance' it is therefore not sufficient to just isolate and sequence genes but a knowledge of all the processes in 'cell biology' and how protein function is regulated, is necessary.

There may be some common elements in the manner in which a plant responds to infection but this varies between plant pathogens, suggesting that plant signals can discriminate between different pathogens e.g. not only between fungi and viruses but between biotrophs and necrotrophs, etc. The metabolic pathways chart should therefore be viewed as a potential response to infection; it does not imply that all such processes are, or can be, activated by every pathogen. In addition, not all of the pathways shown will be up-regulated after infection. For example, after infection, glycoalkaloid accumulation is suppressed in favour of sesquiterpene accumulation. Importantly, many enzymes exist as isozymes which may have different intracellular locations and hence may be differentially regulated. For instance, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) involved in the mevalonate pathway is encoded by three genes. *Hmg1* is strongly induced by wounding, leading to the accumulation of glycoalkaloids, whereas hmg2 and hmg3 are up-regulated by arachidonic acid and P. infestans. Such compartmentalisation plays a crucial rôle in enabling the plant to differentially up-regulate gene products to give a different wound- or pathogen-induced response. The sequential expression of ACC synthase genes suggests that they may be controlled by different signal transduction and gene regulatory mechanisms.

With recent work at SCRI on identifying and sequencing resistance-related genes in potatoes<sup>2</sup>, and, in consequence, the potential to analyse the signal transduction pathway leading to induced resistance, the production of this metabolic chart is timely. Clearly, the molecular interactions and intracellular signalling responses in animal cells have their analogues in plant cells. With the isolation of signalling genes, there will be increased potential to engineer resistance cascades in order to modify resistance responses. This can only be carried out effectively if the complexity of the plant's responses is understood and the resistance-related pathways integrated into a unified structure. It is clear that plants possess a lot of

plasticity in the way that they can react to stress and the environment. Genetic manipulation of response genes has a number of important considerations, not only the primary one in relation to disease resistance, but also the toxicological implications and whether such manipulation can alter the nutritional status of the plant. For example, Laurila et al.<sup>3</sup> showed that new glycoalkaloids are present in potatoes derived from a cross between S. tuberosum and S. brevidens which are not present in either parent. Concepts on plant transformation over the last few years have, of necessity (given the paucity of information available), been on a simplistic basis, suggesting that dramatic changes in resistance can be achieved by insertion (haphazardly within the genome) of a single gene producing greater amounts of an antifungal protein. Once the signalling cascades activated in a resistant plant have been better characterised, we should expect to see much more subtle concepts being considered for engineering increased levels of resistance. Future transformations will therefore involve manipulation of genes involved in signalling, rather than genes associated with the synthesis of antimicrobial proteins.

The chart<sup>1</sup> summarising the metabolic pathways of the diseased potato is nowhere near a complete and full description of the potato's response to infection but it does include much of the information currently available. As we progress from sequencing plant genomes, a knowledge of non-intermediary metabolism will become increasingly important in assigning functionality to genes. Metabolic databases will become important in maximising the impact of genome sequencing projects.

The chart is accessible on the Internet as a Portable Document Format file which can be read using Adobe Acrobat Reader 3.0 and a wall chart printed if access to a large format (e.g. A0) printer is available. Acrobat Reader can be down-loaded free from the Internet. The chart will be up-dated as new information becomes available.

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# Nematode transmission of plant viruses - a 30 year perspective

D.J.F. Brown & D.L. Trudgill

Outbreaks of a lethal 'leaf curl' disease in plantations of Baumforth's Seedling raspberry were first observed in eastern Scotland in 1922. The disease did not affect cv. Lloyd George, but further increased 20 years later when cv. Norfolk Giant began to replace Lloyd George. Healthy plants, used to replace diseased plants, subsequently became infected and plants on the periphery of patches of diseased plants became infected. Thus, the size of the diseased patches extended annually.

During the 1950s, research at the Scottish Crop (Horticultural) Research Institute, demonstrated that sap-transmissible viruses caused this and similar diseases. Techniques were developed to recover and serologically type these viruses and to transfer them between herbaceous plants. At the end of the decade, it was discovered in North America that plant ectoparasitic nematodes could transmit some of these viruses.

Following this discovery, research at SCRI identified that two viruses (raspberry ringspot (RRSV) and tomato black ring (TBRV) nepoviruses) damaging raspberry and strawberry in Scotland and other areas of the UK, were transmitted by the nematode Longidorus elongatus and that two other viruses (arabis

mosaic (ArMV) and strawberry latent ringspot nepoviruses (SLRSV)), more important in England, were transmitted by the nematode *Xiphinema diversicaudatum*. Research elsewhere showed that tobacco rattle tobravirus (TRV), a cause of 'spraing' disease in potato throughout Europe (brown necrotic arcs in the tuber flesh), was transmitted by trichodorid nematodes. This virus and several trichodorid species were identified as being widespread in Scotland.

Control measures developed at SCRI included the use of soil sterilant chemicals, e.g., D-D (dichloropropane-dichloropropene) and the fungicide, Quintozene (pentachloronitrobenzene), both of which control *L. elongatus* and *X. diversicaudatum*. However, early recognition of the problems associated with these highly toxic compounds led to alternative disease control strategies based on epidemiological and ecological studies of the disease.

Raspberry was shown not to be a host for L. elongatus, whereas many weed species were identified as hosts for the vector and as over-winter reservoirs for the viruses. The importance of good weed control, to prevent virus spread, was emphasised by the discovery that the virus particles are retained within the nematode's feeding apparatus, similar to the association of non-circu-



lative viruses in aphids. Acquisition of the virus from weeds was shown to be important as the nematodes lost the virus during each of the four moults and only retained the virus for a maximum of a few weeks.

Infected planting material was shown to be another important source for disseminating these viruses. The introduction of a certification scheme for disease-free soft fruit planting material, pre-planting soil tests for nematodes and viruses, and strict weed control in raspberry plantations, all but eliminated nematode-transmitted virus disease problems in the Scottish raspberry industry. Subsequently, these control measures were adopted world-wide but relaxation of weed control measures in soft fruit plantations in the UK during recent years has resulted in a resurgence of nematode-transmitted virus disease problems.

During the 1960s, many further reports were published world-wide of the apparent ability of different nematode species to transmit a variety of plant viruses. However, many of these reports were based on laboratory experiments that frequently did not include appropriate safeguards to exclude the possibility of transmission by an alternative vector or experimental contamination. Techniques and criteria developed at SCRI during the 1970's, including accurate identification of the virus and the nematode and the use of small numbers of hand-picked nematodes, led to two thirds of the then reported virus and nematode associations being rejected. Those which fulfilled the criteria, revealed the existence of a high degree of specificity between the vector species and its associated virus. As a consequence of these highly specialised techniques, with their requirement for multi-disciplinary collaboration between nematologists and virologists, the SCRI became established as the principal centre for research into nematode transmission of plant viruses.

To determine if virus-vector nematodes were a localised or a widespread problem, funding was obtained to undertake a systematic sampling throughout the UK. This survey revealed the ubiquitous distribution of virus-vector nematodes and showed that, in the UK, between 5 and 25% of vector populations were naturally associated with virus. Funding for a pan-European virus-vector nematode survey followed, providing the first systematic approach to identifying distribution patterns of plant pathogens at a continental level. Results from these surveys revealed that trichodorid and *Xiphinema* virus-vector species were widespread in Europe, whereas the *Longidorus* vector

species have more localised distributions. Subsequently, the European distribution maps of virus-vector species were used for the preparation of Phytosanitary Regulations.

Research at SCRI showed the ability of nematode species to act as virus vectors is inherited. Populations of vector *Xiphinema* spp. were shown to be highly efficient vectors. Up to 100% of nematodes in a *Xiphinema* population could transmit virus, whereas with most vector *Longidorus* species, generally less than 10% of the population transmitted virus. This discovery led to accurate procedures for risk-assessment when sampling prospective soft fruit planting sites. Also, intra-specific variability in the ability of nematodes to transmit isolates of viruses was demonstrated, leading to the recognition that each population of a virus-vector species requires to be tested for its ability to transmit virus.

During the 1980s, 'spraing' disease in Scottish potato crops increased, partly because movement by and transmission of TRV by the vector trichodorid nema-

Nematode	Virus	Serotype
P. anemones	PEBV	TpA56 (English)
	TRV	PaY4 (English)
P. hispanus	TRV	Portuguese
P. minor	TRV	American
P. pachydermus	PEBV	Dutch
• •	TRV	PpK20 (Scottish)
		PaY4 (English)
P. teres	PEBV	Dutch
	TRV	Oregon (Dutch)
P. tunisiensis	TRV	Italian
T. cylindricus	PEBV	English
·	TRV	RQ (Scottish)
		TcB2-8 (Scottish)
T. primitivus	PEBV	TpA56 (English)
	TRV	TpO1 (English)
T. similis	TRV	TsB (Belgian)
		TsD (Dutch)
		TsG (Greek)
T. viruliferus	PEBV	English
	TRV	RQ (Scottish)

**Table 1** Specific associations between *Paratrichodorus* and *Trichodorus* species and serologically distinguishable strains of tobacco rattle (TRV) and pea early-browning (PEBV) tobraviruses.

todes was facilitated by increases in crop irrigation. Studies of the transmission of TRV by trichodorid nematodes had made little progress since the 1960s, mainly because of the lack of techniques for working

Nematode	Virus	Major crops affected
L. apulus	artichoke Italian latent	artichoke, chicory, grapevine
L. arthensis	cherry rosette	cherry
L. attenuatus	tomato black ring	beet, celery, leek, lettuce
L. elongatus	raspberry ringspot	raspberry, strawberry
	tomato black ring	potato, raspberry, strawberry
L. fasciatus	artichoke Italian latent	artichoke
L. macrosoma	raspberry ringspot	cherry, grapevine, raspberry, strawberry,
L. martini	mulberry ringspot	mulberry
P. maximus	raspberry ringspot	grapevine
X. americanum	cherry rasp leaf	apple, cherry, peach, raspberry
	peach rosette mosaic	blueberry, grapevine, peach
	tobacco ringspot	cherry, grapevine, soybean
	tomato ringspot	apple, grapevine, <i>Prunus</i> spp., <i>Ribes</i> spp., <i>Rubus</i> spp.
X. bricolense	tomato ringspot	see above
X. californicum	cherry rasp leaf	see above
	tobacco ringspot	see above
	tomato ringspot	see above
X. diversicaudatum	arabis mosaic	apricot, blackcurrant, hop, grapevine, peach, raspberry, strawberry,
	strawberry latent ringspot	blackcurrant, grapevine, peach, raspberry, strawberry
X. index	grapevine fanleaf	grapevine
X. intermedium	tobacco ringspot	see above
	tomato ringspot	see above
X. rivesi	cherry rasp leaf	see above
	tobacco ringspot	see above
	tomato ringspot	see above
X. tarjanense	tobacco ringspot	see above
	tomato ringspot	see above

 Table 2
 Longidorus, Paralongidorus and Xiphinema virus-vector species, their associated viruses and major crops affected.

with such small animals (<1mm long). Micro-techniques developed at SCRI, which used individual trichodorids in virus transmission experiments, led to specific associations being identified between different trichodorid species and serologically distinguishable strains of TRV and pea early-browning (PEBV) tobraviruses (Table 1). The new understanding derived from this research explained anomalies such as the non-correlation in the field between trichodorid population density and levels of TRV infection. Often, large population densities were found to be comprised of several species, only one of which, frequently present in small numbers, was the vector species. Conversely, small field population densities were of a single, efficient vector species. Also, in multi-species populations, two or more of the species could be associated each with their particular strain of TRV. The association of species with different strains of TRV also explained the observation that potato cultivars grown at different field sites showed differences in their reaction to TRV, i.e., at some sites a cultivar might show few symptoms of infection whereas at other sites it would be highly infected. Most recently, it has been shown that some potato cultivars e.g. Home Guard, King Edward and Wilja, may be infected symptomlessly but act as a source of the virus if planted at sites, formerly free of TRV, where the appropriate vector trichodorid species is present. Current research is examining the impact of trichodorids and TRV infection on potato growth.

The techniques developed at SCRI formed the basis for collaborative studies to investigate the transmission of viruses by nematodes in North America. Transmission of four nepoviruses by several species within the *X. americanum* group of nematodes (which is comprised of 45 parthenogenetically-reproducing, morphologically-similar, putative species) has been reported. Using the micro-techniques developed at SCRI, it was demonstrated that, whereas European longidorid vector species specifically transmit one or at most two serologically distinguishable strains of virus, several American species transmit most, if not all four, of their associated nepoviruses (Table 2).

Associated studies revealed that virus-vector populations of *X. americanum* species from North America

had only three juvenile stages whereas species from other areas of the world, which are not associated with viruses, have four juvenile stages. This discovery is being investigated for its utility as a biological marker to identify potential virus-vector species and populations in this group of nematodes. These nematodes and their associated viruses are regarded as important Phytosanitary quarantine organisms, and work under an international EU-funded project on detection and identification of *X.americanum*-group virus-vector nematodes has just started.

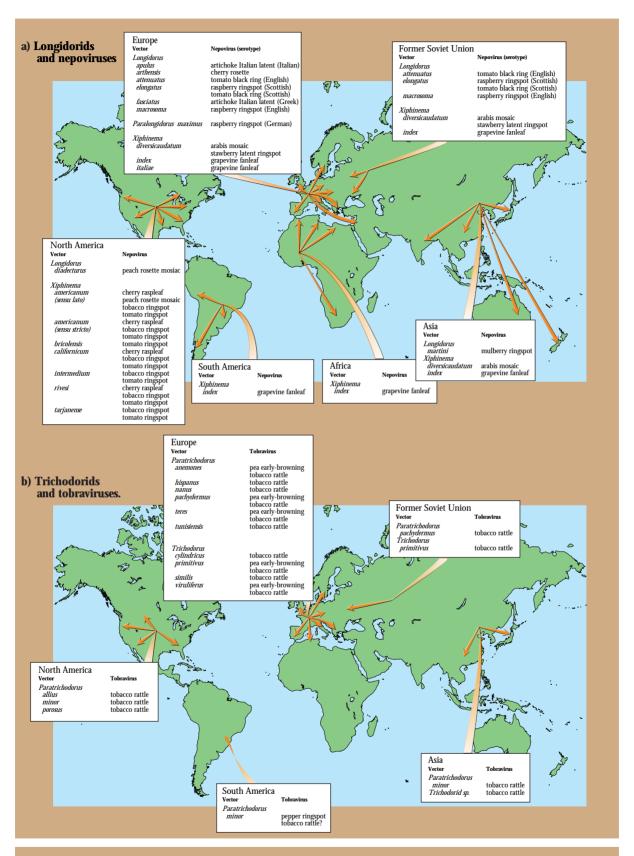
The development of molecular virological techniques during the 1990's has resulted in new, fundamental approaches to understanding the unique recognition phenomena between nematodes and their associated viruses. Investigations of the genetic determinants for specificity of transmission of tobravirus are supported by three post-graduate research students from Greece funded by the EU and the Greek State Foreign Scholarship Fund. Non-structural genes on the RNA-2 of tobraviruses have been identified as helper components in vector transmission. Vector-transmitted isolates of tobacco rattle and pea early-browning tobraviruses, each incorporating a green fluorescent protein, are being used in in vitro investigations to determine the vector trichodorid feeding mechanism(s) involved in virus transmission and subsequent virus spread from the infection site (= epidermal root cell) (Fig. 1).

Nematode transmission of viruses is an evolving area of study and, with increasing international interest, new virus and vector associations are being recognized world-wide (Fig. 2). Much of this work involves SCRI staff collaborating with international colleagues, i.e., a Royal Society and an internationally-funded research collaboration to investigate nematode-transmitted viruses in China and Brazil, respectively. Also, control of nematode-transmitted virus diseases, with few



**Figure 1** Initial infection site (arrowed) and subsequent systemic cellular spread in root of *Nicotiana clevelandii* of green fluorescent protein-tagged tobacco rattle tobravirus transmitted by *Paratrichodorus pachydermus*.

exceptions, has changed little since the 1960's. However, with societal emphasis on reducing agropesticide inputs, more benign nematode and associated virus-induced crop disease control methods are urgently required. The movement to lighter soil types for potato production and a consequent requirement for crop irrigation, has resulted in an increased probability of 'spraing' disease caused by tobacco rattle tobravirus. Current research at SCRI is focused on reducing the dependence on chemicals for controlling this disease by seeking to establish cultural methods to prevent virus transmission by the vector nematode, developing natural resistance in potato to tobacco rattle tobravirus and, in collaboration with colleagues from Leiden University, the Netherlands, investigating transgenic resistance. Also, an EU-funded project for improved detection and molecular-based identification of vector trichodorids has just begun. This productive interdisciplinary research relies on the continued, unique collaboration between nematologists and virologists at SCRI and their international colleagues.



**Figure 2** Main world-wide occurrence of virus-vector species in association with viruses: a) longidorids and nepoviruses; b) trichodorids and tobraviruses.

# Molecular ecology of the peach-potato aphid and a relative

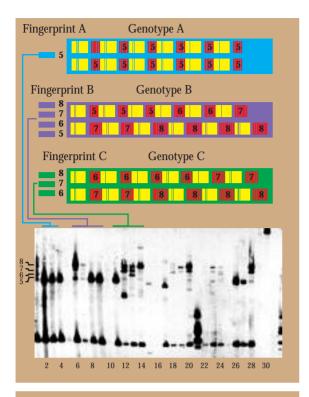
B. Fenton, J.A.T. Woodford & G.M. Malloch.

The peach-potato aphid (Myzus persicae) is one of **1** the most widespread and important virus vector aphids. In Scotland, its impact is particularly high in the seed potato industry as it spreads many destructive potato viruses such as potato leaf roll virus (PLRV). Like all aphids, it can reproduce asexually (without mating) during the summer months and this allows numbers to build up very quickly on plants. The process of asexual reproduction generates many genetically identical offspring (a clone) from a single female. Winged forms are also generated, usually as a result of overcrowding, and these move freely between fields carrying viruses. To stop this spread, farmers have to control the vector using intensive prophylactic spraying regimes, aided by monitoring of aphid numbers by agency advisers. Despite the obvious importance of aphid movement to agriculture, little is known about this part of their ecology. To understand what is happening, discriminating features either for individuals or populations are required. Previous studies, using methods such as allozyme electrophoresis, found very little variation between different peach-potato aphid clones. The only allozymes found to vary were the esterases which are known to be involved in insecticide resistance. This led to the suggestion that the genetic composition of this species consisted of a small number of genotypes or clones.

The population structure of these clones, and therefore the accuracy of this model, could not be determined without a more sensitive method for differentiating individual genotypes. To provide a means of doing this, SCRI has studied this aphid by directly analysing highly variable regions of its DNA. The method has already been applied to analyse biotypes of the large European raspberry aphid (Amphorophora idaei). The technique uses natural variation in the length of the IGS spacers found between the ribosomal genes. Within any individual eukaryote, there are many copies of these regions, and each can be a different length. Figure 1 shows examples of the types of patterns which can be obtained for different clones of the peach-potato aphid. In each lane, genomic DNA from an individual aphid clone isolated from the field, has been digested using a combination of restriction enzymes. When these fragments are separated and transferred to a nylon membrane, they can be detected using a short labelled probe. The combination of different sized fragments gives a unique profile (an IGS fingerprint) for each aphid clone. Those that share all bands are likely to be the same clone.

Using this technique, we have found, for the first time, that a closely related species, *Myzus antirrhinii*,

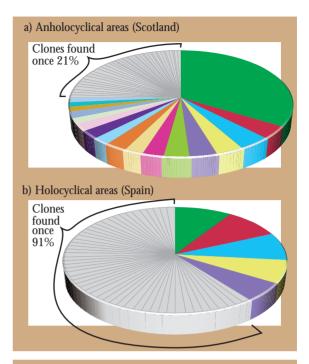




**Figure 1** The main panel shows the results of IGS fingerprinting of *M. persicae* collected from a single field. Lanes 2, 3, 4, 8, 9, 11, 26 and 27 contain a pattern which was present in 30 percent of all samples. Unique genotypes are present in most other lanes except lanes 6 and 7 which contained a 4-banded pattern. A much larger band pattern was found in samples of *M. antirrhinii* (lanes 29 and 30). Above the main panel, a schematic diagram shows how these bands originate from different copies of rDNA. Three genotypes are represented by different colours and their patterns are shown on the left. Each gene can have a different number of repeats (shown in red with the numbers of repeats indicated) between the genes (shown in yellow).

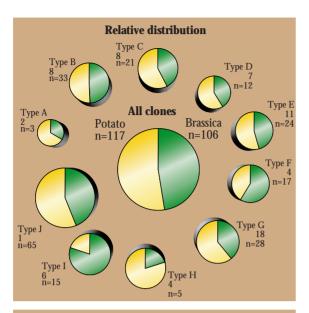
was present in samples collected from crops in Scotland. This aphid is very similar in appearance to *Myzus persicae*. The only physical feature which can give a clue to its presence is that it is always dark green. However, there are *Myzus persicae* clones which are also dark green. But, using the IGS fingerprinting method, it is quite clear that *Myzus antirrhinii* has a distinct pattern (Fig. 1).

The work has also demonstrated that the peach-potato aphid consists of a large number of different genotypes. There are at least 80 among the 276 samples examined. Within these results, there are other interesting trends. The proportion of genotypes is not distributed evenly. A large number (30%) of samples appear to be exactly the same clone (Fig. 2). By comparing peach-potato aphid clones from elsewhere in



**Figure 2** Pie charts illustrating the results of genetic analysis of *M. persicae* from two areas. The lower chart illustrates the distribution of genotypes found in areas of holocyclical reproduction (after Martinez-Tores *et al.* 1997<sup>1</sup>). The upper chart illustrates areas of anholocyclic reproduction (work at SCRI). Each pie segment represents a single genotype and its area represents the frequency with which it was collected. The smallest segment in each case represents a single genotype, found once. There were far more unique genotypes in Spain than in Scotland.

Europe and the world, it was possible to see that this genotype appeared only in Scottish samples. Amongst the other genotypes, 29 were found more than once, but most (49/80) appear only once (Fig. 2). The clonal expansion of genotypes appeared to be greater in Scottish samples when compared to those from Spain. In Spanish samples, only five genotypes were sampled more than once and the greatest proportion of a single genotype was 8% (Fig. 2). These differences in distribution patterns are most likely to be due to the frequency of sexual reproduction (holocycly). The peach-potato aphid, as its name suggests, needs peach trees to complete its sexual life cycle. Peach trees are scarce in Scotland, compared to Spain, and tend to be restricted to sheltered environments such as glasshouses. Therefore, the aphid must survive winter by continued asexual propagation (anholocycly) on hardy secondary hosts or suitable hosts in glasshouses. The details of this part of the survival of the aphid are not fully understood, but an ability by the aphid to find



**Figure 3** Fingerprints from aphids collected on potato or brassica were analysed and grouped into those that were most similar (groups A - J). The total numbers collected are shown in the central pie, with those from potato in yellow and those from brassica in green. Each group is also represented as a pie and is divided into those from the two crops. The size of each pie represents the total number of samples in that group (this is also given numerically beside each). The total number of genotypes in the groups is shown as the central number beside each pie. For example, Type J has only 1 genotype but this was found in 65 samples.

suitable plants and/or to withstand winter temperatures will be necessary. There are already field observations that demonstrate that some clones are better at over-wintering than others. The aphid type which we find in 30% of Scottish peach-potato aphid samples, has been found in December and February, when peach-potato aphid numbers are extremely low. While the numbers we could examine were small, these observations tend to suggest that this clone is particularly well adapted to over-wintering in Scotland. In the following seasons, this clone will predominate because it is already reproducing before any long distance migrants arrive.

The representation of different clonal groups on two crop plants was examined using the fingerprinting technique. Each group had representatives on both potato and brassica (Fig. 3), suggesting that there were no genotypes associated with a specific crop as has been found in some cereal aphids.

Future work will use the molecular markers, which we have developed, to try to determine exactly where different genotypes can be found over-wintering. This will allow a much clearer model of aphid numbers to be constructed and this, in turn, should enable more targeted use of insecticides and other control measures.

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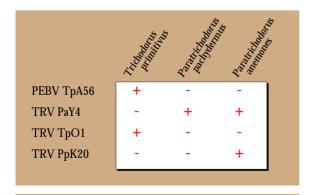
<sup>1</sup> Martinez-Torres, D., Carrio, R., Latorre, A., Simon, J. C., Hermoso, A. & Moya, A. (1997). *Journal of Evolutionary Biology* 10: 459-477.

## Molecular biology of the tobravirus genome

S. MacFarlane, D.J.F. Brown, N. Vassilakos, A. Mooney, C. Schmitt, A-M. Mueller, D. Prior & K. Oparka

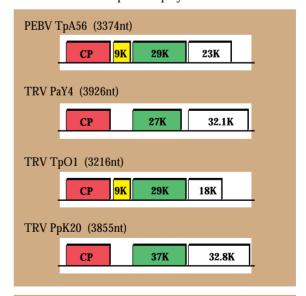
The three viruses that make up the tobravirus genus, tobacco rattle virus (TRV), pea early-browning virus (PEBV) and pepper ringspot virus (PRV), collectively are able to infect an extremely wide range of plant species and, with the exception of PRV which until now has only been detected in South America, they occur widely in the world. In Scotland, TRV is mostly associated with a disease of potato known as spraing, whereas PEBV is found primarily as a disease of peas occurring in Eastern England. SCRI has been, and continues to be, a major centre for research into these viruses, where we are studying several of the properties which are unique to this group of viruses and which contribute to their success as plant pathogens.

Tobraviruses are one of only two types of plant viruses (the other being nepoviruses) which are moved (transmitted) from plant to plant by nematodes. Part of our research programme aims to understand what special features these viruses possess to allow transmission to occur. In addition, we are studying how different virus isolates maintain their highly specific relationships with usually only one or two nematode species. Detailed investigation of the virus genes specifying nematode transmission involves in vitro mutagenesis of a cDNA copy of the virus genome, and inoculation of plants with synthetic virus RNAs carrying the different mutations. To carry out this work, full-length, infectious cDNA clones of RNA2 of four viruses (PEBV TpA56, TRV PaY4, TRV PpK20 and TRV TpO1) have been constructed. Virus derived from the cDNA clones of the first three viruses can be transmit-

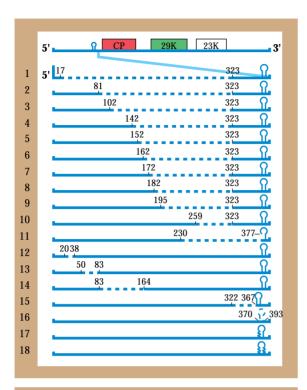


**Figure 1** Transmissibility of tobravirus isolates by three different nematode species.

ted in the glasshouse by nematodes but transmission of cloned TRV TpO1 has not yet been confirmed (although the wild-type virus stock is transmissible). Transmission tests of all four viruses using three different nematodes species has identified a complex pattern of interactions (Fig. 1). Comparison of the nucleotide sequence of each of these viruses has revealed interesting similarities in their genes, and some notable differences. Unlike many of the previously sequenced TRV isolates, which have been maintained in the glasshouse for a long time and have lost the ability to be transmitted by nematodes, these four nematode-transmissible isolates encode at least two genes in addition to the coat protein gene (Fig. 2). Downstream of the coat protein gene in PEBV TpA56 and TRV TpO1 there is a possible gene for a 9K protein. Results of previous experiments suggested that the PEBV 9K protein might be involved in the nematode transmission process. Identification of a gene encoding a similar protein in a second tobravirus increases the likelihood that it is functional, and additional experiments are underway to clarify whether the PEBV 9K gene does indeed have a rôle in transmission. Next to the PEBV 9K gene, there is a third gene encoding the 29K protein. Mutagenesis experiments indicated that this protein plays an essential rôle in



**Figure 2** Genome organization of four nematode-transmissible tobravirus isolates.



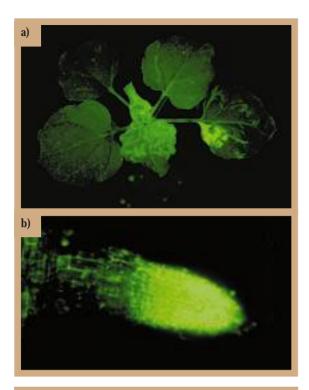
**Figure 3** Depiction of mutations introduced into PEBV RNA2 to study sequences necessary for RNA replication and coat protein expression.

nematode transmission. Proteins having many sequence similarities to the PEBV 29K protein are encoded by each of the other three viruses (TRV PpK20 37K, TRV TpO1 29K, TRV PaY4 27K) and have been shown or are expected to be essential for nematode transmission. The last gene encoded by each virus is much more variable in sequence, and possibly also in function. Previously, experiments showed that a frameshift mutation of the PEBV 23K gene prevented transmission, whereas a small, internal deletion of the gene reduced transmission to a low

level. We have now constructed and analysed a mutant in which the entire 23K gene has been deleted. This mutant can be nematode-transmitted but at a much reduced frequency. It is not clear how the 23K protein functions, as the deletion mutant appears to spread and accumulate within the plant in the same way as does the wild-type virus. Experiments using antibodies raised against the 23K protein have shown that it is expressed in both leaves and roots of infected plants. Interestingly, the 23K protein appears to be glycosylated, which may be important for it to function correctly in the transmission process. The equivalent genes of the other virus isolates that are being studied (TRV PpK20 33K, TRV TpO1 18K, TRV PaY4 32K) have no amino acid sequence similarities. Deletion of the TRV PpK20 33K gene did not diminish the frequency of nematode transmission, and recent experiments revealed that deletion of the TRV PaY4 18K gene also does not affect transmission by Paratrichodorus anemones nematodes. Our tests have shown that TRV PaY4 is transmitted by both P. anemones and P. pachydermus, whereas TRV PpK20 is transmitted only by *P. pachydermus*. One of our future aims is to investigate which parts of the TRV PaY4 coat protein and/or 27K protein enable this virus to interact with two, different vector nematodes.

Tobraviruses control the expression of the coat protein gene in an unusual way. All of the genes on RNA2, including the 5' proximal, coat protein gene, are translated from subgenomic RNAs. This arrangement appears to be unique to tobraviruses, for in all other viruses examined to date, the 5' proximal gene is expressed directly from the full-length, genomic RNA. It was previously shown that sequences at the 5' terminus of RNA2 (the 5' non-coding region, 5' NCR) are involved in the specific recognition, and subse-





**Figure 4** Expression of green fluorescent protein by a modified tobacco rattle virus. GFP fluorescence in (a) systemically infected leaves and (b) lateral root tip of *N.ben-thamiana*.

quent multiplication, of RNA2 by the viral polymerase protein. This region also contains as yet undefined sequences that are required for production of the coat protein subgenomic RNA (sgRNA). A series of PEBV RNA2 mutants has been constructed which have deletions or base changes in the 5' NCR (Fig. 3). The mutants were tested for the ability to replicate in whole plants and in protoplasts, and for the capacity to express viral coat protein and the 29K and 23K nematode-transmission proteins. These experiments indicated that sequences present in the first 81 nucleotides of RNA2 are essential for it to be replicated, although deletion of some of the downstream sequences (between bases 81 to 170) reduces the efficiency of RNA2 replication. It also has been confirmed that the presence of a putative stem-loop structure located immediately upstream of the start site of the coat protein sgRNA, is essential for coat protein expression but is not involved in expression of the 29K and 23K proteins.

Plant viruses have become useful tools in agricultural biotechnology, for example by providing active

sequences (cauliflower mosaic virus 35S promoter, TMV  $\Omega$  translational enhancer, tobacco etch virus proteinase) or as high copy number, extrachromosomal gene expression systems (TMV and PVX). TRV possesses a number of properties which makes it a promising candidate as a gene vector. Firstly, although it has two genomic RNAs, RNA1 provides all the functions for virus multiplication, gene expression and movement in the plant. Thus, RNA2 can be extensively modified without affecting the viability of the virus. Secondly, our previous work has shown that the highly active coat protein promoter (for synthesis of the sgRNA) is separate from sequences involved in replication of the virus, and that the PEBV promoter is active when inserted into TRV and vice versa. A TRV-based gene expression vector has been constructed in which both of the non-structural (37K and 33K) genes have been deleted from RNA2 of TRV PpK20. In addition, a fragment carrying the PEBV coat protein promoter has been introduced immediately downstream of the TRV coat protein gene. As a first step in assessing the potential of this system, the gene for the green fluorescent protein (GFP), from the jellyfish Aequorea victoria, has been inserted next to the PEBV promoter. Inoculation of this construct (together with RNA1) onto plants results in infection by the virus throughout the leaf and root systems, and the concomitant expression of GFP. The GFP is expressed at a sufficiently high level that the green fluorescence is visible by eye when the plant is illuminated by UV light (Fig. 4). In contrast to some other plant viruses, the tobraviruses have evolved a very efficient mechanism for movement into and spread throughout the root system (a reflection of their strategy of nematode transmission). By studying the pattern of GFP fluorescence using the confocal laser microscope, we have shown that TRV moves to the root tip and emerges from the plant vascular system at the position where the phloem tissue is unloading solutes. This work is likely to progress in several directions. The practical use of this modified virus as a gene delivery system will involve the replacement of the GFP gene with other genes encoding, for example, proteins involved in the protection of plants against pests or pathogens. In addition, an exciting area for future scientific study will be the investigation of the virus genes which allow tobraviruses to move so efficiently into roots but which are lacking in many other types of plant virus.

#### John W. Crawford

An outstanding feature of biological systems is the significance of context for their functioning. Reductionism is a paradigm of the physical sciences where significant progress has been made with systems that can be 'decomposed' into a number of simpler subsystems. These can be regarded as essentially isolated and the whole system can be understood as a simple superposition of the behaviour of the parts. By contrast, biological systems may not be similarly decomposed because such weakly interacting subsystems cannot usually be defined. Their functioning is a consequence of the significance of couplings within and between different levels of organisation, and with the system's environment. Most importantly, these interactions are generally not linear in nature and many of the conceptual devices developed in the physical sciences such as reductionism, linear superposition and determinism are not universally appropriate. The work under the Plants, Soils and Environment theme is strongly integrative in character, and addresses these issues in the context of several related topics.

The soil-plant-microbe complex is the epitome of a natural system strongly influenced by its environment, in this case the physico-chemical environment of the soil. Our research has explicitly demonstrated the two-way interaction between soil architecture and biological functioning. These interactions provide the capacity for self-organisation in the system, and explicitly demonstrate emergent behaviour arising at the system scale as a consequence of fine-scale interactions.

Plants interact with each other locally *via* competition for heterogeneously distributed resources, and both locally and distally through geneflow. The balance

between coexisting individuals within a community depends on the distribution of phenotypic traits among individuals, as well as the fate of novel genes or individuals that enter the population. Thus, the dynamics of populations are driven by the properties of individuals and by couplings which operate across a broad range in spatial and temporal scales. This complexity presents significant challenges to experimental and theoretical insight, especially where the environment impacts significantly on the dynamics, since the conceptual tools for defining and understanding the equilibria and stability of such systems are lacking.

Our research focuses on determining the nature of the couplings between individuals and on the significance of individualistic behaviour for community-scale dynamics. Experimental approaches are being developed in tandem with novel theory to provide the necessary synthesis to tackle the underlying complexity. The fundamental component of the research programme of this theme underpins SCRI's strategic

research on major environmental issues carried out in partnership with policy makers and industry. Targets include the fate of organic compounds in soil, solute transport in river catchments, reduced-input control of plant-pathogenic nematodes, geneflow in spatially fragmented populations including mahogany and Scots pine, dynamics of *E.coli* O157, and the environmental impact of genetically modified organisms.



## Physics and physiology of plant growth in the soil

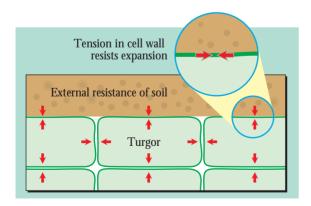
A.G. Bengough, C. Croser, J.M. Kirby<sup>1</sup>, I.M. Young, J. Pritchard<sup>2</sup> & B.M. McKenzie<sup>3</sup>

Soil that is too hard, too dry, or too wet provides an adverse environment for root growth. Crops with root systems that have been restricted, may not obtain sufficient water or nutrients for optimum yield. This represents an enormous economic cost, but also a very real human problem, as some of the most physical-

ly degraded soils are found in the poorest countries of the world.

Relatively little attention has been paid to the selection of plants with root systems adapted to poor soils. Indeed, most physiological experiments study growth in hydroponics, or in other unrestrictive growth media. By understanding the physiological and biophysical processes of growth in soil, we can provide a rationale for evaluating potential gains from selecting plants with root systems that are better adapted for particular conditions. By determining the physiological responses of roots to adverse conditions we can also relate laboratory experiments to real field conditions.

Root growth occurs as a result of cell expansion. This is a process driven by the hydrostatic pressure, or tur-



**Figure 1** Schematic diagram showing pressures acting in the epidermis of a root growing in soil.

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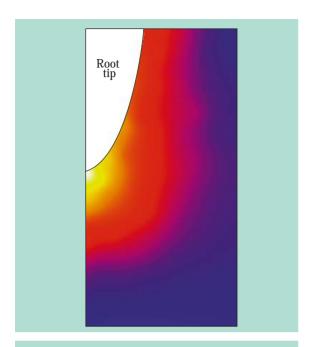
gor, within the cells of the root. Large pressures are exerted, of up to 1 MPa, which is about five times greater than the air pressure within an average car tyre. This pressure explains why roots are capable of cracking concrete and lifting paving stones. The rate of root growth depends on the stiffness of the cell walls in the growing zone of the root, the

turgor pressure, and the external pressure of the soil (Fig. 1).

When roots grow in compacted soil, the external soil pressure can exceed 1 MPa, slowing the root elongation rate. The roots become fatter and the surface of the root may become distorted where particles of soil exert large point pressures on the root surface (Fig. 2). We have found that the growing zone of the root becomes shorter in compacted soil, so that there is a shorter region of root that is expanding actively. When the root grows out of the hard soil into looser soil, the elongation rate increases only slowly for a



**Figure 2** Roots grown in hard soil (top) become thicker and more distorted than those grown in loose soil (bottom).

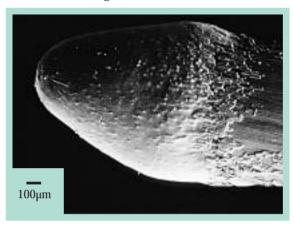


**Figure 3** Stress distribution predicted around a simulated pea root (blue represents low stress, yellow high stress).

period of several days. This shows that the root elongation is not just limited by the external pressure of the soil, but by some physiological response of the root that is present even after the pressure is removed. We measured the turgor pressure in the pea roots that had been impeded, using a pressure probe, and found that the pressure was the same as in unimpeded roots. This shows that the pressure driving cell expansion is the same in the two sets of roots, and suggests that elongation is slower because the tension in the cell wall is greater, due to a stiffening of the cell wall. Tension in the walls of expanding cells often exceeds 100 MPa (about two orders of magnitude greater than the tension in the wall of a car tyre), and it is now thought that cell walls play a major role in controlling cell expansion.

Given that roots can generate large pressures, is there any design of root that transmits this pressure to the soil in a more efficient manner? To answer this, we need to determine the stress distribution in the soil, and how it is affected by the geometry and physical properties of the root surface. In collaboration with CSIRO, Canberra, we are using critical state soil mechanics with finite element models to determine the stresses around a root (Fig. 3). Preliminary findings have shown that doubling of the root diameter can decrease the peak stress at the tip of the root by a factor of two. This means that the thickening of roots in hard soil will decrease the pressure on the centre of

the root cap - a result which is of considerable importance regarding the ability of roots to penetrate hard soils. The coefficient of friction between the soil and the root surface has a major effect on the resistance experienced by the root. We have shown experimentally that roots experience much less friction than metal probes, and we believe that sloughing of root cap cells plays a major role in decreasing this component of friction (Fig. 4).



**Figure 4** Maize root tip covered in mucilage and sloughing root cap cells (reproduced with permission from *J. Exp Bot* **48**, p891).

Shoot growth is often decreased in compacted soils, indirectly via a reduction in water and nutrient uptake, and directly via root-shoot signalling. The effect of soil hardness is difficult to separate from the effects of water content, because a dry soil is normally a hard soil. To separate out these effects, we measured the leaf extension of wheat and barley grown in sand, and found that compressing the sand (increasing the resistance to root growth) decreased the leaf elongation rate within minutes. The sand was saturated with aerated nutrient solution at atmospheric pressure, and so only the strength of the growth medium changed. The mechanism behind this response is unknown but means that plants grown in hard soils can somehow sense the strength of the soil and communicate this information to the shoot.

We have made considerable progress in understanding the physics and physiology of plant growth in soil. This understanding also gives us valuable information on the mechanics of plant tissues that may be applied in apparently unrelated areas, such as that of fruit and vegetable texture and handling properties - a subject of considerable interest to consumers and the food processing industry.

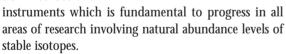
## Developing an interpretation for $\delta^{15}N$ in plants

L.L. Handley, A. Johnston, R. Neilson, D. Robinson & C.M. Scrimgeour

The Unit for Stable Isotope Studies in Biology uses all levels of isotope enrichment as research requires, but specifically investigates the conceptual, methodological and instrumentation approaches to complex problems using the naturally-occurring levels of the biologically important stable isotopes (i.e.  $^{15}\mathrm{N}/^{14}\mathrm{N}$ ,  $^{13}\mathrm{C}/^{12}\mathrm{C}$ ,  $^{18}\mathrm{O}/^{16}\mathrm{O}$ ,  $^{2}\mathrm{H}/^{14}$  and  $^{34}\mathrm{S}/^{32}\mathrm{S}$ ). Here we report mainly on research into the interpretation of isotopic compositions of C ( $\delta^{13}\mathrm{C}$ ) and N ( $\delta^{15}\mathrm{N}$ ) in plants and plant N sources. This research lies in four major areas of plant research: (1) the use

of  $\delta^{15}N$  as a genetic trait for correlation with molecular markers; (2) explaining the isotopic signatures of  $^{15}N/^{14}N$  (and

 $^{13}C/^{\hat{1}2}C)$ terms of plant physiological mechanisms and plant N nutrition; (3) linking physiological mechanisms to molecular markers via interpretation of isotope values; and (4) the continuing development of new chemical preparation methods and



Our biological isotope research is done in a variety of environments, e.g. microbial cultures, highly controlled glasshouse studies and designed sampling of the natural environment in terrestrial and aquatic systems.

Two major questions underpin all of our research on  $\delta^{15}N$ : (1) what are the extent and causes of  $\delta^{15}N$  variations in plants and plant N sources; and (2) how can their mechanisms be quantitatively modelled?

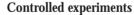
Elements artificially enriched in their heavier stable isotope have been used for many years as tracers in biological systems. These can be used to describe the amounts of an element which move from a source to a sink. The naturally-occurring levels of stable isotopes (identified by the  $\delta$  notation), and especially those of N, C, O, H, and S, can be used to investigate processes within complex biological systems. These processes can be opaque to more traditional techniques, such as measuring changes in mass amounts, and difficult to study using heavy-isotope-enriched tracers. If enough information is known about the system under investigation, the naturally-occurring levels of stable isotopes

can be used with a socalled 'fractionation' model to provide a combined description of the processes and source-sink relationships of the element. In moving from the use of enriched tracers to natural abundance levels, the conceptual framework

changes dramatically, and the limits to interpretation are different. Additionally, the required instrumentation and sample preparations are, at once, more sophisticated and less well developed.

Enriched tracer methods have a conceptual framework, well-established mechanistic interpretations and comprise off-the-shelf technology for biological studies. Interpretation of natural abundance level iso-

topes has no such firm basis yet, and constitutes a new area of basic research, whose results will provide a new means of understanding some of the more subtle biological processes.



 $\delta^{15}N$  of barley as influenced by genotype and abiotic stresses. Wild and cultivated barley genotypes were ranked by their  $\delta^{15}N$  values after growth in highly controlled hydroponics on  $NO_3^-N$  as the sole N source. This work  $^{1,2}$  showed that barley shoots expressed large differences in  $\delta^{15}N$  which were correlated with genotype, with salt stress and with genotype interacting with stress. Whole plant  $\delta^{15}N$  for barley genotypes subjected to the stresses of drought and N



deficiency, also expressed a large range of values, and a variety of intra-plant partitioning patterns of  $\delta^{15}N,$  which were correlated with genotype, stress and the interaction of these. While  $\delta^{13}C$  has been used as an index of stress tolerance (water loss *versus* carbon gained) in a great many studies, this was the first systematic demonstration that plant  $\delta^{15}N$  is genetically and environmentally determined, rather than being directly and solely a function of the  $\delta^{15}N$  of the plants' external N source.

As a first step in explaining the mechanisms underlying the plant  $\delta^{15}N$  values obtained from these experiments, Robinson et al.3 developed a theoretical model explaining the patterns of intra-plant  $\delta^{15}N$  resulting from primary assimilation of NO<sub>3</sub>-N. This model is based on three simple rules: (1) when an N pool divides without transformation, there is no change in  $\delta^{15}$ N values of pools (this reflects that little fractionation is associated with transport processes); (2) when an N pool divides by enzyme-catalysed transformation (e.g. during NO<sub>3</sub> reduction catalysed by nitrate reductase), the resulting  $\delta^{15}N$  values depend on  $^{15}N/^{14}N$ fractionations as approximated by a Rayleigh-type equation; and (3) when two N pools mix (e.g. when reduced N transported from shoots to roots mixes with that already present in roots), the resulting  $\delta^{15}N$ value is a mass-weighted average of  $\delta^{15}N$  values of the component pools.

Despite the simplicity of these rules (and the restriction to plant growth on  $NO_3^-N$ ), this initial  $^{15}N/^{14}N$ fractionation theory for use of a single chemical type and isotopic source of N (NO<sub>3</sub>-N of a known and constant  $\delta^{15}N$ ) is more complex than that for e.g. plant  $\delta^{13}$ C which can be largely explained by a single, simple equation<sup>4</sup>. The new model for  $\delta^{15}$ N requires 30 sets of equations, cannot be condensed into a single expression, and must be solved numerically. The processes it describes include: NO<sub>3</sub> reduction in roots and shoots; transport from roots to shoots of unreduced and reduced N; transport in the opposite direction of reduced N; and the efflux of reduced and unreduced N from roots, the only plausible mechanism for a net <sup>15</sup>N/<sup>14</sup>N discrimination between the plant and its N source. It provides estimates of these processes which match the calculated  $\delta^{15}N$  values of certain N pools (e.g. shoot and root total N) to those which have been measured in a particular experiment. In reality (even in potted plants in glasshouses), the available N pool is much more complex, commonly comprising NO<sub>3</sub>-N, NH<sub>4</sub>-N and various types of organic N, as well as influences on assimilation by other organisms such as  $N_2$ -fixing microbes, various pathogens, mycorrhiza-forming fungi (see below) and abiotic stresses. This means, *inter alia*, that the net plant response in terms of  $\delta^{15}N$  is composed of a time-and-mass-averaged, net  $\delta^{15}N$  value related to the assimilatory processes of all of the types of N used by the plant, and as moderated by environmental and biotic influences. This is presently uninterpretable in the field. Understanding the mechanisms related to  $NH_4^+N$  nutrition is the next goal, to be followed by modelling the assimilation of mixed N sources (chemical and isotopically mixed) and then relating these models to perennial as well as annual plants. In the meantime, controlled experiments have been done to understand better biotic influences on plant  $\delta^{15}N$ .

In a separate experiment, Robinson & Conroy<sup>5</sup> found that the plant itself influences the  $\delta^{15}N$  of its own rhizosphere during growth and does this in proportion to the adequacy of available soil water.

Biotic influences on plant  $\delta^{15}N$  One of the applications of  $\delta^{15}N$  in terrestrial plant-soil studies was to use the  $\delta^{15}N$  of a potentially  $N_2$ -fixing plant to calculate how much of its N came from  $N_2$ -fixation by symbiotic microbes. This, and other uses of  $\delta^{15}N$  as a 'pseudo-tracer' of N sources, has come under periodic criticism from many researchers. We conducted a glasshouse experiment<sup>6</sup>, comparing estimates of the amount of N fixed using three enrichment levels of N (natural abundance, 0.5 atom % and 5 atom %) with a *Rhizobium*-clover symbiosis. Even in closely controlled glasshouse studies,  $\delta^{15}N$  was unacceptably variable and inaccurate as a 'pseudo-tracer'.

It has long been assumed that  $N_2\text{-fixation}$  incurs no isotopic fractionation and that the  $\delta^{15}N$  of atmospheric  $N_2$  fixed by microbes associated with vascular plants is nil. We have shown recently that  $N_2\text{-fixation}$  can be accompanied by  $\delta^{15}N$  values ranging from 0% to -4.4% and that the net  $\delta^{15}N$  value is correlated with the type of  $N_2\text{-fixing}$  enzyme (nitrogenase) which is most active. This new information will have a considerable impact on perceptions of the influence which  $N_2\text{-fixation}$  has in determining net  $\delta^{15}N$  in plants, ecosystems and palaeo-studies.

Following a field report by Högberg<sup>8</sup>, that foliar  $\delta^{15}N$  at an African site correlated with type of ectomycorrhiza, we conducted two controlled experiments<sup>9,10</sup> using arbuscular mycorrhizas (AM). These showed that infection by AM-forming fungi could influence the whole plant  $\delta^{15}N$  by as much as 3‰ and that the

largest influences on plant  $\delta^{15}N$  (studied in these experiments) were species of fungus and external N concentration. Amount of fungal infection had no effect on plant  $\delta^{15}N$ . New experiments will test the extent of  $\delta^{15}N$  variation induced by drought and fungal infection on plant  $\delta^{15}N$ .

 $\delta^{15}N$  (and  $\delta^{13}C$ ) were used to assess the effects of biotic stress caused by pathogens, where *Petunia hybrida* was infected with a systemic virus, nematodes and a combination of virus and nematodes. Pathogen-induced effects were not confined to the sites of virus infection. All treatments resulted in a depletion of shoot and root  $^{15}N$  compared with uninfected controls, and pathogen loading altered the  $\delta^{15}N$  of whole plants, shoots and roots. In the double infection treatment,  $\delta^{15}N$  patterns suggested that N nutrition was impaired.

Controlled experimentation has, thus, established minimum ranges of  $\delta^{15}N$  variation and some of their proximate causes. This work underscores the futility of using  $\delta^{15}N$  as a 'pseudo-tracer' of N sources in plant, soil and animal studies and emphasises the process-based nature of  $\delta^{15}N$ .

#### Field-based studies

The overall approach of the Stable Isotopes Unit is iterative, from field observation to controlled experiments (designed on the basis of field results), and then field verification of experimental results. Our first large field study was done over three full years, examining the spatial and seasonal  $\delta^{15}N$  of the vegetation of a field in the first stages of primary succession<sup>11</sup>. The plants comprised legume-rhizobia symbioses and non-N<sub>2</sub>-fixers, woody components and a herbaceous sward, xerophytes and mesophytes. This study established that the type of chemical N source used by the different plant species and life-forms could not be determined on the basis of  $\delta^{15}N$  from field samples. It also confirmed the findings of an earlier study in Africa<sup>12</sup> that  $\delta^{15}N$  could not be used to calculate the amounts of plant N derived from N2-fixation. In contradiction to reports from another European laboratory, clear seasonal changes in leaf δ<sup>15</sup>N were documented for all of the plants studied.

In a field study of the native juniper tree, *Juniperus communis*<sup>13</sup>, we found that foliage  $\delta^{15}N$  co-varied with both tree gender and soil moisture content. This suggests that juniper may vary its success in different soil water regimes and that the different soil-moistures are reflected in the foliar  $\delta^{15}N$ .

Soils and soil invertebrate communities The tradition of isotope research has been (and still is, to some extent) based on single samples or at least very little replication. With the advent of the new automated mass spectrometers, it is now possible to analyse >100 samples overnight, making statistically-designed field experiments feasible. However, no work had been done to determine optimum sampling patterns for natural abundance level stable isotopes. We quantified the spatial variability of three soil properties (total N, total C and pH) and two stable isotopes ( $\delta^{13}$ C and  $\delta^{15}N$  of whole soil), using geostatistical techniques in upland Scottish pastures given contrasting management regimes (grazed, fertilised and ungrazed, unfertilised)<sup>14</sup>. The results suggested that to obtain statistically independent samples, a sampling distance of  $\geq 13.5$  m is required for  $\delta^{15}$ N of total soil N.

Following the geostatistical results,  $\delta^{15}N$  (and  $\delta^{13}C$ ) analyses of soil, plant and invertebrate samples showed that the land-use treatments greatly affected the trophic interactions of soil invertebrates and primary producers<sup>15</sup>. These data also showed that the total soil  $\delta^{15}N$  changed seasonally and was not, therefore, a constant background value, as previously assumed. Seasonal declines of  $\delta^{13}C$  and  $\delta^{15}N$  were detected in earthworms and slugs and may reflect previously unsuspected invertebrate behaviour.

 $\delta^{15}N$  fractionation modelling for aquatic studies The concentration of nitrate in the River Ythan catchment, north-eastern Scotland, is above 10 mg NO<sub>3</sub>-N l<sup>-1</sup>. Information is sought on the source of the nitrate and the extent to which the nuisance blooms of the green seaweed, Enteromorpha sp., are dependent on the high levels of nitrate entering the estuary. We are using stable isotopes to investigate the movements of nitrogen through soil N pools, streams and the river into the estuary. Theoretically, as the nitrate moves towards the estuary, its  $^{15}N/^{14}N$ isotopic composition should change. By characterising the isotopic composition of the nitrogen in each major pool and the growth and isotopic composition of aquatic plants, the movement of inorganic nitrogen will be modelled.

In parallel with this study is the characterisation of the isotopic composition of the oxygen atom in the nitrate. Naturally derived and industrially produced nitrate have distinct  $\delta^{18}{\rm O}$  values. The addition of inorganic N fertilisers to a number of test fields located close to targeted burns will allow us to track the movement of nitrate by the  $^{18}{\rm O}$  composition. This

approach is further strengthened by the characterisation of the isotopic composition of the inorganic carbon in the river and estuary water. Carbon dioxide is the primary substrate for photosynthesis. By tracking the seasonal changes in the isotopic composition of organic carbon in the seaweeds, we will be able to define the major growing season of the algae and relate this to the acquisition and assimilation of inorganic nitrogen.

#### Chemistry

The foregoing projects exploiting stable isotopes depend on our ability to measure stable isotope levels in a range of samples. For the large number of plant and invertebrate samples studied in our laboratory (up to 25,000 per year), we use continuous flow systems with elemental analyser sample converters (see analytical facilities entry). This approach allows us to make most of the measurements we need, but there is a clear need for isotope data which can not be met with existing methods. This is addressed by an active research effort aimed at developing novel instrumentation and sample preparation methods, to meet both immediate and future needs.

We have successfully completed an EU-funded project to extend continuous flow methods to hydrogen and oxygen isotopes. Full exploitation of this methodology may be some time in the future, but it has already proved robust in collaborative research on the energy expenditure of foraging bumble bees, using stable isotope labelled water. The sample conversion methods developed in this project will also find application in compound specific analysis of soil N species. This is a difficult problem, to which solutions will be wel-

comed in our laboratories and in the wider community of stable isotope users.

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## Research services

## **Administration Department**

#### R.J. Killick

The Administration Department is responsible for not only the day-to-day smooth running of the Institute, but also for longer-term assessments and planning. Its work falls into two overlapping areas; personnel and finance. The overlap comprises the 66% of net expenditure on staff costs, not counting the labour cost in contracted-out services. Virtually 80% of our Grant-in-Aid is required for core staff pay costs.

Not surprisingly then, considerable importance is attached to the Personnel Section's work. Apart from recruiting staff, it is responsible for their induction and probationary oversight and for monitoring absences, especially those attributed to ill-health. It also arranges training, accommodation of visitors and new staff and various other welfare-type issues. Since the delegation of pay and grading in 1994, it also handles job evaluations upon request, of which there were 11 during the year. At any one time, there are about 60 research students working with the Institute and Personnel takes an active rôle in monitoring their progress.

The Institute is subject to considerable financial pressure; the Grant-in-Aid, which still represents two thirds of its income, has increased by only 3.1% in 5 years. Thus the prudent management of our income and expenditure is a critical activity, albeit one subject to increasing tensions as budgets shrink in real terms. Capital grant from SOAEFD was £437,000 of which a substantial portion was spent on computing equipment.



## **Analytical facilities**

W.W. Christie

#### **Laboratory Accreditation**

Several laboratories within the Chemistry Department operate a formal Quality System, certified to BS EN ISO 9002 by SGS Yarsely International Certification Services Ltd. The stable Isotopes Facility and Lipid Analysis Unit of MRS Ltd were registered in December 1996, and the Gas chromatograph-Mass spectrometry Laboratories were registered in June 1997.

A Quality Plan for a generic Quality System for SCRI has been adopted and will be implemented throughout the Institute. A copy is included in the Institute's Corporate Plan. The Quality Plan 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 electronic archival facility has been set up within the Chemistry Department, based on the use of a compact disc (CD) writer installed in a personal computer. 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, and at maximum transfer rates, a CD can be written in less than 40 minutes. Two copies are made, one for the owner of the data, one for the archive.

#### **Stable Isotope Facility**

Stable isotopes are now basic tools 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. With these, we can tackle most of the biologically-important, low atomic number elements - <sup>13</sup>C, <sup>15</sup>N, <sup>18</sup>O and <sup>34</sup>S in a wide range of solid, liquid and gas

samples. All the instrumentation is based on continuous-flow isotope-ratio-mass spectrometers that are fully automated and operated through computer data systems. Automation allows a high through-put of samples, essential for many biological experiments where large data sets are required. For solid samples, the Europa Scientific Tracermass and 20-20 mass spectrometers are interfaced to Roboprep CN and ANCA-NT SL combustion sample converters. A Roboprep G+ gas purification unit is used for gas analysis. Plant samples of one to five milligrams are used, containing 25 to 100mg 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 sample types. 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 commissioned programme.

#### **Mass Spectrometry**

The Institute is particularly well equipped in the field of mass spectrometry (MS), with three state-of-the-art instruments devoted to structural analysis of organic compounds. Housed in a purpose-built laboratory suite, all systems have integrated computer control, library search capabilities and distributed data processing facilities. The core instrument is a Hewlett Packard 5989B MS ENGINE research-grade quadrupole instrument. The mass spectrometer has electron impact and chemical (positive/negative) ionisation modes with a mass range of 2000 amu, together with an autosampler and distributed processing software which will permit off-line data processing and reduce operating costs. It also has a particle beam liquid chromatography (LC)/MS interface which will complement existing LC/MS instrumental techniques. This instrument can provide mass and structural data on a wide range of organic compounds.

In addition, a bench top instrument is dedicated to the analysis of organic volatiles. This consists of a Perkin Elmer automated thermal desorption system (ATD) linked to a VG TRIO-1000 quadrupole gas chromatograph (GC)-MS and permits detailed char-

#### Research services

acterization of the profiles of organic volatiles generated by biological systems.

A Finnigan SSQ 710C dedicated LC-MS instrument, with atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) interfaces, completes the facility. This has an ability to analyse samples whose high molecular weight, lack of volatility or polarity, precludes analysis on the other instruments. APCI and ESI are soft ionization techniques and generally only produce molecular ions, e.g. [M-H]<sup>+</sup> or MH<sup>+</sup>, 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 fatty acids. The facilities are operated by experienced and expert staff, ready to tackle and solve most structural problems. They are actively seeking full laboratory accreditation status and working practices are commensurate with recognised standards.

During 1997, the Institute's ISO 9002 certification was extended to include the analytical operation of the two GC/MS systems. This involved the successful inhouse updating of instrument computer and archival facilities to appropriate levels, together with the required documentation, including a quality plan and standard operating procedures.

#### **Biomathematics & Statistics Scotland**

R.A.Kempton & J.W.McNicol

**D** ioSS is a leading international centre in the field Bof mathematics and statistics applied in the biological sciences. Originally established in 1987 as the Scottish Agricultural Statistics Service (SASS) to support the R&D programmes of the Department of Agriculture for Scotland (now SOAEFD), it provides research, consultancy and training to the SABRIs, SAC and other agricultural, biological and environmental organisations in Scotland, through a network of 30 specialists based in Edinburgh, Aberdeen, Ayr and Dundee. BioSS has its headquarters on the King's Buildings science campus of the University of Edinburgh, but for administrative purposes, it operates as a Unit of SCRI and its Director (Rob Kempton) reports to the Director of SCRI. BioSS publishes an annual 3-year Corporate Plan and a Biennial Report.

The BioSS programme covers four main objectives as follows: consultancy 46%; research 33%; technology transfer 16%; training 5%. These objectives are closely linked and synergetic. Thus, research into development and novel application of statistical/mathematical methods supports and is stimulated by consultancy work; statistical and mathematical methods, which have proved their usefulness in public-sector research, can be promoted more widely through technology transfer; and the training programme, though representing a small proportion of BioSS overall activity, provides an efficient mechanism for upgrading scientists' knowledge and skills, complementing the provision of individual advice.

BioSS research ranges widely to cover most of the R&D programmes sponsored by SOAEFD. Particular strengths are in design and analysis of crop, animal and sensory experiments; analysis of images from microscopes, medical scanners and remote sensing; modelling epidemics in plants and animals; statistical genetics and bioinformatics; and environmental modelling, including wildlife management and monitoring water quality. There is also a growing involvement in

the social sciences, oriented to consumer, psychological and behavioural studies. This research involves extensive interaction and collaboration with scientists in SABRIs and SAC, and with UK universities and international research laboratories. BioSS also runs a successful postgraduate research programme with 10 PhD students currently linked to five Scottish universities.

The BioSS unit at SCRI is led by Jim McNicoI and consists of three core-funded staff. In addition, two research assistants undertaking PhDs are funded through SOAEFD's Flexible Fund, and a postdoctoral research scientist, funded by the BBSRC initiative for Genetics of Agriculturally Important Traits, is currently being appointed. The Unit has particular strengths in statistical genetics and bioinformatics. Current research topics include methods for mapping quantitative trait loci in tetraploid species such as potato; identifying the degree of similarity among species based on molecular marker data; and phylogenetic methods, including identification of mosaic sequences (formed by recombination), in bacteria and viruses. Other research is in spatial and temporal modelling, including the estimation of trends and competition in field experiments using smoothing methods and models for vegetation dynamics. Staff based at BioSS Headquarters also collaborate with SCRI scientists in projects relating to the interpretation of images from magnetic resonance microscopy, the modelling of crop epidemics, and the sensory assessment of soft fruit. One result of this collaboration is the publication of 27 papers in refereed journals and 10 conference presentations over the last 3 years (1995-7).

BioSS received a core grant of £635k from SOAEFD in 1997/8. Receipts from SOAEFD Flexible Fund and work for Scottish Agricultural Science Agency are estimated at £187k and external income at £170k. The income of the Dundee Unit in 1997/8 was £74k, in addition to its core funding

## **Engineering and Maintenance Department**

S. Petrie

The Engineering and Maintenance Department **■** 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 Department 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 Department 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 Department'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 Department 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.



Adding the finishing touches to a refurbished laboratory.

During 1997, several areas of the Institute were refurbished to either enable new and expanded areas of work to be carried out or to simply improve the existing facilities. The main projects undertaken were as follows:-

**Building V** This project involved the conversion of an 850 m<sup>2</sup> building, previously used for crop storage, to a purpose-designed facility incorporating fourteen laboratories and six offices to accommodate the Institute's Unit for Soil-Plant Dynamics.

To minimise costs, Engineering and Maintenance staff carried out all the electrical, plumbing and heating services work, the telephone and Category 5 computer network cabling, and all joinery works, including the fitting out of laboratories and all painting works.

This was by far the largest refurbishment project ever undertaken which involved such a scale of dependence on our own staff and required several months of work. During this time, normal duties also had to be carried out and an extremely heavy burden was placed on the staff. It is to their credit, and a measure of their commitment to the Institute, that services were maintained during this time and that such a noticeably high standard of work was achieved within this project.

**Building B** This project involved the refurbishment of much of the ground floor of this  $1300~\text{m}^2$  building. Seven laboratory areas and three offices were upgraded and the confocal and electron microscopes relocated to enable the Institute's microscopy unit to be housed in a single location.

Upstairs, two laboratories were combined to form a single laboratory with over  $80~\text{m}^2$  of floor space. The new laboratory is now being used by a scientific unit within the Virology Department.

This project also relied heavily on engineering staff, with several areas of the work being carried out entirely in-house.

Mylnefield Research Services - New Facility Engineering and Maintenance staff's involvement in this project included site clearance, provision of site services and installation of a structured telephone and data cabling system.

Buildings D and N One of the main laboratories within this building, used by the Fungal and Bacterial Plant Pathology Department, was completely refurbished along with a major upgrading of the Department's quarantine facility within their glasshouse building.

**Building M** Prior to the previously described conversion work within Building V, a suite of elderly cold rooms occupied part of that building. Relocating such rooms was not economically viable and, as a result, a section of Building M was re-wired to house a new suite of seven cold rooms.

**Building P** This in-house project involved the refurbishment of two of the Nematology Department's insect rooms. New air conditioning units were installed, along with more energy efficient and higher output lighting systems.

**Telephone System and Internal Wiring** The Institute's electronic exchange was upgraded and digital telephone lines installed.

Problems were also being encountered with the main telephone cabling within the Institute, due to water ingress. Over 1997, our own engineering staff replaced many of these cables, resulting in much clearer lines throughout the site. Provision for future expansion of the telephone system has also been catered for within the new cables.

Computer Networking Apart from the areas previously described, several additional areas were either added to the Institute's network using Category 5 installation standards or upgraded from the previous network wiring arrangements. This process is ongoing and, despite budget restraints, will continue over the next few years.

The Department is also responsible for negotiating utility contracts with electricity, gas, water and telephone companies, and economies have been gained in these areas through reducing tariffs and lowering consumption where possible.

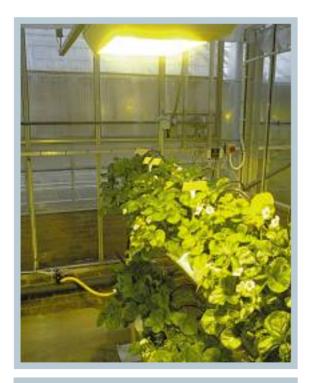
A number of external service contracts have also been discontinued, or the cover provided reduced. Inhouse maintenance cover has been extended to counter-balance such measures and to minimise any reduction in the service provided to staff.

# Estate, Glasshouse & Field Research Department

G. Wood

iodiversity, vegetation dynamics, and spatial dis-Birding, 138 associated with a novel research initiative planned for the next decade. A 10 ha, broad-leaved wood was established by the Novel Crops Unit, with 22,000 trees planted and one mile of deer and rabbit fence erected during the latter months of 1997. The main species of oak, ash and birch occupy an unique lowland site in a field area taken out of intensive arable cropping. Modern research technologies will be utilised in this large-scale, above- and below-ground level, ecosystem study. Techniques, including DNAprofiling, marker geneflow, non-linear mathematical modelling, biogeochemical changes and natural abundances of stable isotopes, will be used to study species competition, utilisation of resources and soil/plant/microbe/invertebrate interactions during the 'bioremediation' of this fertile site.

The SFPC/MRS strawberry breeding programme expanded and changed its approach to the environment under which selections are made. In the field,



New glasshouse strawberry system.

this resulted in the adoption by the Soft Fruit Trials Unit of a system of raised beds, and laying of irrigation tape and plastic mulch, followed by machine planting in twin, staggered rows. In the glasshouse, a new growing system was established for research purposes in a complete wing of four Cambridge cubicles. This required erection of two-tier gantries holding plants in growbags, with totally automatic fertigation, heating, lighting, venting and thermal/shade screen environment control. In the field, these changes were implemented to allow selections to be made under conditions mimicking those in which any new cultivars would subsequently be grown commercially. The changes also served to establish a much closer working relationship between colleagues in the SFPC/MRS and EGFR Departments: a relationship further enhanced by regular briefing and feedback meetings of the respective soft fruit breeding, research, glasshouse plant production and field trials unit team leaders.

George Dow retired after 9½ years of service in the Glasshouse Research Unit. He was largely responsible for the significant improvements achieved over the last 4 years in throughput and quality standards associated with plant production for, and consequential success of, the various blackcurrant, raspberry and strawberry breeding and research programmes at the Institute.

On 14 August 1997, the first joint SCRI/SAC potato field trials Open Day (major sponsor The British Potato Council) was held at the SCRI's site at Gourdie farm. More than 250 people, representing the many and varied interests of the potato industry,



Potato trials open day.

attended the event. They were given a guided tour of the trial plots (including cultivars, fertiliser rates, disease screening, tuber size distribution, and seed multiplication) and were addressed by the various specialists involved with each of the trials. There was time allocated after the tours for individuals to question those undertaking the work in more detail. As well as inspecting the plots, static displays were on view from SCRI, SAC, SASA, and others, on various aspects of potato research and development being conducted by these organisations. The success of the event was due to support from several commercial and grower organisations, the SSCR, and also, in no small part, to David Jack and colleagues in the Field Research Unit of the EGFR Department, Tim Heilbronn of the SLIS Department, and Philip Burgess, Alistair Donald and colleagues from SAC.



Opening of the CEUG Conference (I to r) Director; Councillor Peter Mulheron, Perth & Kinross Council; Peter Gill

The annual meeting of the UK Controlled Environment User Group (comprising engineers and manufacturers, university, commercial and research organisation users) was held at the Institute on 16 and 17 September 1997. This was the first time in the 30+ year history of the Group that this event had been held in Scotland at one of the SABRI centres. Business, paper and exhibit sessions spanned the 2 days. The conference was almost single-handedly organised by Peter Gill (Glasshouse and Controlled Environment Unit Manager) and was supported by SET, Perth and Kinross Council, and Dundee and Angus Tourist Board. More than 50 people attended the very successful event, at which highlights included papers on new technologies in enclosed environment research for experimentation in space, and controlled ecological life support systems, given by Professor T W Tibbitts (University of Wisconsin, USA) and Professor M A Dixon (University of Guelph, Canada), respectively.

Agronomy research field trials by the Arable Trials Unit concentrated on winter and spring barley in 1997. In a spring malting barley small plots trial, cultivars Landlord and Optic were comparable with cultivar Chariot in terms of yield. Yields of just over 7 tonne ha<sup>-1</sup> were achieved with all varieties at seed rates calculated to produce from 300 to 350 plants m<sup>-2</sup>. On a large field scale, however, Optic was 2 to 3 days later, outyielded Chariot by on average over 0.5 tonne ha<sup>-1</sup>, and had lower grain nitrogen levels. In the winter malting barley small plots trial, cultivar Regina outvielded Melanie by over 0.5 tonne ha<sup>-1</sup>, although the former had higher grain nitrogen and screening levels. In this trial, both those cultivars were superior to cultivars Rifle and Spirit and the standard cultivar Halcyon was in a poor last place. In a seed rate trial using Melanie, highest yields were attained at a seed rate calculated to produce 350 plants m<sup>-2</sup>. Yields decreased but grain N levels increased at the lowest and highest target densities used in this trial (200 and 450 plants m<sup>-2</sup>, respectively). The effects of nitrogen fertiliser top-dressings on Melanie were tested at a range of total rates split between applications made at the start of spring growth and at the end of tillering. Dressings from 105 to 140 kgN ha<sup>-1</sup> raised yields to close to or above 8 tonnes ha-1. Grain N levels, however, became unacceptably high as the fertiliser rate increased above 120 kgN ha-1. The trial highlighted the need for further investigations on the timing of nitrogen fertiliser applications to achieve an acceptable yield and low grain N levels suitable for malting. A small plot trial to test the effect of a novel plant hormone biostimulant chemical (AXISTM, Mandops (UK) Ltd) on yield of Melanie showed no difference between the treated and untreated plots. However, on a large-scale field trial, utilising 4 ha areas of Melanie, the area treated once with AXISTM at the 3-leaf growth stage outyielded the untreated area by over 0.5 tonne ha<sup>-1</sup>.

This whole series of investigations served to emphasise, yet again, the inherent dilemma of field trialling: small plot trials may detect an effect which does not materialise when put into practice on a large field scale, and large-scale field trials may show a difference that was not detected by small-scale plot trials.

### Scientific Liaison & Information Services

W.H. Macfarlane Smith

The Scientific Liaison and Information Services (SLIS) remit includes the promotion of SCRI and its science wherever appropriate opportunities present themselves. These range from events such as SCOTCROP and POTATO HARVEST '97, through articles in the popular press, magazines and scientific journals to radio and television appearances. As a result, public knowledge of the Institute's work has widened, with consequent benefits for SCRI's reputation. However, the costs of participation in some of these activities has increased to such an extent that SLIS constantly seeks new and cost-effective means of publicising the Institute's many successes.

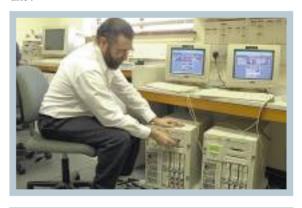
The number of scientists (both public and private sector), students, journalists, etc. visiting the Institute continues to grow. SCRI was delighted to welcome the new Minister of State for Agriculture, the Environment and Fisheries in Scotland, Lord Sewell, who visited shortly after his appointment to familiarise himself with the Institute's research.



Dr. Karl Oparka explaining to Lord Sewell the rôle of plasmodesmata in plant virus transport

The appointment of Dr David Marshall to lead the Unit of Bioinformatics and Information Technology (formerly Information Technology Services) has brought new strengths to the Institute. A major programme of upgrading both hardware and software has now commenced. Over a period, the Local Area Network (LAN) cabling will be upgraded to C5 standard and extended. New servers have been purchased and installed.

Communication, both within the Institute and between SCRI, the Scottish Office and other Institutes, will be improved by standardisation on the software packages, Windows NT and Office 97. This necessitates a substantial investment in upgrading and purchasing of new computers. The number of computer users continues to rise in both the scientific and service areas. Much of the Institute's science is now heavily dependent on the use of such equipment. As a further aid to publicising SCRI, a start has been made on expanding and improving our Web pages (http://www.scri.sari.ac.uk). This will be followed by the creation of Intranet pages to deal with many standard internal procedures and information (e.g. chemical safety data). A review of possible problems with computers and other equipment in the year 2000 has been carried out and a programme initiated to ensure that all such equipment is, or will be, '2000-compliant'.



Ian Black installing software on new Compaq Proliant servers.

The activities and future needs of the Library have been reviewed. Library costs continue to increase in excess of inflation, with inevitable pressure on the number of journals taken and books purchased. Measures taken to minimise any reduction in journals subscribed to, include membership of the Research Institutes Serials Consortium (BRISC) and the Research Councils Library and Information Consortium (RESCOLINC). These consortia have been able to negotiate advantageous prices from the suppliers of both printed and electronic journals. As a result of membership of RESCOLINC, Institute staff



Librarians Sarah Stephens and Ursula McKean accessing an electronic journal.

now have electronic access to the complete stable of Academic Press titles. The demands on the Library continue to increase, with additional access required to the databases, BIDS ISI, EDINA BIOSIS and CAB Abstracts. The number of inter-library loans has increased by 20% and there was a 5% increase in the number of new books obtained. We are grateful to all those who made donations, so allowing us to maintain a comprehensive and modern stock of reference material. Inadequate space in the Library is a growing cause of concern and necessitated the creation of an annex to archive old or less used journals and books. While this has resolved the problem in the short term, further measures will be required at a later date.

The services of the Visual Aids Unit continue to be in heavy demand for a wide range of activities from the provision of routine photographs of gels, through photographs and graphics for publications, brochures, and the Annual Report, to the creation of large-scale displays of the Institute's research and commercial products for conferences, agricultural shows and so on. It is pleasing that the standard of presentation from the Unit receives very favourable comments in comparison with displays produced by private sector organisations. While the rate of increase in the number of such jobs is not as great as in previous years, the complexity of each job is now greater than ever before.

Health and Safety at Work issues continue to have a high profile with the advent of both new and modified legislation and the necessity to demonstrate competence by means of certificated training. Inevitably, this has substantial cost implications. Overall responsibility for Fire Safety has been transferred from the Scottish Office to the local Fire Authority.

The major Dundee contribution to the new Millenium will be the creation of a Science Centre, one of a network of such centres in Scotland. SCRI is a partner in this activity, along with Dundee Council, Scottish Enterprise and the Universities of Dundee, St.Andrews and Abertay. Aimed at creating a greater public awareness and understanding of science, the Centre will have a substantial input from SCRI, demonstrating the latest scientific advances in plant, soil and environmental research. The Deputy Director, Professor Wilson, has been involved in bringing the scheme to fruition and, together with SLIS staff, will have an ongoing committment to its successful implementation.

## Media Kitchen

T.M.A. Wilson & W. Ridley

Cognisant of changing work practices and an increasing emphasis on laboratory-based tissue culture and molecular biological research techniques which require consistent, accredited and high quality standards of media, plasticware etc., a central Media Kitchen facility was established at SCRI at the beginning of fiscal year 1996/97. The daily provision of fresh materials to 12 pick-up and drop-off locations around the SCRI laboratory complex began in earnest during the early summer of 1996 and expanded steadily to accommodate the increasing requirements of the various user groups. The outputs of the first full year of operation of the Media Kitchen facility, 1997, are summarised in Figure 1. Overall, through

Tips Eppendorf tubes Agar plates poured Total items\* 1,393,350 520,095 37,011 41,187

\* 1 item = 5ml LB or 1000ml SSCx20

Figure 1 Media kitchen outputs 1997.

central bulk purchasing agreements, the Media Kitchen has created savings of up to 50% on disposable plasticware and media ingredients. Moreover, innovative scientists, visiting workers, trainees, students and support technicians alike, have been freed from repetitive tasks connected with the preparation of standard microbiological, mycological and plant tissue culture, media preparation. By centralising the production facility, by applying stringent quality controls, and by encouraging interactive feedback from user groups around the Institute, Media Kitchen staff have been able to sustain the highest level of service and customer satisfaction throughout the year. The Media Kitchen operates as a research facility under the central administrative overhead, to minimise bureaucracy; nevertheless, each user site is 'shadow tolled' for its throughput of consumables. The day-to-day operation of the facility is supported and greatly enhanced by the efforts of Walter Burry and Jimmy McMillan, who were recruited from the HELM Project Centre in Dundee and provided with skills training. The range of products, services and facilities made available by the Media Kitchen to all SCRI staff has continued to expand during 1997 to meet an ever-increasing demand for this essential core facility. Media Kitchen



Wendy Ridley and Evelyn Warden (foreground) prepare over 60 different sterile microbiological and plant tissue culture media and pre-pour petri dishes for SCRI staff in all research departments.

staff also collect and recycle glassware, as well as autoclaving used microbiological materials. In this way, it has been possible to secure a standard quality of service amenable to accreditation and resistant to the vagaries and additional expense of Departments establishing their own 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 central media and sterile disposable-ware facility, with daily delivery service throughout SCRI, has proved invaluable both to researchers and to those monitoring costs and assessing value-for-money.

## Mylnefield Research Services Ltd

N.W. Kerby

Institute (SCRI) to enhance competitiveness, understand and fulfil the needs of industry. MRS not only markets the resources and expertise of SCRI, but also undertakes near-market research and development. MRS places particular emphasis on developing partnerships and forging stronger relationships with customers.

MRS acts as the gateway to a variety of skills unique within the UK biological, agricultural and horticultural research services, ranging from fundamental studies on genetics, molecular biology and physiology, through agronomy and pathology, to glasshouse and field trials from a single site. As a technology transfer company, MRS is able to market the scientific expertise and resources of SCRI, and promotes the contribution of science and technology to wealth creation and the quality of life.

Innovation - the introduction of something new - is essentially the exploitation of new ideas, concepts and processes that generate competitive advantage. We aim to improve competitiveness and enhance the future prosperity of SCRI by reducing reliance on Government funding.

#### **Responsibilities of MRS**

- Marketing SCRI's scientific expertise
- Protecting and managing Intellectual Property (IP)
- Developing new markets for SCRI's and MRS's IP
- Licensing
- Providing an awareness of new funding opportunities
- Diversifying the funding base
- Assisting scientists in preparing research proposals
- Negotiating contracts
- Managing external contracts
- Promoting SCRI as a centre of scientific excellence

#### **Mission Statement**

Mylnefield Research Services Ltd will exploit commercially the scientific expertise and resources of the Scottish Crop Research Institute while protecting its charitable status and intellectual property.

**Finance** From the time of incorporation, MRS has been self-sufficient in providing its own accommodation and staffing, achieved without start-up funding, Government subsidy or venture capital.

MRS's major sources of income (1996-1997) are:-

- Contract research (47%)
- Collaborative research (45%)
- Royalties and licence fees derived from commercialising IP (3%)
- Analytical services and consultancy (5%)

Income generated from royalties and analytical services (e.g. Lipid Analysis Unit) is increasing, whereas income from collaborative research as a percentage of total income has and will continue to decline.

Contract research covers projects that are fully funded by a commercial partner to achieve specific targets and accounts for approximately 47% of the total income. Collaborative research projects are funded from the EU and governmental sources which include MAFF, DTI, DoE, The Scottish Office, and Research Councils, sometimes with additional support from industry. Collaborative research projects differ from contract research projects in that the research is often

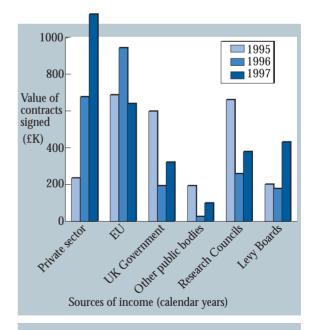


Figure 1 Collaborative and contract research awards.

conducted at more than one site and that the terms and conditions of the funding allow all the partners to exploit IP generated during the project. The portfolio of services that MRS can offer is complex and reflects SCRI's wide range of integrated activities.

The value of contracts from the private sector has steadily increased as we have successfully diversified the sources of funding (Fig. 1).

Since our first financial year (1992) income, which reflects business activity, has increased by 148% (Fig. 2). The rate of growth has slowed down but new measures and initiatives, introduced through investment and consolidation, will ensure that growth is sustained.

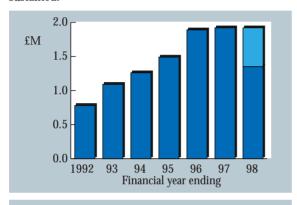


Figure 2 MRS Income.

**Market Development** The marketing action plan of MRS encompasses:-

- continuing to maintain favourable contacts with key customers
- expanding our global network of potential buyers
- prioritising areas where SCRI and MRS can demonstrate their greatest strength for income generation
- developing autonomous units with the ultimate aim of creating independent new companies

Internally, the objectives of MRS are to:

- work closely with SCRI scientists, networking to maintain effective communication
- develop new 'in-house' autonomous units providing commercial services
- realise commercial opportunities which might originate in SCRI or from the market
- protect and manage IP
- facilitate the writing and submission of research proposals

**Project Management** The active role that MRS takes in project management has been acknowledged and appreciated by our customers and has been an important factor in obtaining further funding from existing

customers. Currently, MRS manages 4 DTI-LINK Schemes and 5 EU Projects.

By working closely with its customers, MRS more fully understands their needs and can identify potential areas of further collaboration. We facilitate the flow of information between projects. Where possible, and without compromising confidentiality, we identify synergies between existing projects and integrate academic disciplines to enable the customer to gain the greatest benefit from our combined (SCRI/MRS) resources and knowledge. This is evident by the range and number of industry consortia we have generated.

Protection, Management and Exploitation of Intellectual Property This is a particular strength of MRS. MRS's strategy is consonant with the 1993 Science White Paper (Realising Our Potential - A Strategy for Science, Engineering and Technology Cm2250) which emphasises the importance of wealth creation and technology transfer. SCRI has embraced the need for wealth creation and significant numbers of the staff are actively seeking protection for their innovative research. Frequently, MRS receives requests from other bodies for advice on IP and best practice for commercialising science and technology.

Lipid Analysis Unit A MRS Lipid Analysis Unit was established in October 1995, providing reliable routine analytical services, specialised analyses and contract research projects. The Unit employs one full-time and one part-time technician. It has a current annual turnover in excess of £63,000 with world-wide coverage from customers in the health food, confectionery, pharmaceutical and food industries. The Unit was awarded the ISO 9002 quality assurance standard in 1997, emphasising our commitment to quality. Since 1996, the Lipid Analysis Unit has successfully organised two 2-day courses on the chemistry and analysis of fatty acids. MRS has already begun advertising for the next course that will be held in September 1998.

Collaborative Research MRS places considerable emphasis on developing successful partnerships between the academic/research community of SCRI and Industry to provide wealth creation and to enhance our quality of life. This is exemplified by our participation in four new LINK projects commissioned during 1997.

What is LINK? LINK is the UK Government's principal mechanism for supporting collaborative research between UK industry and the science base. It

Country	Title	Inventor	Application No	Status
PCT	ANTI-VIRAL	J M S Forrest	PCT90/01638	National Phased
Europea	n MATERIAL	D Stewart	90915787.7	Granted National Phase
Belgiun	.	W E G Müller	BE90915787.7	Granted
Switzer	and		CH90915787.7	Granted
France			FR90915787.7	Granted
UK			GB90915787.7	Granted
Canada			CA2071546-4	Pending
Russia			RU5052241.14	Accepted
USA		J []	US143500	Granted
PCT	SPLICEOSOMAL	J W S Brown	PCT9501443	National Phased
Europea	n PROMOTER	G C Clark	EP95922617.6	Pending
USA		G G Simpson	US750654	Pending
Canada			CA2192971	Pending
Australi	a		AU27449/95	Pending
New Ze	aland		NZ288283	Pending
PCT	METHOD FOR	S N Chapman	PCT9502457	Pending
Europea	n CHIMERIC PROTEIN	S P Santa Cruz	EP95394228.8	Pending
Australi	n	K J Oparka	AU36598/95	Pending
Canada		T M A Wilson	CA2202761	Pending
New Ze	aland		NZ294014	Pending
USA		J	US844045	Pending
Europe		A Dolan	EP96303105.9	WTG Search
	PCR PRIMERS	J M Duncan		
	_	D E L Cooke		
UK	POLLEN SPECIFIC	G C Machray	9705694.9	Pending
	PROMOTER	P Hedley		
		R Meyer		
		A Maddison		
PCT	CHIMERIC	T M A Wilson	PCT9701065	Pending
	PSEUDOVIRUSES IN BACTERIA	S N Chapman		
UK	METHOD (PHAGE Typing)	I Toth	GB9809414.7	Preliminary Filing
UK	BARLEY MARKERS	WTB Thomas	GB9805087.5	Pending
	FOR ETHYL CARBAMATE	W Powell		
	PRODUCTION AND	J S Swanston		
	FERMENTABILITY	J		

Figure 3 Patented SCRI technology (granted and pending).

aims to enhance the competitiveness of UK industry and quality of life through support for managed programmes of pre-competitive science and technology in market or technology sectors, and by encouraging industry to invest in further work leading to commercially successful products, processes, systems and services.

#### Marketing

MRS has adopted a wide variety of approaches to marketing the expertise of the Institute. These include organising face-to-face meetings with key personnel in the industrial, scientific and political sectors,



#### Research services

<b>Tra</b> Count	 <b>Marks</b> Trade Mark	Class/Classes	App/Regn No	Status	Renewal Date
UK UK UK UK	SCRI MYLNEFIELD MRS	Research, analytical and consultancy services in chemistry, biology and agriculture	2029548 2029550 2029551 2041244	Registered Registered Registered Registered	04.08.2005 04.08.2005 04.08.2005 13.10.2005
EU UK USA	OVERCOAT OVERCOAT OVERCOAT	Microorganisms, viruses and proteins in industry and agriculture	EUT000433078 2102099 208908	Pending Accepted Pending	08.06.2006
UK	DISCOVERY	Agricultural, horticultural and forestry products	2017679	Registered	18.04.2005

Figure 4 Trademarks.

giving presentations at seminars and workshops, and attending tradeshows, conferences and meetings in strategic areas of interest.

MRS has produced a number of promotional brochures that cover individual plant varieties, services available and general areas of interest. These brochures are mailed out to potential customers and made available to visitors to the Institute.

We recognise the importance, cost and complexity of successful marketing and market research. We are investigating new strategies to continue to diversify the funding base and attract new customers.

#### **Premises**

In June 1997, MRS moved into a purpose-built Portakabin. In addition to offices, filing rooms and toilets, the premises include a boardroom capable of seating up to 12 people with an adjacent kitchen, and

	Variety	PVR	_		Variety	PVR
Strawberry	Symphony	EU Switzerland	[	Potatoes	Buchan Glamis	EU
Raspberries	Glen MARS Glen Lyon	EU UK			Brodie Stirling Brodick	
Blackberry	Loch Ness	UK France Netherlands USA (plant patent)			Kirrie Claret Othello Spey	
Blackcurrants	Ben Connan Ben Tirran	Denmark  USA (plant patent)  UK  USA (patent pending)  France  UK  Denmark		Brassicas	Invitation Brora Highlander Airlie Kenmore Virtue	UK
	Ben Loyal Ben Alder	Netherlands UK UK			Interval Hot Stuff Massif Caledonian	

Figure 5 Plant variety rights.

LINK Scheme: Biological Treatment of Soil and Water

Project Title: Integrating Microbial Processes in Soil at Successive Scales

Duration: 48 months Total Value: £853,753

Partners: Rhone Poulenc Agriculture Ltd., QuantiSci, MRS Ltd.

Principal Scientists: Dr J Crawford, Dr I Young, Dr K Ritz

LINK Scheme: Biological Treatment of Soil and Water

Project Title: Novel Antibody-Like Particles for the Detection, Monitoring and Elimination of Pollutants in Water

Duration: 36 months Total Value: £555,340

Partners: Yorkshire Water Services Ltd., Environmental Sensors Ltd., WRc plc, MRS Ltd.

Principal Scientists: Dr Lesley Torrance

LINK Scheme: Agro-Food Quality

Project Title: A Genome Based Approach to Improving Barley for the Malting and Distilling Industries

Duration: 39 months Total Value: £567,386

**Principal Scientists:** 

Partners: Home Grown Cereals Association, Advanta Holdings (UK) Ltd., Scotch Whisky Research Institute, MRS Ltd.

Dr WTB Thomas, Prof W Powell

LINK Scheme: Hort LINK

Project Title: Genetic Modification of the Commercial Strawberry for Improved Disease Resistance

Duration: 33 months Total Value: £339,002

Partners: Kentish Garden Marketing, Zeneca, MRS Ltd.

Principal Scientists: Dr Julie Graham

has been in considerable demand for project management meetings, meetings with prospective customers and internal review meetings. Sponsorship has been obtained from a number of key customers to develop the area around the building into a recreational amenity to be enjoyed by all the staff of SCRI. Our thanks go to Darby Brothers, VHB, Greenvale Produce, John Hargreaves and Sons, and Sharpes International for their contributions to this facility.

#### **Employees**

In 1997, Dr Jonathan Snape joined MRS as Commercial Manager. A graduate of Cambridge and Birmingham Universities, Dr Snape has gained experience of technology transfer and research project management while working for Unilever in Japan. The administrative staff of MRS was further strengthened by the appointment of Lesley Beaton as Administrative Assistant. In 1997, the following scientists were employed by MRS; Sharon Canavan, Dr Wendy Craig, Dr Yuchao Han, Emily Cobb, Jonathan Tonberg and Sheena Rowbottom.

#### Acknowledgement

MRS could not operate without the full and generous support of SCRI staff. We fully acknowledge that our success is largely due to their innovative ability and quality.

## **Scottish Society for Crop Research**

#### D. L. Hood

Trustees:- Mr A G M Forbes

Mr G B R Gray Mr I E Ivory Mr A Pattullo

Chairman:- Dr D A S Cranstoun Vice-Chairman:- Mr J M Drysdale

Members of Committee of Management:-

Mr J Arbuckle Mr A C Bain Mr D Craib Mr R G Galloway Mr A Logan Mr G Rennie

Secretary and Treasurer:- Mr D L Hood

Registered Office:- c/o Scottish Crop Research

Institute, Invergowrie, Dundee DD2 5DA

Membership Numbers:- 315

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.



RJ McNicol detailing quality attributes of SCRI strawberry cultivars to SSCR members.

The Society has recently concluded its sponsorship of a 3-year programme aimed at developing spring and winter barley varieties specifically suited to Scotland, northern Britain, and other northern climates, including Scandinavia.

The Society provides administrative support for The Peter Massalski Prize, which is a fund established in memory of Dr Peter R Massalski through the generosity of his parents Professor and Mrs T B Massalski. This is awarded biennially to the person(s) under 36 years old who is considered to have done the most meritorious research while working at SCRI.

Previous recipients include Dr K J Oparka, Dr B P Forster, Dr K Ritz and Dr R Waugh, and Dr C G Simpson.

The annual Soft Fruit Walk is a feature of Society life and recently the Potato Crop Walk has been held jointly with the Scottish Agricultural College at the Institute's Gourdie Farm site.

Crop Sub-Committees meet regularly throughout the year and their Chairman or Technical Secretary reports to the Management Committee of the Society on their developments.

The funds of the Society are professionally managed by the Trustees working in conjunction with the Secretary and Treasurer together with the Auditors and Stockbrokers.

#### Occasional Publications and Bulletins

The following publications are available (at the price shown), and can be ordered from the Secretary of the Scottish Society for Crop Research.

#### **SCRI Bulletins**

Brassicas and alternative crops for Scotland. Proceedings of the Scottish Society for Crop Research (in association with Sharpes International Seeds Ltd), Stakis Earl Grey Hotel, Dundee, 24 November 1995. Perry, D.A. (ed.). SCRI Bulletin No. 10. 1995. Scottish Crop Research Institute, Dundee, 35pp. £10

Prospects for cereals. Proceedings of the Scottish Society for Crop Research, Stakis Earl Grey Hotel, Dundee, 20 November 1991. Perry, D.A. (ed.). SCRI Bulletin No. 9. 1992. Scottish Crop Research Institute, Dundee, 32pp. £10

Potatoes, getting them right. Proceedings of the Scottish Society for Crop Research, Stakis Earl Grey Hotel, Dundee, 22 November 1989. Fox, R.A. (ed.). SCRI Bulletin No. 8. 1990. Scottish Crop Research Institute, Dundee, 44pp. £10

Soft fruit. Proceedings of the Scottish Society for Crop Research, University of Dundee, 4 February 1987. SCRI Bulletin No. 7. 1987. Scottish Crop Research Institute, Dundee, 24pp. £10

Cereals. Proceedings of the Scottish Society for Crop Research, Invercarse Hotel, Dundee, 27 January 1987. SCRI Bulletin No. 6. 1987. Scottish Crop Research Institute, Dundee, 38pp. £10

Seed potatoes for export. Proceedings of the Scottish Society for Crop Research, Angus Hotel, Dundee, 20 November 1985. SCRI Bulletin No. 5. 1986. Scottish Crop Research Institute, Dundee, 43pp. £10

Forage brassicas. Proceedings of the Scottish Society for Crop Research, Invergowrie, 25 September 1984. SCRI Bulletin No. 4. 1984. Scottish Crop Research Institute, Dundee, 26pp. Out of print.

Cereal requirements for Northern Britain. Proceedings of the Scottish Society for Crop Research, Kellogg Hall, Bush Estate, 17 November 1983. SCRI Bulletin No. 3. Scottish Crop Research Institute, Dundee, 35pp. Out of print.

Soft fruit. Proceedings of the Scottish Society for Crop Research, Invergowrie, 23 November 1982. SCRI Bulletin No. 2. 1983. Scottish Crop Research Institute, Dundee, 41pp. Out of print.

Producing quality seed potatoes in Scotland. Proceedings of the Scottish Society for Crop Research, Invergowrie, 26 November 1981. SCRI Bulletin No. 1. 1982. Scottish Crop Research Institute, Dundee, 52pp. Out of print.

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Raspberry Cultivar trial 1985-1990. Gordon, S.L. & Cormack, M.R. Occasional Publication No.10. 1996. Scottish Crop Research Institute, Dundee, 24 pp. £10

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Strawberry cultivar trial 1975-1978. Cormack, M.R.& Brown, J.McD. Occasional Publication No. 7. 1979. Scottish Horticultural Research Institute, Dundee, 10pp. Out of print.

Highbush blueberries. Cormack, M.R. Occasional Publication No. 6. 1979. Scottish Horticultural Research Institute, Dundee, 18pp. £5

Calabrese cultivar screening 1970-1976. Taylor, H. Occasional Publication No. 5. 1979. Scottish Horticultural Research Institute, Dundee, 19pp. Out of print.

Scottish Horticultural Research Institute publications 1952-1977. Bogen, B. Occasional Publication No. 4. 1978. Scottish Horticultural Research Institute, Dundee, 70pp. Out of print.

European Plant Parasitic Nematode Survey. Instructions for participants. Brown, D.J.F., Boag, B. & Taylor, C.E. Occasional Publication No. 3. 1978. Scottish Horticultural Research Institute, Dundee, 16pp. Out of print.

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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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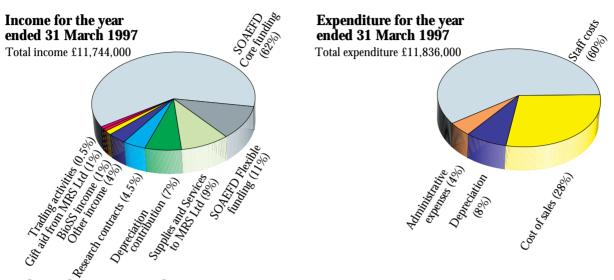
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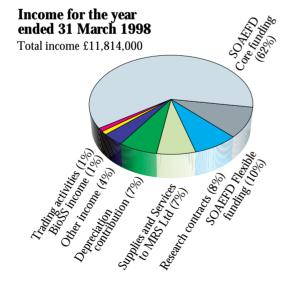
# Summary of the Accounts

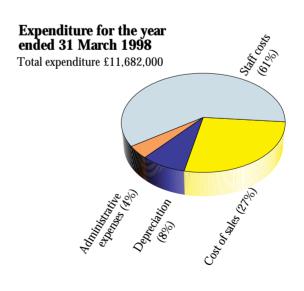


#### Balance sheet at 31 March 1997 Total value £12,485,000 Assets Liabilities

Fixed assets	93 %	
Stocks	1 %	
Debtors	6 %	

Capital reserve 91 %
Income & expenditure account 1 %
Current liabilities 8 %





#### Balance sheet at 31 March 1998 Total value £12,457,000

Assets			Liabilities
Fixed assets	92 %		Capital

Fixed assets	92 %	
Stocks	1 %	
Debtors	7 %	

Capital reserve	90 %
Income & expenditure account	3 %
Current liabilities	7 %

#### The Governing Body



Back row, I to r: Prof. PC Young, Mr. P Whitworth, Mr. AC Bain, Mr. JM Drysdale, Dr. JM Sime, Mr. JB Forrest, Mr TPM Thomson (retired)

Front row, l to r: Prof. JR Hillman, Mr. JE Godfrey, Mr. AN MacCallum(Chairman), Prof. DL Lee (retired), Mr. K Hopkins (Absent: Prof. J Belch, R Cogdell, Prof. J Evans, Prof. B King, Dr. JM Sime, Prof. JS Marsh, AR Slabas)

Chairman: A.N. MacCallum, B.Sc., F.D.I.C., is Group Chief Executive of Don & Low (Holdings) Ltd, Forfar, Industrial Textile Manufacturers, a position he has held since 1986. He graduated from Glasgow University with a degree in Chemistry. Prior to his appointment at Don & Low, he held management positions with Baxters of Fochabers; Devro Ltd of Glasgow; Guard Bridge Paper Company in St Andrews; and Unilever. He was Chairman of CBI Scotland from 1991-93. He currently holds directorships with a number of companies, including the newly formed North Water Authority, and is Chairman of Montrose Harbour Board. He joined the Governing Body of SCRI in 1995.

**A.C.** Bain is a soft fruit grower from Invergowrie. He has served two terms as Chariman of the Scottish Soft Fruit Discussion Society, and two terms on the board of the Scottish Nuclear Stock Association. He is currently on the Committee of Management of the

Scottish Society for Crop Research. He was the founder President of the Rotary Club of Dundee Camperdown. He was appointed to the Governing Body of SCRI in 1997.

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. She has been Chairman of the UK Forum on Angiology since 1995, and is Medical Adviser to the Raynaud's and Scleroderma Association. 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 appointed to the Governing Body of SCRI in 1997.

J.M. Drysdale is a specialist cereal grower, who farms near Kirkcaldy, Fife, and is Chairman of the Tayforth Marketing Group. He is currently on the Committee of Management of the Scottish Society for Crop Research. He was appointed to the Governing Body of SCRI in 1997.

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 Commission from 1989 to 1997. He is Vice-Chairman of the Commonwealth Forestry Association, a member of the Foresight Panel on Agriculture, Horticulture and Forestry, 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 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.

J.E. Godfrey, B.Sc., A.R.Ag.S, gained his degree in agriculture from the University of Reading, and is a director of family farming companies in Lincolnshire and Yorkshire. He is Chairman of Sentry Farming Group plc, managing farms in the UK, Poland and Czech Republic. He is a member or adviser to numerous agricultural committees, including The Centre for Agricultural Strategy, University of Reading; The Royal Agricultural Society of England; Food Chain Group of the Foresight Programme; and Humberside Training and Enterprise Council. He is a director of World Potato Congress Inc. He joined the Governing Body of SCRI in 1992, and became Vice-Chairman in 1997.

K. Hopkins, F.C.A., joined Reeves & Neylan, Chartered Accountants, in Canterbury, Kent, in 1971, and moved to open the Scottish Practice in 1978. He 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. Mr Hopkins specialises in capital taxes, agricultural law and cooperatives, writes for the agricultural press, and lectures throughout Scotland. He is Treasurer for District 1010 of Rotary, Treasurer of Strathmore Cricket Club, 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 Director of the Dundee Healthcare Trust, Governor of the Unicorn Preservation Society, and Chairman of the Committee of Principals of Scottish Centrally-Funded Colleges. 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.

Emeritus Professor J.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, Chairman of the Agricultural Wages Board, Chairman of RURAL Council, Governor of the Royal Agricultural College, Member of the Agriculture, Horticulture and Forestry Foresight Panel, Member of Ministers Advisory Group. He was appointed to the Governing Body of SCRI in 1998.

Dr. J.M. Sime, M.Sc., Ph.D., F.R.S.C., C. Chem., is the Chief Executive of the BioIndustry Association, a position he has held since 1995. Prior to this appointment, he held R&D, general management, and strategic marketing positions with Beecham and then SmithKline Beecham, in the UK, USA, Japan, Indonesia, Australia and New Zealand. He is a member of the CBI Biostrategy Committee and of the Management Board of the Advanced Centre for Biochemical Engineering at University College, London. He was appointed to the Governing Body of SCRI in 1997.

Professor A.R. Slabas, B.Sc., D.Phil., is Director of Research, Department of Biological Sciences, University of Durham, where he currently leads a group of 20 involved in various aspects of plant lipid metabolism. He has extensive collaborations with industry, including Monsanto, Unilever, and Nickerson Biocem. He is a member of the UK Technology Foresight Programme Panel Committee on Health and Life Sciences; the Agricultural Systems Directorate Management Committee; the Eukaryotic Cell Link Management Committee; and the BBSRC Innovative Manufacturing Committee. He joined the Governing Body of SCRI in 1995.

#### The Governing Body

P. Whitworth, H.N.C., retired from United Biscuits as Technical Director, Snacks in March 1996. He has been associated with the production of potato crisps and savoury snacks for over 35 years. 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.

Professor P.C. Young, B.Tech., M.Sc., M.A., Ph.D., Wh.F., C.Eng., M.I.E.E., F.I.M.A., F.R.S.S., is Director of the Centre for Research on Environmental Systems and Statistics, Lancaster University. He was Head of the Environmental Science Department at Lancaster, 1981-87; Professorial Fellow at the Australian National University, Canberra 1975-81: and Lecturer in Engineering/Fellow of Clare Hall, Cambridge University, 1970-75. His main research interests are in mathematical modelling, time series analysis, forecasting and automatic control. He has worked in a wide range of application areas but his research on agricultural systems includes modelling and advanced control of the micro-climate in horticultural glasshouses; and the data-based mechanistic modelling of biological, horticultural and ecological systems. He has been a Member of the Council, Freshwater Biological Association. He was appointed to the Governing Body of SCRI in 1997.



Assistant to Director T.J.W. Alphey, B.Sc., Ph.D., C.Biol., M.I.Biol

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. 1,2,3,13 Band 1 Director Deputy Director Professor T.M.A. Wilson, B.Sc., Ph.D., C.Biol., M.I. Biol.<sup>2</sup>

Secretary & Financial Manager

R.J. Killick, B.Sc., M.B.A., M.A., Ph.D., C.Biol., M.I.Biol.

Band 3 (Regr. Jan)

#### Cell & Molecular Genetics Department (CMG)

Head: W. Powell, B.Sc., M.Sc., Ph.D., D.Sc. <sup>4,5,10</sup> J.W.S. Brown, B.Sc., Ph.D. <sup>6</sup> R. Ellis, B.Sc., Ph.D. <sup>6</sup> B.P. Forster, B.Sc., Ph.D. <sup>6</sup> G.C. Machray, B.Sc., Ph.D. W.T.B. Thomas, B.Sc., Ph.D. C.G. Simpson, B.Sc., Ph.D. J.S. Swanston, B.Sc., Ph.D., C.Biol., M.I.Biol. Gillian Clark, H.N.C., B.Sc. B. Harrower, H.N.D., B.Sc. Jackie Lyon	Band 3 Band 4 Band 4 Band 4 Band 4 Band 5 Band 5 Band 7 Band 7 Band 7	Clare McQuade, B.Sc. G.R. Young, H.N.C. A. Booth, H.N.C. Diane Davidson R. Keith Jennifer Watters, H.N.D. A. Wilson J.D. Fuller Patricia E. Lawrence Alice Bertie	Band 7 Band 7 Band 8 Band 8 (P/T) Band 8 Band 8 (P/T) Band 8 Band 9 (P/T) Band 9 Band 9 (Regr. Dec) Band 9 Band 10
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#### Cellular & Environmental Physiology Department (CEP)

#### Chemistry Department (Chem)

Head: W.W. Christie, B.Sc., Ph.D., D.Sc., C.Chem., F.R.S.C. B.A. Goodman, B.Sc., Ph.D., C.Chem., F.R.S.C. D.W. Griffiths, M.A., Ph.D, C. Chem., M.R.S.C. G.W. Robertson, B.Sc., C.Chem., M.R.S.C.	Band 4 Band 5 Band 5	H. Bain, H.N.C., L.R.S.C. G. Dobson, B.Sc., Ph.D. Winifred M. Stein, H.N.C., B.Sc. Fiona Falconer, H.N.C. Jean Wilkie	Band 6 Band 6 Band 6 Band 8 Band 10
C.M. Scrimgeour, B.Sc., Ph.D. <sup>6</sup>	Band 5	Quality Assurance Officer: T. Shepherd, B.Sc., Ph.D.	

#### **Crop Genetics Department** (CG)

Head: G.R. Mackay, B.Sc., M.Sc., C.Biol., F.I.Biol. 4.5 J.E. Bradshaw, M.A., M.Sc., Ph.D.6 M.F.B. Dale, B.Sc., Ph.D.6 R. Waugh, B.Sc., Ph.D.6 G. Bryan, B.Sc., M.Sc., Ph.D. I. Chapman, B.Sc. M.J. De, Maine, B.Sc., M.Phil. S. Millam, B.Sc., Ph.D.6 G. Ramsay, B.Sc., Ph.D. W. De Jong, B.Sc., Ph.D. K. Harding, B.Sc., Ph.D. Alison K. Lees, B.Sc., Ph.D. Ruth M. Solomon-Blackburn, B.A., M.Sc.	Band 3 Band 4 Band 4 Band 4 (Tr. from CMG Jul) Band 5 Band 5 Band 5 Band 5 Band 5 Band 6 Band 6 Band 6 Band 6 Band 6	Helen E. Stewart, C.Biol., M.I.Biol. Jane McNicoll, H.N.C., B.Sc. Jane Shearer, B.Sc. G.E.L. Swan D. Todd, B.Sc. R.N. Wilson, N.C.H. Nicky Bonar, H.N.C. M.P.L. Campbell S.McDonald, B.Sc., M.Sc. Moira Myles, O.N.C. Sharon Neilson A. Margaret McInroy Gail Simpson	Band 6 Band 7 Band 7 (Appt. Jan) Band 7 Band 7 Band 7 Band 7 Band 7 Band 8 (Tr. from CMG Jul) Band 8 Band 8 (Tr. from FBPP Apr) Band 9 Band 9 Band 10 Band 10
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Visiting Professor in the University of Strathclyde
 Visiting Professor in the University of Dundee
 Visiting Professor in the University of Edinburgh
 Honorary Senior Lecturer in the University of St. Andrews
 Honorary Senior Lecturer in the University of St. Andrews
 Honorary Senior Lecturer in the University of Dundee

Honorary Lecturer in the University of Dundee
 Honorary Research Fellow in the University of Dundee
 Honorary Professor of Botany, Florida International University
 Honorary Lecturer in the University of Glasgow
 Honorary Professor, Oregon State University

Honorary Fellow in the University of Edinburgh
 Honorary Lecturer in the University of Aberdeen
 Visiting Professor in the University of Glasgow
 Professor, Universities of Cordoba and Malaga



#### Fungal and Bacterial Plant Pathology Department (FBPP)

Head: J.M. Duncan, B.Sc., Ph.D.5	Band 3	Lizbeth J. Hyman, B.A., M.Sc.	Band 6
G.D. Lyon, B.Sc., M.Sc., Ph.D., D.I.C.6	Band 4	R. Lowe	Band 6
A.C. Newton, B.Sc., Ph.D.6	Band 4	I. Toth, B.Sc., Ph.D. <sup>12</sup>	Band 6
P. Birch, B.Sc., Ph.D.	Band 6	Jacqueline Heilbronn, H.N.C.	Band 7
D. Cooke, B. Sc., Ph.D.	Band 6	Naomi A. Williams, H.N.C.	Band 7

#### Nematology Department (Nem)

Head: D.L. Trudgill, B.Sc., Ph.D., C.Biol., F.I.Biol., F.S.O.N. <sup>5</sup> B. Boag, B.Sc., Ph.D. <sup>6</sup> D.J.F. Brown, B.A., Ph.D., C.Biol., M.I. Biol., F.R.S.N., F.S.O.N. M.S. Phillips, B.Sc. W.M. Robertson, H.N.C. F.L.S.	Band 3 Band 4 Band 4 Band 4 Band 4	A. Kumar, B.Sc., Ph.D. J.T. Jones, B.Sc., Ph.D. R. Neilson, H.N.C., M.Sc. Ailsa Smith, B.Sc. Anne M. Holt Alison Paterson Sheena S. Lamond	Band 5 Band 6 Band 7 Band 7 Band 8 (P/T) Band 10 (P/T) Band 8
W.M. Robertson, H.N.C., F.L.S.	Band 4	Sheena S. Lamond	Band 8
Vivian Blok, B.Sc., M.Sc., Ph.D.	Band 5 (Prom. Jul)		

#### Soft Fruit & Perennial Crops Department (SFPC)

Head: R.J. McNicol, B.Sc. <sup>5</sup> A.T. Jones, B.Sc., Ph.D. <sup>5</sup> B. Williamson, B.Sc., M.Sc., Ph.D., D.Sc. <sup>6</sup> A.N.E. Birch, B.Sc., Ph.D., C.Biol., M.I.Biol. R.M. Brennan, B.Sc., Ph.D. B. Fenton, B.Sc., Ph.D., C.Biol., M.I.Biol. S.C. Gordon, H.N.C.	Band 3 Band 3 (IMP) Band 4 Band 5 Band 5 Band 5 Band 5 Band 5	Julie Graham, B.Sc., Ph.D. G. Thow, B.Sc., Ph.D. Alison Dolan, H.N.C. Gaynor Malloch, D.C.R., B.Sc. Wendy J. McGavin, B.Sc. Sandra L. Gordon, H.N.C. Kay Smith, Dip. H.E. Departmental Administrator: Maureen Murray	Band 5 Band 6 Band 7 (P/T) ) Band 7 Band 7 Band 8 Band 8 Band 8
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#### $Virology\ Department\ (Vir)$

Head: P.F. Palukaitis, B.Sc., Ph.D. <sup>5</sup> K.J. Oparka, B.Sc., Ph.D., C.Biol., M.I.Biol. <sup>5</sup> M.A. Mayo, B.Sc., Ph.D., C.Biol., M.I.Biol. <sup>5</sup> H. Barker, B.Sc., Ph.D. J.M.S. Forrest, B.Sc., Ph.D. I.M. Roberts, H.N.C., Dip.R.M.S. D.J. Robinson, M.A., Ph.D. <sup>6</sup> Lesley Torrance, B.Sc., Ph.D. J.A.T. Woodford, M.A., Ph.D. <sup>6</sup> G.H. Duncan, H.N.C. S.A. MacFarlane, B.Sc., D.Phil. B. Reavy, B.Sc., D.Phil. S. Santa Cruz, B.Sc., Ph.D.	Band 3 Band 3 (IMP) Band 3 (IMP) Band 4 Band 4 Band 4 Band 4 Band 4 Band 5 Band 5 Band 5 Band 5 Band 5	M. Taliansky, Ph.D., D.Sc. T. Canto, B.Sc., Ph.D. Maud M. Swanson, B.Sc., Ph.D. Kathryn M. Wright, M.A., Ph.D. A. Ziegler, B.Sc., Ph.D. G.H. Cowan, H.N.D. Sheila M.S. Dawson, H.C. Kara D. McGeachy, H.N.C. Jill Middlefell-Williams, H.N.C. D.A.M. Prior, H.N.C. Fiona Carr Gillian L. Fraser	Band 5 (Appt. Jan) Band 6 (Appt. Jan) Band 6 Band 6 Band 6 Band 7 Band 8 (P/T) Band 8

#### $\textbf{Scientific Liaison \& Information Services Department} \ (\textbf{SLIS})$

Head: W.H. Macfarlane Smith, B.Sc., Ph.D., C.Biol., M.I.Biol., F.I. Mgt. D.F. Marshall, B.Sc., Ph.D.	Band 4 Band 4 (Appt. Oct) Band 5	I. Black, H.N.C. S. Clark, H.N.C. S.F. Malecki, A.B.I.P.P.	Band 7 Band 7 Band 7
T.G. Geoghegan, A.B.I.P.P., A.M.P.A. T.D. Heilbronn, B.Sc., M.Sc.	Band 6	Ursula M. McKean, M.A., Dip. Lib.	Band 7 Band 7
I.D. Hendronn, B.Sc., M.Sc. I.R. Pitkethly, H.N.D.	Band 6	G. Menzies Janette Keith	Band 11 (P/T)
P. Smith. B.Sc.	Band 6		,
Sarah E. Stephens, B.Sc., M.A., A.L.A.	Band 6	Safety Coordinator: Kathryn M. Wright, M.A., Ph.D.	Band 6

#### $Administration\ Department\ ({\tt Admin})$

Secretary & Financial Manager: R.J. Killick, B.Sc., M.B.A., M.A., Ph.D., C.Biol., M.I.Biol. Financial Controller: I.F. Harrington, C.A. Accountant: S.L. Howie, C.A. Assistant Secretary: D.L. Hood, B.Admin., Dip. Ed., L.T.I., A.I.I.M.	Band 3 (Regr. Jan) Band 4 (Appt. Jun) Band 5 Band 6	Sheena Forsyth Kristy L. Grant, B.A. Theresa Ower, B.A. Sarah-Jane Simms, H.N.D. Elizabeth L. Stewart Barbara V. Gunn	Band 8 Band 8 Band 8 Band 8 Band 8 Band 9
Personnel Officer: I. Paxton, H.N.C., M.Sc., M.I.P.D.	Band 6	Media Kitchen	
Anne Pack Catherine Skelly Dianne L. Beharrie, Dip. Ed. Joyce Davidson Rhona G. Davidson Pam Duncan	Band 7 Band 7 Band 8 Band 8 Band 8 Band 8 Band 8	Wendy Ridley Evelyn Warden W. Burry J. McMillan	Band 7 Band 9 Band 11 (HELM) Band 11 (P/T) (HELM)

#### $Engineering \ \& \ Maintenance \ Department \ (\text{EM})$

Head: S. Petrie, B.Sc.	Band 4	R. Pugh	Band 9
D. Gray, H.N.C.	Band 6	W. Scott	Band 9 (Appt. June)
A. Low	Band 7	C. Conejo	Band 10
I.C. McNaughton, H.N.C.	Band 7	J. Flight	Band 10
K. Henry	Band 8	N. McInroy	Band 10
R.D. McLean	Band 8	D.L.K. Robertson	Band 10
G.C. Roberts	Band 8	J. Rowe	Band 10
R. White	Band 8	M.J. Soutar	Band 10
J. Anderson	Band 9	J. Oldershaw	Band 11
D. Byrne	Band 9	Departmental Administrator:	
E. Lawrence	Band 9	Wendy A. Patterson, H.N.D.	Band 8 (P/T)
C.G. Milne	Band 9	,	, ,

#### Estate, Glasshouse & Field Research Department (EGFR)

Head: G. Wood, B.Sc., Ph.D., F.E.T.C. P.A. Gill, H.N.D. J.R.K. Bennett W.D.J. Jack, B.Sc. D.S. Petrie B.D. Robertson, N.E.B.S.M., H.N.C., Dip. Mgt., M.B.A. A. Grant A.W. Mills R. Ogg D.G. Pugh	Band 4 Band 6 Band 7 Band 7 Band 7 Band 7 Band 8 Band 8 Band 8 Band 8 Band 8	L.A. McNicoll Alison Dobson I. Fleming A.C. Fuller J. Mason T.A. Mason, N.E.B.S.M. Gillian Pugh J.K. Wilde J. Abernethy M. Torrie	Band 9 Band 10 (Appt. Sept) Band 10 Band 11 (P/T) (HELM) Band 11 (P/T) (HELM)
Angela M. Thain J.T. Bennett	Band 8 Band 9	<b>Departmental Administrator :</b> Wendy A. Patterson, H.N.D.	Band 8 (P/T)
B. Fleming	Band 9	, , , , , , , , , , , , , , , , , , ,	

#### **Biomathematics and Statistics Scotland (BioSS)**

King's Buildings, University of Edinburgh Director: R.A. Kempton, M.A., B.Phil. <sup>11</sup> G.J. Gibson, B.Sc., Ph.D. C.A. Glasbey, M.A., Dip. Math. Stats., Ph.D., D.Sc., M.I.S.I. <sup>11</sup> E.A. Hunter, B.Sc., M.Phil. <sup>11</sup> M. Talbot, F.I.S., M.Phil. <sup>11</sup> Janet M. Dickson, B.Sc. Elizabeth J. Austin, M.A., D.Phil. J.A.N. Filipe, B.Sc., M.Sc., Ph.D. A.D. Mann, B.Sc. I.M. Nevison, M.A. Muriel A.M. Kirkwood, D.A.	Band 3 Band 4 Band 3 (IMP) (Prom. Jul) Band 4 Band 5 Band 6 Band 8	Ayr Unit D.A. McNulty, B.Sc., Ph.D.  Aberdeen Unit, RRI Head: G.W. Horgan, B.A., M.Sc. Carol A. Reid, B.Sc., Dip. Acc., Ph.D. Karen A. Robertson, B.Sc.  Aberdeen Unit, MLURI Head: D.A. Elston, B.A., M.Sc. D.J. Hirst, B.Sc., Ph.D. Elizabeth I. Duff, B.Sc. T.S. Smart, B.A., P.G.C.E., M.Sc.	Band 6 Band 5 Band 6 Band 7  Band 4 Band 5 Band 6 Band 6
Karyn Linton Diane Glancy Amy G. Stewart Secretary: Elizabeth M. Heyburn, M.A.	Band 9 (P/T) Band 10 (P/T) Band 10 (P/T) Band 7	Dundee Unit Head: J.W. McNicol, B.Sc., M.Sc. Christine A. Hackett, B.A., Dip. Math. Stats., Ph.D. F.G. Wright, B.Sc., M.Sc., Ph.D.	Band 4 Band 5 Band 5

#### **Short-term Contracts**

SOAEFD Flexible Funding		Chemistry	
BioSS		Claire Fernie, B.Sc.	Band 7
Nicole H. Augustin, M.Sc.	Band 6	Crop Genetics	
I.J. McKendrick, B.Sc., Ph.D.  Maria I. Durban Postuara P.Sc. Din Math. State	Band 6 Band 7	Jane S. Miller, B.Sc., Ph.D.	Band 6 (Appt. May)
Maria L. Durban-Reguera, B.Sc., Dip. Math. Stats.  Cell and Molecular Genetics	Dallu 1	Fiona M. Walls, H.N.D., B.Sc., M.Sc. Mary McGregor	Band 8 (Appt. May) Band 11 (P/T)
J. Provan, B.Sc., Ph.D.	Band 6	Fungal and Bacterial Plant Pathology	
J. Hamilton, B.Sc.	Band 7	Alia Dellagi, B.Sc., Ph.D.	Band 6 (Appt. Nov)
Karen McLean, B.Sc.	Band 7	D.C. Guy, H.N.D.	Band 7
Cellular and Environmental Physiology	D 150 0 0	Nematology	D 10
A.M. Johnston, B.Sc., Ph.D. C. Clegg, B.Sc., M.Sc., Ph.D.	Band 5 (Appt. Oct) Band 6	Alison Prior, B.Sc. Jane Wishart, B.Sc.	Band 7 Band 7
Lynda Deeks, B.Sc., Ph.D.	Band 6 (Appt. Sep)	A. Stevenson, B.Sc.	Band 10 (P/T)
E. Grist, B.Sc., M.Sc., Ph.D.	Band 6	Soft Fruit & Perennial Crops	
Joanna Chessell, B.Sc.	Band 7 (Appt. Sep)	Linzi Ross, H.N.D.	Band 10 (Appt. Sep)
A.M. Cooper, H.N.D. G. Henderson, B.Sc., M.Sc.	Band 7 Band 7	Virology	
Sigrun Holdhus, Cand. mag.	Band 7	Rachel Toth, B.Sc., Ph.D.	Band 6
D. Kiezebrink, B.Sc., M.Sc.	Band 7	DTI/LINK	
Sarah Tiller	Band 7	Cell and Molecular Genetics	
M.O. Henry, B.Sc. Alexandra Holmes, H.N.D., P.G.Dip. Biotech.	Band 7 (Appt. Oct) Band 10	Rhonda Meyer, B.Sc., Ph.D.	Band 6 (Appt. Oct)
Fiona C.M. Milne, B.Sc.	Band 10 (Appt. Oct)	Jennifer Ritchie, O.N.C.	Band 10 (Appt. Nov)
Elise Flipse, Ir., Ph.D.	Band 6	Virology	D 1.0
Lesley McAllister, B.Sc.	Band 8 (Appt. Jun)	Karen Harper, B.Sc., Ph.D.	Band 6
Fungal and Bacterial Plant Pathology	Pand 7 (Annt New)	MAFF	
Vanessa Maughan, B.Sc.	Band 7 (Appt. Nov)	Cell and Molecular Genetics	D 1.0
Soft Fruit & Perennial Crops Phil Irving, B.Sc., P.G.Dip.	Band 7	A. Ibrahim, B.Sc., Ph.D. Jennifer Watters, H.N.D.	Band 6 Band 8 (P/T)
Kathryn Watt, B.Sc.	Band 7	Cellular and Environmental Physiology	Dalid O (1/1)
Nematology		M. Young, H.N.D., M.Sc.	Band 6
Lindsey Millar B.Sc.	Band 6 (Appt. Dec)	Crop Genetics	
Irene E. Geoghegan M.Sc. Diane Harkins	Band 7 Band 10	Caroline Thompson, B.Sc., Ph.D.	Band 6 (Appt. Oct)
Virology	Dalid 10	Fungal and Bacterial Plant Pathology	
D.A.C. Jones, B.Sc., Ph.D.	Band 6	D. Cullen, B.Sc., Ph.D.	Band 6 (Appt. Sept)
Wendy Smith	Band 7 (Appt. Jun)	Soft Fruit & Perennial Crops	Pand 7 (Arest Oct)
BBSRC		Trudi Gillespie, B.Sc. <i>Virology</i>	Band 7 (Appt. Oct)
Cell and Molecular Genetics		Michelle Liney	Band 7
Linda Cardle, B.Sc., Ph.D.	Band 6	HortLink	
M. Macaulay, H.N.C., B.Sc.	Band 7	Soft Fruit & Perennial Crops	
Chemistry Samantha Gill, B.Sc., M.Sc.	Band 7	Heather McCafferty, Ph.D.	Band 6 (Appt. Oct)
Virology	Daliu 1	McCains PLC	. 11
C. Lacomme, B.Sc., Ph.D.	Band 6	Crop Genetics	
Lisa Smolenska, B.Sc.	Band 7	Hayley Baldie	Band 9 (Appt. Aug)
British Potato Council		ODA	
Cellular and Environmental Physiology		Soft Fruit & Perennial Crops	
Venetia Mahoney	Band10	Karen B. Howat, H.N.D.	Band 8
Crop Genetics		SmithKline Beecham R&D Fund	
Sally J. Monnington, B.Sc. Mairi J. Nicolson, B.Sc.	Band 6 (Appt. Nov) Band 10 (Appt. Nov)	Cellular and Environmental Physiology	
Fungal and Bacterial Plant Pathology	Daliu 10 (Appt. Nov)	M.R. MacLeod, B.Sc., Ph.D.	Band 6
K. Bell, B.Sc., Ph.D.	Band 7 (Appt. Dec)	Miscellaneous funding	
Jane Roberts, H.N.C.	Band 10	Soft Fruit & Perennial Crops	
Nematology		P. Lanham, B.Sc., Ph.D.	Band 6
M. Elliot	Band 7 (Appt. Dec)	BioSS	
CEC		Grietje Holtrop, M.Sc.	Band 6 (Appt. Oct)
Cell and Molecular Genetics			
P. Hedley, B.Sc., Ph.D.	Band 6		
L. Ramsay, B.Sc., Ph.D.  Cellular and Environmental Physiology	Band 6		
J. Foster, B.Sc., Ph.D.	Band 6		
Paula M. Hebden, B.Sc.	Band 8		
J. Pelloux, B.Sc., Ph.D.	n/a (Appt. Dec)		
Nancy Van Overstraeten, B.Sc., Ph.D.	n/a (Appt. Oct)		

# Resignations

Name	Dept.	Band	Month
Cl A . l	CC	7	A . 1
Sharon Anderson	CG	7	April
R. Boath	Admin	4	February
K.J. Brown	BioSS	7	August
K. Clacher	SFPC	7	July
J. Duffin	Admin	6	July
Lisa Duncan	NEM	6	October
G. S. Lacey	EGFR	10	September
C. McCreadie	EGFR	10	August
M.J. Metcalf	BioSS	6	July
R. Murray	EGFR	10	November
V.M. Trenkel	BioSS	6	November
S.A.R. Williams	BioSS	7	February

#### **Staff Retirements**

Name	Dept.	Band	Month	
G. Dow	EGFR	10	July	
A. Young	CMG	6	November	
Joyce Young	CMG	10	May	

# Voluntary and Flexible Retirements

Name	Dept.	Band	Month
M.F. Franklin	BioSS	4	March
Margaret Garland	CEP	8	March
Eva Bennett	CG	8	March
Frances Gourlay	CG	7	March

# Mylnefield Research Services Ltd

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. (Appt. Sep) Administrative Executive Officer: Anne Cameron, H.N.C. Administrative Assistant: Lesley Beaton, H.N.C. (Appt. Oct)

Personal Secretary : Linda Butler

Sharon Canavan (Appt. Apr)
Emily Cobb, H.N.D. (Appt. Oct)
Wendy Craig, B.S.c., Ph.D. (Appt. Apr)
P. Davie, O.N.C.
Patricia Dobson
Jane E. Fairlie, O.N.C.
Yuchao Han, B.Sc., M.Sc., Ph.D.
R.E. Harrison, B.Sc., Ph.D.
Angela Ingram, B.Sc.
Angela Ingram, B.Sc.

S. Nikki Jennings, B.Sc. C. Jones, B.Sc. Susan Mitchell, B.Sc. A. Mudie, B.Sc. Jacqueline Murphy, B.Sc., Ph.D. Claire Reid, B.Sc. Sheena Rowbottom, O.N.C., H.N.C. (Appt. Oct) Joanne Russell, B.Sc., Ph.D. J. Tonberg, B.Sc. (Appt. Aug) Mary Woodhead, B.Sc., Ph.D.

#### Honorary Research Professors

Professor P. Broda, M.A., M.Sc., Ph.D., D.Sc., Hon.D.Sc.
Professor H. Griffiths, 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., AR.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., F.R.S.E.

#### Honorary Research Fellows

R.A. Brown, B.Sc., M.Sc., Ph.D.
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.
P.D. Smith, B.Sc., Ph.D., C.Math., F.I.M.A.

# 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
H. Barker	Member Member	Association of Applied Biologists (Virology Group) Working group to produce FAO/IPGRI "Technical Guidelines for the Safe Movement of Potato Germplasm"
A.G. Bengough	Joint Co-ordinator Member	British Soil Water Physics Group Scottish Soils Discussion Group Committee
Vivian Blok	Committee Member	AAB Nematology Sub-group
B. Boag	Member	UK National Committee for Biodiversity
R. Brennan	Adviser	SmithKline Beecham Blackcurrant R&D Committee
D.J.F. Brown	Co-Chairman Member	Russian Society of Nematology International Symposium American Society of Nematology Ad Hoc Committee, International Federation of Nematology Societies
	Member	European & Mediterranean Plant Protection Organization Ad Hoc Committee. Xiphinema americanum group nematodes
W.W. Christie	Member Member	Steering Committee, 23rd Congress of the International Society for Fats Research Committee of the European Section of the American Oil Chemists' Society
D.E.L. Cooke	Member	British Society for Plant Pathology Council
J.W. Crawford	Member	BBSRC EBS Committee
H.V. Davies	Member	EU Scientific Committee on Plants (DG24)
	Member Member	Kluwer Academic Press Scientific Advisory Board (Plant Science) EU FAIR Evaluation Panel
G. Dobson	Treasurer	Royal Society of Chemistry Lipid Chemistry Group
J.M. Duncan	Member	Phytophthora Committee, International Society of Plant Pathology
B.P. Forster	Co-ordinator	Chromosome 4, International Barley Chromosome Mapping
C.A. Glasbey	Member	Council of Royal Statistical Society
on a diaboty	Member	EPSRC Mathematics College
B.A. Goodman	Member	Organising Committee of the Crocon Network, funded by the EU under the ALFA Programme for cooperation with South American countries in "Scientific and Technological Training".
T.D. Heilbronn	Finance / Publicity Officer	Association for Crop Protection in Northern Britain
J.R. Hillman	Chairman	Agriculture, Horticulture & Forestry Sector Panel, UK Technology Foresight Programme
	Chairman	SCRI/SASA/SAC Liaison Group
	Chairman Deputy Chairman	Tayside Biocentre Group Board of Directors, Mylnefield Research Services Ltd
	Member	Committee of Heads of Agricultural and Biological Organisations in Scotland
	Member	SOAEFD Joint Consultative Committee for Management Board
	Member Member	ECRR Board of Management SNSA Adviser to Committee
	Member	Senate, University of Dundee
	Member	University of Strathclyde Sub-Board for the Degree of B.Sc. in Horticulture
	Member Member	SSPDC Management Committee Tayside Economic Forum
	Member	PSRE Network Steering Committee
	Member	Perth & Kinross Agricultural Forum
	Member Adviser	Board of Directors, BioIndustry Association International Foundation for Science, Stockholm
D.J. Hirst	Member	ECN Statistical Technical Advisory Group
D.L. Hood	Secretary & Treasurer	Scottish Society for Crop Research
G.W. Horgan	Committee Member	Royal Statistical Society
E.A. Hunter	Member	ASU Conference 1997 Scientific Committee
R.A. Kempton	Vice President	British Region, International Biometric Society
1	Member	Award Fund Committee. International Biometric Society
	Member Chairman	Organising Committee, 8 <sup>th</sup> IMA Conference on Mathematics in Medicine and Biology, Oxford Local organising committee IBS British Region 50 <sup>th</sup> Anniversary Conference
R.J. Killick	Member	Scottish Management Advisory Committee
IC.J. KIIIICK	Member	BBSRC Pay Advisory Group and Pay Negotiation Team
	Company Secretary	Mylnefield Research Services Ltd
W.H. Macfarlane Smith	Member	BBSRC Joint Committee on Health & Safety
	Member Member	BSPB Oilseed & Industrial Crops Group ECRR PR Officers' Group
	Member	SABRI Safety Officers' Group
	Member	NPTC Plant Variety Development Panel
S. MacFarlane	Member	Association of Applied Biologists (Virology Group)
G.R. Mackay	Chairman	GILB Steering Committee
DVI M.V	Chairman	Potato Section, EUCARPIA
D.K.L. MacKerron	Secretary Chairman	Potato-Crop Sub-Committee, SSCR Potato Crop Network, GCTE Focus 3
	Citatilian	Totato Crop Pretwork, GCTE Pocus 3

Name	Position	Committee or Organisation
D.F. Marshall	Member Member Chairman Member Member Member Member	Plant and Animal Genome Analysis (PAGA) Initiative, BBSRC Resource Allocation and Stress in Plants (RASP) Initiative, BBSRC NASC Steering Committee, BBSRC Genomics Strategy Review, BBSRC Quality of Science Institute Review PMS, BBSRC Genome Analysis of Agriculturally Important Traits (GAIT) Initiative, BBSRC Agri-Food Directorate Network Group, BBSRC
M.A. Mayo	Executive Committee Chairman Chairman Member	Interaction Committee on Taxonomy of Viruses (ICTV) Plant Virus Subcommittee (PVS) of ICTV Study Groups of ICTV PVS on Sequivirdae, Tenuibviruses, Tymoviruses, Marafiviruses, Satellites IUBS/IUMS International Commission on Bionomenclature
U.M. McKean R.J. McNicol	Member Member Adviser Adviser Adviser Member Member Member Member	Scottish Agricultural Librarians' Group HDC Soft Fruit Trialling Sub-Committee Soft Fruit Sub-Committee, SSCR SNSA Committee SSFG Ltd Board Soft Fruit Committee of Horticulture Research International CHABOS - Control of Food Intake Committee Lifestyle Project (Berry Project, Finland)
S. Millam	UK Representative UK Representative	COST 822 WG5 COST 824 WG2
I.M. Morrison	JSFA Representative Member Member Member	SCI Publications Committee SCI Agriculture and Environment Committee COST 814-II Alternative Fibre Crops Energy and Industrial Cropping Group, National Farmers Union of Scotland
A.C. Newton	Web Server Manager Committee Member Committee Member National Delegate	British Society for Plant Pathology Local Arrangements Committee of the Seventh International Congress of Plant Pathology (ICPP98) United Kingdom Cereal Pathogen Virulence Survey Management Committee of EU COST Action 817
K.J. Oparka	Member Member Conference Organiser	SEB Plant Biology Committee International Organising Committee, Plant Membrane Transport Conference, Cambridge 1998 Juan March Fundacion, International Workshop on Plasmodesmata and Macromolecular Trafficking, Madrid 1998
P.F. Palukaitis	Advisor	ATCC Plant Virus Collection
W. Powell	External Examiner External Examiner External Examiner	B.Sc. Genetics, University College of Wales, Aberystwyth M.Sc. Cell and Molecular Basis of Crop Improvement, UCW, Aberystwyth M.Res. Plant Science, UCW, Aberystwyth
G. Ramsay	Member	UK Plant Genetic Resources Group
K. Ritz	Secretary	BBSRC/SOAEFD Soil:Plant:Microbe International Initiative
W. Robertson	Organising Committee SCRI Coordinator Committee Member	ESN Symposium 1998 EC Biotechnology Project Arena CHABOS Committee on control of Helminths
D.J. Robinson	Member	Advisory Committee on Releases to the Environment
G.R. Squire	Chairman Member Project Co-ordinator Member	CHABOS Working Group on Vegetation Dynamics CHABOS Working Group on Soil Conservation SOAEFD Co-ordinated Programme in Vegetation Dynamics CHABOS Working Group on Environmental Pollution and Biological Radiation
S.E. Stephens	Joint Chair Member Member Working Group Member Forum Group Member	Scottish Agricultural Librarians' Group Information Services Group - Scottish Library Association Tayside and Fife Library and Information Network British Research Institutes Serials Consortium (BRISC) Research Councils Library and Information Consortium (RESCOLINC)
M. Talbot	Chairman Member Member	Statistics Group of UK Plant Varieties and Seeds Committee Statistics Committee of International Seed Testing Association Management Board of European Network for Information Technology in Agriculture
L. Torrance	National Representative	Management Committee EC-COST 823
I.K. Toth	Organising Committee	Crop Protection in Northern Britain 1999
D.L. Trudgill	President	Governing Body of European Society of Nematology
T.M.A. Wilson	Committee Member Co-organiser Member Member Member	Scientific Programme Committee for 7th International Congress of Plant Pathology Joint Royal Society/RSE Symposium on 'A Century of Research' Dundee Science Centre Consortium Steering Committee Programme Committee, VIIth International Congress of Plant Pathology, Edinburgh Church of Scotland, Society Religion and Technology Project 'Ethics of Genetic Engineering of Non- Human Life'
F.G. Wright	Member	BBSRC collaborative computational project CCP11 in Biosequence and Structure Analysis, Steering Group
I.M. Young	Member	British Standards Soil Quality Committee



# **Postgraduate Students**

Name	Dept.	Subject
D.J. Allcroft	BioSS	Mathematical modelling of short-term behaviour in farm animals.
M. Armstrong	Nem	Molecular heterogeneity in potato cyst nematodes.
Nicole Augustin	BioSS	Statistical spatio-temporal models with applications in vegetation dynamics.
N. Aziz	CMG	Genetic engineering of crops.
Suzanne Baker	FBPP	The effect of biotic and abiotic stress on the molecular processes underlying <i>ml-o</i> resistance in barley.
O. Brendel	CEP	<sup>13</sup> C and genetic variation in native Scots pine.
G. Cowan	Vir	Production and application of antisera to non-structural proteins of potato mop-top virus.
Elaine Davidson	CEP	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.
G. Dunlop*	CEP	Linking germination traits of oilseed rape to DNA markers.
Maria Durban-Reguera els.	BioSS	Modelling spatial trends and local competition effects in field trials, using generalised additive mod
M. Ehwaeti	Nem	Root-knot nematodes, biology and control.
S.J. Ferris	BioSS	The investigation and control of carryover effects in observer perception and recording.
J. Forster	CEP/CMG	Genetic manipulation of nitrate reductase activity in potato.
Liliana Franco-Lara	Vir	Development of transgenic resistance to potato leafroll virus in <i>Solanum phureja</i> .
Shahid Hameed	Vir	Properties and diversity of geminiviruses in Pakistan.
J.I. Hamilton	CMG	Molecular characterisation of RNA binding proteins in pre-mRNA splicing.
B. Harrower*	CMG	Genetic variation of PCN as revealed by molecular markers.
G. Henderson	CEP	Modelling soil-water/structure functions.
Phil Irving	SFPC	Molecular analysis of insect guts.
C. Jones	CEP	Molecular basis of ripening in <i>Rubus</i> .
Irene Karanastasi	Vir	Plant virus sequences involved in particle assembly and transmission by nematodes.
D. Kiezebrink	CEP	Modelling soil and water structure functions to assess the efficiency of pesticides in agricultural soils against plant-pathogenic nematodes.
P. Lava Kumar	SFPC	Assessment of the genetic variation within and between populations of <i>Aceria cajani</i> , the mite vector of the agent of sterility mosaic of pigeonpea in different regions of Asia.
S.G. Lane	VIR	Studies on recombinant antibodies to water pollutants.
Fevronia Lioliopoulou	Vir	Studies on molecular interactions between PMTV and its vector, <i>Spongospora subterranea</i> f.sp. <i>subterranea</i> .
Gaynor Malloch*	SFPC	Genetic variation in the family Byturidae.
M. Maule	BioSS	Stochastic modelling in plant epidemiology and ecology.
Hazel McGovern	CEP	The influence of soil biota on soil structural conditions.
Grainne H. McGuire	BioSS	The statistical modelling of the genetic structure of bacterial populations.
D. Milbourne	CMG	Molecular marker-assisted targeted breeding for potato cyst nematode and late blight.
Sarah Miller	FBPP	Assessment of the potential to control potato diseases by resistance elicitors.
Adele Mooney	Vir	Replication of pea early browning virus.
A. Munir	Nem	Management of potato cyst nematodes in Pakistan.
R. Neilson*	Nem	The rôle of soil fauna in nutrient cycling as indicated by stable isotopic analysis.
Ederlinda Pascual	Chem	Oxidation processes in coffee.
A.A.F.L.K. Perera	CMG	Molecular diversity in coconut.
Alexandra Popovich	SFPC	Development of a rapid screening system for gene function.
Alison Prior	NEM	Functional characterisation of a secreted protein from potato cyst nematode, <i>Globodera pallida</i> .
J. Provan	CMG	Development of simple sequence repeat markers in potato.
A. Richardson	CEP	Coniferyl alcohol oxidases in lignifying tissues of higher plants.
Alison Roberts	CEP	Plasmodesmata and virus transport.
Lee Robertson	Nem	Nematode secretions involved in plant pathogenesis.
Caroline D. Robinson	BioSS	Bayesian methods for segmenting X-ray CT images of sheep.
Louise Shepherd	CEP	Production of novel starches in potato.
Geetha Shilvanth	SFPC	Enhancement of resistance to <i>Botrytis</i> 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	CEP	Carbohydrate metabolism during ripening in the fruit of strawberry.
Nicole Soranzo	CMG	Molecular ecology of Scots pine.
Kiri Stanley	SFPC	Towards an understanding of the molecular mechanisms of lectin toxicity to aphids through gut glycoprotein interactions.
D. Todd*	CG	The genetic effects and consequences of selection for processing potential in the early generations of a potato breeding programme.
N. Vassilakos	Vir	Genetic determinants of complimentarity and exclusivity of vector transmission of tobraviruses.
E. Vellios	Nem	Molecular elucidation of interaction between plant tobravirus gene products and virus-vector trichodorid nematodes.
Gemma White	CMG	Population genetics of Mahogany.
Jane Wishart	NEM	Characterisation of <i>Meloidogyne</i> species using molecular and immunological techniques.
C-P. Witte	CEP	Modification of urea metabolism in transgenic potato.
C. Zhang	CMG	Improvement of Chinese wheat cultivars.

 $<sup>^{</sup>st}$  Permanent member of staff

# **Short-Term Workers and Visitors**

Name	Country of	Dept.	Month/yr	Length
	origin		of arrival	of stay
Julia Alexander	UK	CG	Jul 97	2 months
M. Alphey	UK	CG	Jul 97	2 months
Anna Avrova	Russia	FBPP	Apr 97	1 year
S. Ayivor	Ghana	CG	Apr 97	5 months
P. Barreiro	Spain	BioSS	Jul 97	6 weeks
A. Basu	India	CMG	Oct 97	4 months
P. Bassett	UK	BioSS	Jul 97	10 weeks
Amal Belakbir	Morocco	CEP	Jun 96	1 year
H. Bishop	UK	CG	Jul 97	2 months
M. Bonkowski	Germany	CEP	Jun 96	1 year
D. Bourdin	France	VIR	Oct97	2 weeks
C. Bragard	Belgium	VIR	Oct 96	16 months
J. Bujarski	USĂ	VIR	Mar 97	3 months
T.B. Butt	Pakistan	VIR	Jun 97	4 months
Monica A. Cardoso	Brazil	CMG	Jan 97	1 month
J. Carter	USA	SFPC	Apr 97	6 months
O. David	France	BioSS	Nov 97	2 weeks
E. Dekkers	The Netherlands	FBPP	Mar 97	5 months
A. Edwards	UK	CG	Jul 97	1 month
	Sudan			
I. Eujayl		CMG	Apr 97	3 months
M. Florence	India	CEP	Apr 97	3 months
L. Forsyth	UK	CG	Jul 97	2 months
A. Gal-On	Israel	VIR	Jul 97	3 weeks
M. Ghosh	India	CMG	Dec 97	4 months
Marina Hemery	France	NEM	Jul 97	6 weeks
M. Henry	UK	SFPC	Mar 97	3 months
Mairi Hunter	UK	SFPC	Aug 97	1 year
P.M. Johns	New Zealand	CEP	Sep 97	1 week
Bridget King	Canada	CG	Jun 97	3 months
Safaa Kumari	Syria	VIR	Aug 97	3 months
G. Langford	New Zealand	SFPC	Jul 97	3 weeks
R. Lister	UK	NEM	Apr 97	5 months
A. Lowe	UK	CMG	Mar 97	2 months
Lucy Mackinnon	UK	CG	Jun 97	3 months
Vanessa Maughan	UK	FBPP	May 97	3 months
C.N. Mayers	UK	VIR	Sep 97	3 weeks
Louise McConnachie	UK	SFPC	Mar 97	3 months
Samantha McGinnis	UK	SFPC	May 97	5 months
B. McKenzie	Australia	CEP	Jan 97	6 months
	The Netherlands	CEF		5 months
Klaas Meijer	UK	CG	Apr 97	
Lynne Meikle			Jun 97	2 months
G. Muluvi	Kenya	CMG	Aug 97	4 months
F. Nabugoomu	Uganda	BioSS	Aug 97	6 weeks
Kulpash Nurkiyanova	Kazakhstan	VIR	Oct 97	1 year
Pi Nyvall	Sweden	CEP	Jun 97	4 months
Vlada Peneva	Bulgaria	NEM	Nov 97	5 months
Christiana Petz	Germany	NEM	Nov 97	2 weeks
Nathalie Piroux	France	CEP	Apr 97	5 months
Sahandra Ranomenjanahary	Madagascar	VIR	Aug 97	3 months
P. Read	UK	FBPP	Mar 97	4 months
Linzi Ross	UK	CEP	Jun 97	3 months
I. Scotti	Italy	CMG	Nov 97	6 months
R. Sharp	UK	BioSS	Sep 97	1 year
Dorothy Stein	Australia	CMG	Aug 97	3 weeks
Tracey Sturgeon	UK	CG	Jun 97	4 months
S.A. Subbotin	Russia	NEM	Nov 97	1 week
A. Tattersall	UK	CG	Apr 97	4 months
G.W. Yeates	New Zealand	CEP	Apr 97 Jul 97	1 week
		VIR	Jul 97 May 97	1 week 10 weeks
B. Zange	Germany	VIK	iviay 97	TO Meerz
J. Zheng	China	NEM	Nov 97	5 months

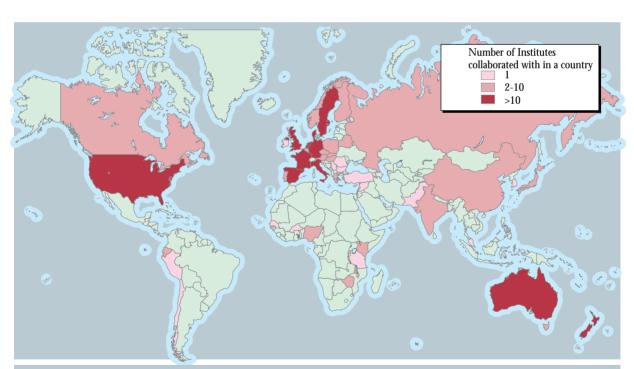
# **Editorial Duties**

Name	Position	Journal Title
H. Barker	Editorial Board Editor	Annals of Applied Biology Description of Plant Viruses
A.G. Bengough	Editor Editor	Annals of Botany British Society of Soil Science Newsletter
B. Boag	Editorial Board Editorial Board	Annals of Applied Biology Nematologia Mediterranea
R. Brennan	Editorial Board Associate Editor	Journal of Science of Food & Agriculture Journal of Horticultural Science & Biotechnology
D.J.F. Brown	Honorary Chief Editor Editorial Board	Russian Journal of Nematology Nematologia Mediterranea
J.W.S. Brown	Advisory Board	The Plant Journal
W.W. Christie	Editorial Board Editorial Board Managing Editor	Chemistry and Physics of Lipids Lipid Technology The Oily Press Ltd
D.E.L. Cooke	Editor	Molecular Plant Pathology On-Line
J.W. Crawford	Editor	Geoderma
J.M. Duncan	Associate Editor	The Journal of Horticultural Science and Biotechnology
C.A. Glasbey	Associate Editor	Biometrics
J.R. Hillman	Publication Committee Editorial Board Editorial Board	Journal of Horticultural Science Agricultural Systems Journal of Agricultural Science
A.T. Jones	Co-Editor	AAB Descriptions of Plant Viruses
R.A. Kempton	Editorial Board Editorial Board	Heredity Journal of Agricultural Biological & Environmental Statistics
D.K.L. MacKerron	Associate Editor Member of Editorial Board	Journal of Horticultural Science Euphytica
M.A. Mayo	Editorial Board Member Editorial Advisory Board Editorial Board	Encyclopaedia of Virology Encyclopaedia of Life Virology
J.W. McNicol	Editor	Annals of Applied Biology
I.M. Morrison	Executive Editor	Journal of the Science of Food and Agriculture
A.C. Newton	Senior Editor	Molecular Plant Pathology On-Line
K.J. Oparka	Editor International Advisory Board	Plant Physiology Journal of Experimental Botany
P. Palukaitis	Senior Editor Associate Editor Editorial Board	Molecular Plant-Microbe Interactions Virology Journal of General Virology
M.S. Phillips	Associate Editor	Journal of Nematology
W. Powell	Editorial Review Board	Molecular Ecology
D. Robinson	Editorial Advisory Board Consulting Editor	New Phytologist Plant and Soil
D.J. Robinson	Editorial Board Member Editor Editor	Journal of Virological Methods Molecular Plant Pathology On-Line Descriptions of Plant Viruses
G.R. Squire	Advisory Board Editorial Board Advisory Board	New Phytologist Experimental Agriculture Crop Physiology Abstracts
D.L. Trudgill	Advisory Board Editorial Board Editorial Board Associate Editor	European Journal of Plant Pathology Nematologica Fundamental and Applied Nematology Journal of Nematology
R. Viola	Editorial Board	Acta Botanica Neerlandica
T.M.A. Wilson	Editor	Journal of General Virology
I.M. Young	Associate Editor	European Journal of Soil Science

## Awards and Distinctions

Name	Dept.	Degree/Award/Distinction/Appointment
H.V. Davies	СЕР	Professorships, Universities of Cordoba and Malaga
H.V. Davies	CEP	Appointed to EU Scientific Committee on Plants
P.F. Palukaitis	VIR	Honorary Senior Lecturer, University of Dundee
S. Millam	CG	Honorary Lecturer, University of Dundee
I.K.Toth	FBPP	Honorary Lecturer, University of Aberdeen
I.M. Young	CEP	Honorary Lecturer, Department of Biological Sciences, University of Dundee
J .E. Angel-Diaz	VIR	Ph.D., University of Dundee
C. Regalado	CEP	Ph.D., University of Dundee
S. Ayivor	CG	M.Sc. Biotechnology (UAD)
A. Tattersall	CG	M.Sc. Biotechnology (UWE)
G.H. Cowan	VIR	M.Sc., University of Dundee
I.E. Geoghegan	Nem	M.Sc., University of Dundee

## International Collaboration and Consultancies



Research is executed within an international framework that encourages information transfer. The extent of SCRI's international commitment between 1993 and 1998 is reflected in the collaborative research that was undertaken with 318 Institutions in 54 countries. Within the UK, SCRI collaborated with over 200 organisations.

# SCRI Research Programme 1997-1998

SOAEFD funded research programme showing: SOAEFD project number; Title (prefixed ROA for ROAMEd corefunded 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/405/93	ROA Structure and function of the genomes of tobraviruses (specifically tobacco rattle and pea early browning viruses), with particular reference to virus variation, transmission and pathogenicity	Robinson D J
SCR/421/94	ROA Biosynthetic control of fibre constituents during development and differentiation of fibre cells and genetic modification of these processes	Davies H V
SCR/422/94	ROA Processing of plant fibres by novel and environmentally acceptable methods	Davies H V
SCR/423/94	ROA Physiological and developmental regulation of plasmodesmata	Oparka K J
SCR/424/94	ROA Relating soil structure to biological function	Young I M
SCR/426/94	ROA Fundamental studies on longidorid and trichodorid nematode vectors in relation to the aetiology of nepo- and tobraviruses which are transmitted to a range of arable and fruit crops	Brown D J F
SCR/427/94	ROA Characterisation of nematode cuticular surfaces of <i>Globodera</i> , <i>Heterodera</i> and <i>Meloidogyne</i> involved in pathogenesis	Robertson W M
SCR/428/94	ROA Investigate inheritance of low temperature sugar stability and develop effective selection strategies to produce superior potato germplasm for processing	Mackay G R
SCR/429/94	ROA Genetic architecture of diploid potatoes and production of enhanced germplasm	Bradshaw J E
SCR/432/94	ROA Integrated approaches for rapid and efficient gene transfer and characterisation in potato	Millam S
SCR/434/94	ROA Dissection of regulatory mechanisms governing invertase gene expression in potato	Machray G C
SCR/435/94	ROA To clone the <i>Hero</i> gene of tomato which confers resistance to potato cyst nematode by transposon tagging	Kumar A
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 <i>Rubus, Ribes</i> and <i>Fragaria</i>	McNicol R J
SCR/446/95	ROA Molecular study of genetic variation in plant parasitic nematodes in relation to virulence and plant resistance especially in relation to potato cyst nematodes (PCN) and root knot nematodes	Phillips M S
SCR/449/95	ROA Advanced information techniques for the study and management of vegetation systems	MacKerron D K L
SCR/450/95	ROA Variation and stability of traits governing plant development and resource capture in relation to environment and plant competition	Marshall B

SCR/451/95	ROA Genetic and environmental analysis of epidemics of <i>Erysiphe graminis</i> on barley and oats, <i>Phytophthora fragariae</i> on strawberries and raspberries and <i>Erwinia</i> spp. on potatoes	Newton A C
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/455/95	ROA DARE Dynamics and connectivity in discontinuous plant populations, using wild raspberry and feral oilseed rape as model systems	Crawford J W
SCR/456/95	ROA Genetics and ecophysiology of abiotic stress tolerance in <i>Hordeum vulgare</i> (barley) and <i>Arabidopsis thaliana</i>	Forster B P
SCR/457/95	ROA Development and evaluation of novel methodology involving modern chromatography and mass spectroscopy for stable isotopes and antinutritional, quality and other biologically active compounds	Christie W W
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/465/96	ROA Application and exploitation of molecular markers in barley genetics	Powell W
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/478/96	ROA Physiological mechanisms underlying the environmental responses of crops in Northern Britain: stable isotope studies of carbon, nitrogen and water relations in barley and contrasting dicot model populations	Handley L L
SCR/479/96	ROA Maintenance, improvement, evaluation and exploitation of biodiversity in germplasm collections of potato	Bradshaw J E
SCR/481/96	ROA Evaluation, improvement, maintenance and exploitation of bio- diversity in germplasm collections of brassicas for improved pest resis- tance (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/485/96	ROA Molecular and biological factors which control the transmission of luteoviruses (in particular potato leafroll virus) and potyviruses (in particular potato virus Y) by their aphid vectors	Mayo M A
SCR/486/96	ROA Identification and development of control strategies for fungal diseases of fruit crops, especially the use of specific enzyme inhibitors for control of <i>Botrytis cinerea</i> in fruit	Williamson B
SCR/487/96	ROA Mathematical analysis of dynamics and scaling in heterogeneous and hierarchially coupled systems II: complex biochemical networks	Crawford J W

# Research Projects

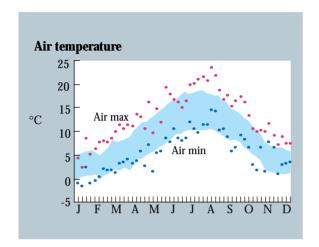
SCR/494/97	ROA Genetic control of pathogenicity, host specificity and race structure at the molecular level in the fungal pathogens <i>Phytophthora infestans, Phytophthora fragariae</i> and related <i>Phytophthora</i> species	Duncan J M
SCR/495/97	ROA Transcriptional and post-transcriptional regulation 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 deploy- ment of new types of resistance for germplasm enhancement	Barker H
SCR/498/97	ROA Genetic modification of soft fruit and identification of tissue specific promoters for future gene targeting	McNicol R J
SCR/499/97	ROA Free radical processes in plants and plant-derived foods	Davies H V
SCR/500/97	ROA Analysis and disruption of the host-parasite interaction of the potato cyst nematode <i>Globodera pallida</i>	Jones J T
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/502/97	ROA Enabling technology for plant genome characterisation	Waugh R
SCR/503/97	ROA Produce and maintain pathogen-tested stocks of soft fruit cultivars and index for infection material imported into SCRI	Jones A T
SCR/416/93	FF Foodweb analysis of below ground ecosystems using natural abundance of stable isotopes	Handley L L
SCR/440/94	FF Investigation of <i>in vitro</i> splicing in plants and characterisation of snRNP and splicesomal complexes	Brown J W S
SCR/443/95	FF Research into nutritional aspects of genetically manipulated potatoes, <i>Solanum tuberosum</i>	Mackay G R
SCR/458/95	FF Determining the origin and genetic structure of late blight outbreaks on Scottish seed and ware potatoes and assessing the hazard of sexual reproduction by <i>Phytophthora</i> to the seed industries of Scotland	Duncan J M
SCR/459/95	FF Development of tests to distinguish potato cultivars and their transgenic variants	Machray G C
SCR/461/95	FF Native Scots Pine: establishing a scientific basis for its conservation	Powell W
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/504/97	FF Comparison of serological and PCR tests on dormant tubers and attempts to identify sources of virus in Scottish fields	Barker H
SCR/505/97	FF Molecular approaches to manipulate the development and composition of strawberry fruit	Davies H V
SCR/803/94	FF Fundamental studies to develop plant virus-like particles expressed in <i>Escherichia coli</i> as vaccine or therapeutic agents	Wilson T M A
SCR/805/94	FF Control of certain invertebrate pests of agricultural importance using gut membrane proteins as targets for antibodies	Fenton B

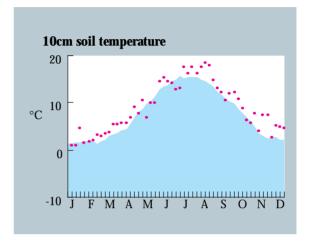
# Research Projects

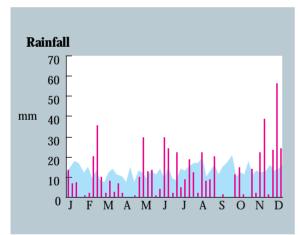
SCR/808/94	FF Development of molecular biological and physiological techniques in studies of the interaction between microbes, nutrient cycling and vegetation among a range of agriculturally important pastures, to enable scaling from microcosm to field.	Ritz K
SCR/815/94	FF Prediction of starch processing potential in relation to cereal and potato production under Scottish conditions.	Morrison I M
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 <i>C. elegans</i> 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

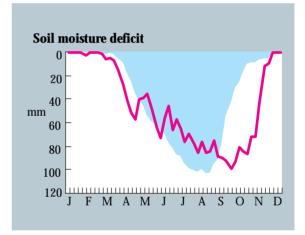
# **leteorological Records**

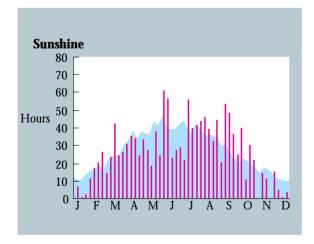
D.K.L. MacKerron
Detailed meteorological records are kept regularly at SCRI. The graphs shown are for weekly values for 1997 and the long term average for 1961-1990(\_\_\_\_\_).

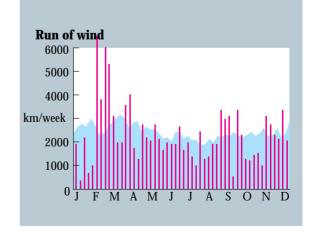












# Cumulative Index 1990 - 1997/8

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

	_	
Quality in potatoes: G.R. Mackay & M.F.B. Dale	J,	9
Anti-nutritional factors in faba beans, forage brassicas and potatoes: J.E. Bradshaw, et al	) 1	12
Malting quality of barley: J.P. Camm <i>et al.</i>	<b>0,</b> 1	16
Low temperature hardiness and avoidance of frost damage in woody perennials: R. Brennan		
Progeny testing for resistance to diseases and pests of potato: R.L. Wastie et al	1, 1	13
Identifying and exploiting resistance to potato late blight: R.L. Wastie, et al	1, 1	16
Breeding for resistance to barley powdery mildew: W.T.B. Thomas et al	1, 1	20
Breeding for resistance to premature fruit shedding: R.J. McNicol	1, 2	23
Conservation and utilisation of germplasm collections of potato and faba bean: M.J. Wilkinson et al	2, 1	13
Breeding to exploit heterosis in swedes: J.E. Bradshaw	2, 1	17
The use of <i>Hordeum spontaneum</i> Koch in barley improvement: R.P. Ellis <i>et al.</i>	2, :	20
Applications of biotechnology to soft fruit breeding: Julie Graham	2, 1	23
Breeding potatoes for warm climates: G.R. Mackay et al	3, 2	20
Endosperm cell walls - barriers to malting quality: J.S. Swanston et al	3, 7	24
Case studies in the investigation of potential industrial oil crops: S. Millam et al	3, 2	26
Potato breeding at SCRI: from wild species to finished cultivars: J.E. Bradshaw <i>et al.</i>	4, :	36
Increasing the applicability of tissue culture methods for the improvement of industrial oil crops: S. Millam et al		
Aspects of environmental risk assessment for genetically modified plants with special reference to oilseed rape: A.M. Timmons et al199	4,	43
Genetic improvement of trees: R.J. McNicol & M. Van de Ven		
Breeding potatoes at SCRI for resistance to PCN: J.E. Bradshaw et al	5, :	30
The adaptation and use of primitive cultivated potato species: M.J. De, Maine et al	5, :	34
Dissecting the Vicia faba genome: G. Ramsay et al		
Investigation of feral oilseed rape population: Y. Charters et al	5,	40
The targeted and accelerated breeding of potatoes: G.R. Mackay et al		
Breeding swede, forage rape and kale cultivars with resistance to clubroot (Plasmodiophora brassicae): J.E. Bradshaw et al	6, 4	45
Non-transgenic applications of plant tissue culture in potato: S. Millam et al		
New brassica cultivars 199		
New potato cultivars	6, :	54
New swede cultivars		
New soft fruit cultivars	5,	43

#### Breeding and genetics (1997/8 onwards)

Applied Potato Genetics & Breeding: the way ahead for potato breeding: J.E. Bradshaw et al	)7,	76
Genome bioinformatics at SCRI: engineering the datastream: D.F.Marshall & L.Cardle	7,	81
Potato Genomics: Development of markers for potato genetics and breeding: R. Waugh et al	7,	86
Blackcurrant breeding and genetics: R.M. Brennan <i>et al.</i>	7,	89

# Soft fruit and perennial crops

1990-1995: Relevant articles appear under Plant genetics, Fungal & bacterial diseases, Virology etc.		
Genetically modified food: J. Graham	1996,	58
Rubus breeding and genetic research: R.E. Harrison et al	1996,	63
Interactions between plant resistance genes, pest aphid populations and beneficial aphid predators: A.N.E. Birch et al	1996,	68
Transgenic resistance to raspberry bushy dwarf virus in Nicotiana species: J.E. Angel-Diaz et al	1996,	73
The increasing importance and control of wingless weevils as pests in temperate world horticulture: S.C. Gordon et al	1996,	75

# **Molecular biology**

Genetic markers: W. Powell <i>et al</i>	.1990,	25
Components of the plant pre-messenger RNA splicing machinery: J.W.S. Brown & R. Waugh	.1990,	28
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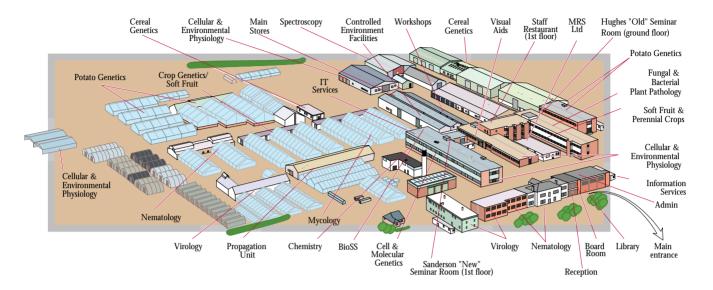
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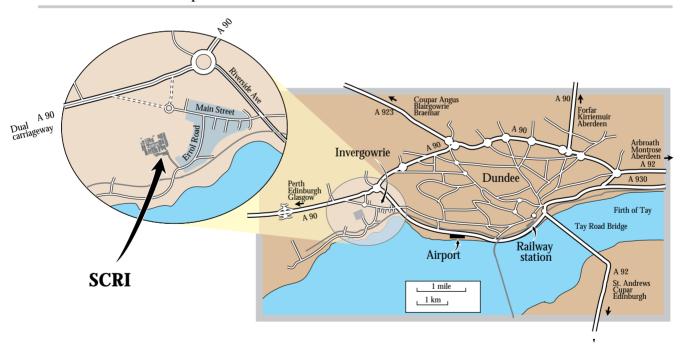
AAB	Association of Applied Biologists	ISPP	International Society for Plant Pathology
ACRE	Advisory Committee on Releases to the Environment	IVEM	Institute of Virology and Environmental Microbiology
ADAS	Agricultural Development and Advisory Service	MAFF	Ministry of Agriculture Fisheries and Food
BBSRC	Biotechnology & Biological Sciences Research Council	MLURI	Macaulay Land Use Research Institute
BCPC	British Crop Protection Council	MRI	Moredun Research Institute
BioSS	Biomathematics and Statistics Scotland	NERC	National Environmental Research Council
BSPB	British Society of Plant Breeders	NFT	National Fruit Trials
BTG	British Technology Group	NFU	National Farmers Union
CAPS	Cleaved Amplified Polymorphic Sequence	NIR	Near Infra-Red
CEC	Commission of the European Communities	NMR	Nuclear Magnetic Resonance
CHABOS	Committee of Heads of Agricultural and Biological	NPTC	National Proficiency Test Council
	Organisations in Scotland	ODA	Overseas Development Administration
CIP	International Potato Centre - Peru	ORSTOM	Organisation for research in science and technology overseas
COST	European Co-operation in the field of Scientific and Technical Research	PCR	Polymerase Chain Reaction
		PMB	Potato Marketing Board
EAPR	European Association for Potato Research	PVRO	Plant Variety Rights Office
ECRR	Edinburgh Centre for Rural Research	RAPD	Randomly Amplified Polymorphic DNA
ECSA	European Chips and Snacks Association	RFLP	Restriction Fragment Length Polymorphism
EHF	Experimental Husbandry Farm	RRI	Rowett Research Institute
ELISA	Enzyme linked immunosorbent assay	SABRI	Scottish Agricultural and Biological Research Institutes
EPPO	European Plant Protection Organisation	SAC	Scottish Agricultural College
ESTs	Expressed Sequence Tagged Sites	SASA	Scottish Agricultural Science Agency
FF	Flexible Funding (SOAEFD)	SCRI	Scottish Crop Research Institute
FLAIR	Food-Linked Agro-Industrial Research	SEB	Society for Experimental Biology
GILB	Global Initiative on Late Blight	SET	Scottish Enterprise Tayside
GIUS	Glasshouse Investigational Unit for Scotland	SNSA	Scottish Nuclear Stocks Association
H-GCA	Home-Grown Cereals Authority	SOAEFD	Scottish Office Agriculture, Environment and
HDC	Horticultural Development Council		Fisheries Department
HPLC	High Performance Liquid Chromatography	SSCR	Scottish Society for Crop Research
HRI	Hannah Research Institute	SSFG	Scottish Soft Fruit Growers Ltd
IACR	Institute of Arable Crops Research	SSPDC	Scottish Seed Potato Development Council
ICTV	International Committee for the Taxonomy of Viruses	STS	Sequence Tagged Sites
IOBC	International Organisation for Biological Control	UNDP	United Nations Development Programme
ISHS	International Society for Horticultural Science	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.