Scottish Crop Research Institute

Annual Report 1996/97

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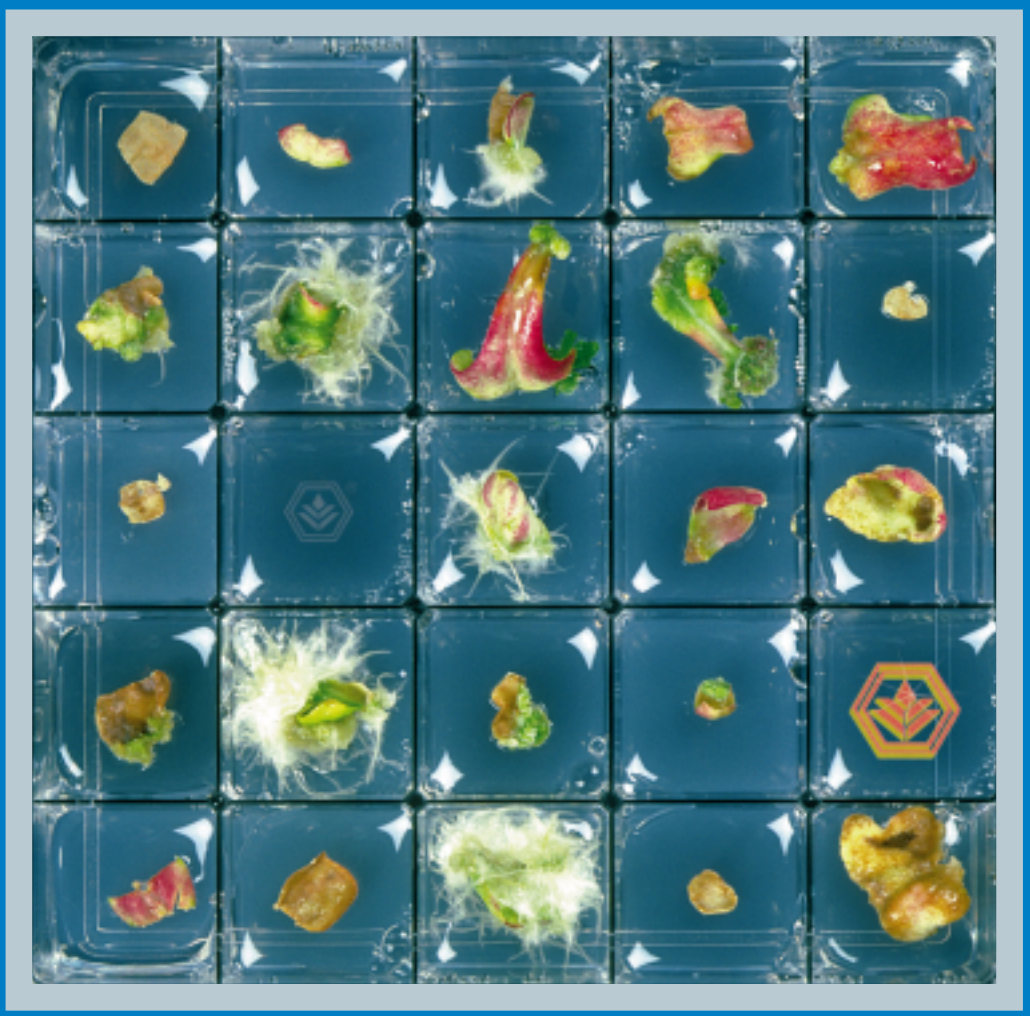

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The Scottish Crop Research Institute (SCRI) is a major international centre for research on agricultural, horticultural and industrial crops, and on the underlying processes common to all plants. It aims to increase knowledge of the basic biological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

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

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

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

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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.

Table 1 The mission and aims of SCRI.

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.

SCRI is a major international centre for basic, strategic and applied research on agricultural, horticultural and industrial crops and on the underlying biological processes common to all plants. It is the only such Institute in Scotland, and 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 important to northern Britain, is not to be found elsewhere within any civil or private sector agribusiness centre in the UK or Europe.

A broad multidisciplinary approach to fundamental 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, 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 Mylnefield Research Services (MRS) Ltd (Managing Director, Nigel W Kerby), the commercial arm of the Institute, are successful in gaining competitive research contracts from government departments and agencies, Levy Boards, grower organisations, international agencies, the European Union, commercial companies, local government, and some Charities, Research Councils and Trust funds,

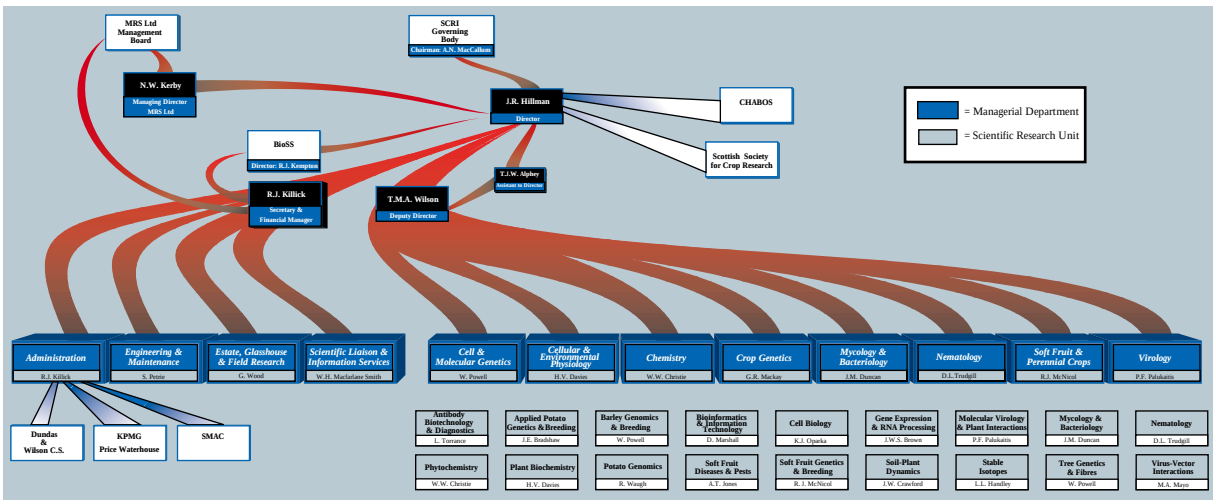


Figure 1 SCRI Departmental and Research Unit structure.

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 end-user/researcher discussion sessions;

* financing science-based advisory publications for the benefit of its members;

* stimulating crop-based sub-committees to support targeted research projects [*e.g.* 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 Service and Forestry Commission, comprise the Committee of Heads of Agricultural and Biological Organisations in Scotland (CHABOS).

The final conclusions and recommendations of the 1995-96 Prior Options Review of Public Sector Research Establishments (PSREs) conducted on SCRI by SOAEFD, on behalf of the UK Office of Science and Technology (OST) and the Cabinet Office Efficiency Unit, were announced in the House of Commons on 29 January 1997. The review focused on five key questions and on the privatisation guidance issued by OST, Office of Public Service and the Treasury. The key questions were as follows. Is the function needed? Must the public sector be responsible for funding the function? Must the public sector provide the function itself? What is the scope for rationalisation? How will the function be managed? The outcome of the review was that the status of SCRI would not alter, the function of the Institute was recognised as being essential to the UK Science Base and the Institute would therefore retain its separate identity. However, further reviews and efficiency scrutinies were presaged for all the UK PSREs.

BioSS (Director, Rob 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. A Visiting Group, comprising Professor J M McGlade (Chairman), Professor L Billard, Professor P J Green and Professor B J T Morgan, reviewed BioSS on 30 and 31 January 1996. The Group was accompanied by Mr B Harris and Ms F Anderson of BBSRC's Swindon Office, and Dr A J Rushworth and Dr T W Hegarty of SOAEFD. The Visiting Group was impressed with the BioSS staff and their achievements, and commended this unique organisation for succeeding in maintaining the difficult balance between the provision of consultancy and training to meet specific requirements, and the pursuit of original research of more general interest. A series of 11 constructive recommendations were made to help BioSS achieve its full potential and exploit the opportunities that were becoming available.

In April 1995, at the end of the analysis phase of the then UK Technology Foresight Programme, the Agriculture, Natural Resources & Environment Sector Panel (ANRE) which I chaired, published its report with a series of recommendations (see previous Annual Reports). Late in 1995, in phase 2 of the programme, ANRE was split into three Panels: Agriculture, Horticulture & Forestry (AHF); Natural Resources & Environment; and Marine. I chaired the AHF Panel until stepping down in March 1997. The Panel's remit for phase 2 was to disseminate the findings of the first phase and to start to promote networking and partnership building between academia and industry. The panel decided that in order to do this, its first task should be to refine the recommendations of ANRE to make them more specific and relevant to the industries it was responsible for. It therefore formed three sub-groups - Plant Systems, Livestock Systems and Forestry & Wood Products - which conducted mini-Foresight analyses

of their sub-sectors, based on the work of ANRE. These analyses included assessing the UK's position in World and European markets, its academic and industrial strengths, the priorities for R&D, and for infrastructural changes to increase UK competitiveness and quality of life. The sub-groups' activities were described in the AHF Panel's first report published in November 1996. During this phase, the programme was referred to as 'Foresight' to reflect that its deliberation extended beyond Technology. In phase 3, the Panel will focus on discussions with non-technical business decision-makers, engaging the wider business community, whilst continuing with scientific and industry analyses and generating position papers and recommendations. The AHF Panel Chairman is now Dr Ed Dart, and the Panel Secretary is Dr David Rawlins, OST. A Food Chain Group chaired by Mr David Sainsbury and overarching the work of several Panels has been established by OST.

Following the pattern of previous SCRI 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, and 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.

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 continuing support of, and demonstrable commitment to, our research programme and to our development. Regardless of the enormous pressures upon them in recent years, they function rigorously, openly and fairly, as always, to the highest professional standards of British public service I have ever encountered. Grants, contracts, donations, advice and joint participation in our activities from the SSCR, other government departments and their agencies, non-governmental agencies, our sister CHABOS institutions and BBSRC institutes with whom we coordinate our research, grower levy boards, local and regional authorities, commercial companies, farmers and other individuals, and learned societies, are also warmly appreciated.

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

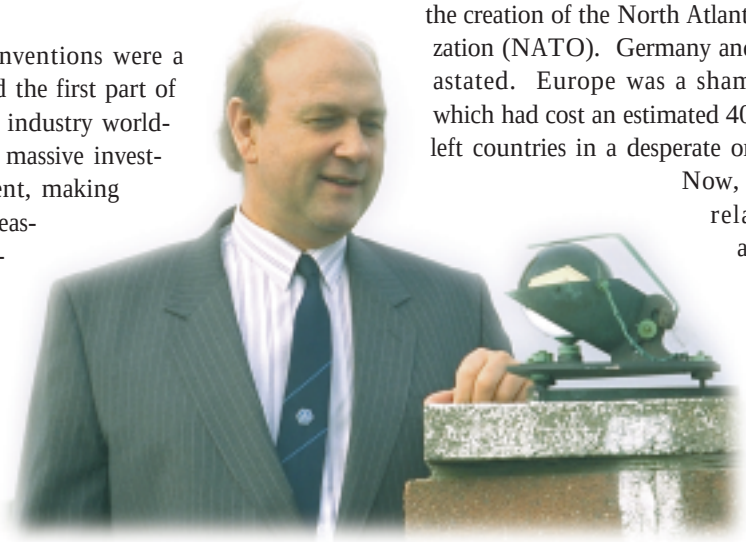
Report of the Director

John R. Hillman

Global perspectives of factors influencing agricultural, biological and environmental sciences, and their associated industries

Preamble

Scientific discovery and inventions were a special feature of 1996 and the first part of 1997. The biotechnology industry worldwide continued to receive massive investments, creating employment, making stunning advances and releasing products into the marketplace. In Europe, problems of food contamination by the proteinaceous agent responsible for Bovine Spongiform Encephalopathy (BSE) and by *Escherichia coli* (*E.coli*) O157 dented public confidence in the enforcement of regulations. Global economic output, agricultural output and the world's population grew. Food aid needs declined, and more attention was given to sustainability issues.



Fifty years ago, the delegates of 51 nations gathered in London for the first United Nations (UN) Organization General Assembly. The UN Educational, Scientific and Cultural Organization (UNESCO) was founded. Winston Churchill delivered his 'Iron Curtain' speech at Fulton, Missouri; communism tightened its grip on Eastern Europe, and the West responded with the creation of the North Atlantic Treaty Organization (NATO). Germany and Japan were devastated. Europe was a shambles after a war which had cost an estimated 40 million lives and left countries in a desperate ordeal for survival. Now, out of the ashes, relative prosperity and peace have been achieved, but a multiplicity of languages still separate the Europeans, along with cultural and

historical differences. Some of the boundaries remain ambiguous, and there continues to be a tendency for fragmentation, regardless of the existence of the European Union (EU), and internalisation of trade, communications and entertainment. Europe retains more of the characteristics of an annelid than a vertebrate.

Whereas 50 years ago the major scientific advances were in chemistry and especially physics, nowadays the biological and related sciences dominate the headlines. Nevertheless, important discoveries in chemistry and physics were made in 1996. For example, element 112 (atomic mass 277) was synthesised by GSI Darmstadt, with the heaviest nucleus (112 protons and 165 neutrons) ever created in the laboratory. Theoretical analysis predicted that although element 112 decays rapidly, the periodic table contains a region of comparatively long-lived superheavy elements beginning at element 114. At the European Laboratory for Particle Physics, Geneva, the first atoms of antimatter, antihydrogen atoms, were produced, thereby confirming the theory of apparent symmetry between normal matter and antimatter. The existence of antiparticles, such as positrons and antiprotons, had been demonstrated in 1932 and 1955 respectively, raising then the possibility of antiatoms and bulk antimatter, identical to atoms and normal matter, except for the reversal in electrical charge and certain other quantum properties.

In August, the US National Aeronautics and Space Administration (NASA) announced that there was evidence for life on Mars having existed more than 3.6bn years ago. This remarkable claim was based on studies of the 1.9kg meteorite ALH 84001, found in 1984 in the Allan Hills ice field of Antarctica and thought to have landed on Earth about 13,000 years ago. It is one of about 12 meteorites whose chemistry matches the unique Martian chemistry found by the Viking spacecraft that landed on Mars in 1976. It was claimed that polycyclic aromatic hydrocarbons of Martian origin, mineral features characteristic of biological activity, and tubular and egg-shaped structures reminiscent of bacteria-like organisms were in the meteorite. Similar claims were made in the UK for another putative Martian-derived meteorite, EETA 79001.

By the end of 1996, several thousand artificial satellites were circling the earth, and even more items of space 'junk' from defunct satellites and related hardware. About 1,000 of the satellites were in geosynchronous orbit. The primary function of many of the satellites is to provide a television service, whereas others deploy observational instruments to monitor meteorological changes, land and sea use, natural resources and transportation for civilian and military purposes.

A boom was noted in science-fiction television and cinema. 'Star Trek' and its derivatives helped spawn dedicated TV channels building on successful earlier shows, more recent series such as 'The X-Files', and the film 'Jurassic Park'. Difficulty in separating fact from fiction, scepticism over regulatory mechanisms, ignorance of scientific terminology and concepts, careful avoidance of taking up scientific careers, a total dependency on the products of science in every aspect of life and lifestyles are the collective attributes of modern adults. Antipathy to science has even permeated those areas of arts-dominated, scientifically ignorant academia concerned with 'imperialistic ideology'. Science has been described by Patrick Riley of University College London as an epistemological philosophy embracing the Humean (after David Hume, the 18th Century Edinburgh philosopher) dichotomy of rational and real knowledge. The scientific process or method involves the validation of ideas, concepts and hypotheses about the real world by experimental comparison of the behaviour of objective reality with that predicted by conceptual models or structures. Put simply, it is a method of acquiring and using knowledge and truth, but is rarely presented as such.

Matthew Parris, occasional commentator on the progress of the Plant Varieties Bill in the House of Commons noted in *The Times* the emergence of a moral, ethical or political authoritarian consensus, a development of 'political correctness', that punishes dissent or perfectly legal activities. Hysteria and emotion, stoked by the activities of tabloid media, can be seen to suppress critical analysis and silence legitimate questions. Such developments are an anathema to science.

Education was a global topic in 1996. Common themes were literacy and numeracy deficiencies in school children and school-leavers, multiculturalism, the use of the information superhighway, drug abuse, the need for training and re-skilling programmes for the unemployed and underemployed, the rapid escalation in public-sector costs of higher and further education, and the suitability of graduates and post-graduates for employment. Most countries adopted measures to increase the proportion of school-leavers

entering university, and raising the status of women in higher education.

Economics

During 1996, according to World Bank and International Monetary Fund (IMF) estimates, global economic output increased by 3.8%, slightly faster than in 1995, despite the disappointing economic performance of many EU nations. For the countries of the Organization of Economic Co-operation and Development (OECD), real gross domestic product increased by just 2.2%, reflecting the mid-cycle economic dip.

In the EU, lower interest rates and steady exchange rates should have aided economic activity, but the effects of the various policies to achieve economic and monetary union (EMU), outlined in the Maastricht Treaty on European Union, led to reduced average economic growth of just 1.6%, a decline from the weak level of 2.5% in 1995. The key convergence criteria for qualification are (i) public-sector deficit to be at or below 3% of Gross Domestic Product (GDP), (ii) outstanding public debt to be no more than 60% of GDP, (iii) inflation rate to be no more than 1.5% above average of the three best-performing countries, (iv) long-term interest rates to be similar to the average of the three best-performing countries, (v) currency exchange rates should have been within the normal Exchange Rate Mechanism (ERM) band for at least two years.

More substantial upturns in activity, compared with the EU, were noted in Australia, Japan, New Zealand, the USA, and most of the Least-Developed Countries (LDCs). Growth rates in South and East Asia were close to 8%.

Several African countries remained amongst the poorest in the world, despite a recovery in economic growth rates. In nearly all the More-Developed (or industrialised) Countries (MDCs), policies were in force to help bring about non-inflationary growth, reduce public-sector deficits, deregulation, and liberalisation in the terms of trade. Fiscal policy remained tight in the EU.

Virtually no improvement was noted in the unemployment rate in the OECD countries, where the average rate remained at about 7.3%, excluding those in voluntary and involuntary early retirement or those not formally registered as job-seekers. New Zealand, the UK and the USA, however, benefited from declines in unemployment. A report in the National Institute Economic Review concluded that the only way to reduce, or even control, taxes and public spending is to put the unemployed back to work. The proportion of GDP spent on social security has grown substantially in recent years, straining other parts of the public sector, not least publicly funded research and development (R&D). In the EU, governments, organised labour and various management organisations tried to address the burgeoning costs of social security, including pensions, unemployment benefits, parental leave, and sickness absence costs. Difficult as the problem may be, the costs must be reduced if only to satisfy the entry criteria for EMU.

For the LDCs, real economic growth averaged 6.5% in 1996, slightly higher than in 1995. Improved foreign investments, expanding domestic growth and export volumes, better control of inflation, and the waning impact of the Mexican financial crisis all aided the more favourable economic environment. Although the Middle East displaced Latin America as the region with the highest overall inflation rate, the IMF expected the median inflation rate to decline from 10% in 1995 to 7% in 1996.

Global trade in goods and services was projected by the IMF and other bodies to have risen between 6.4-6.7%, the strongest growth taking place in the LDCs. Low or falling interest and inflation rates, coupled with higher corporate profitability, sustained a 12% gain in dollar terms of the world's stock exchanges. At the end of the year, comments by Alan Greenspan, the US Federal Reserve Chairman, about 'irrational exuberance' in asset values on Wall Street, destabilised the markets somewhat.

A sceptical attitude towards EMU was adopted by the financial markets, principally in respect of time-scales and convergence of economic cycles. The EU Intergovernmental Conference negotiations were retarded by the UK's strong opposition to further strengthening of the dirigiste EU, or to any move in the direction of a federal super-state. Moves to control BSE, and the ban on exports of UK beef and beef products, led to a campaign by the UK of non-cooperation prior to the Florence Summit, aided by the decision of the European Court to force the UK to introduce, with few exceptions, a 48-hour limit for the working week.

Sobering analysis of the latest (1995) data on productivity and employment in the manufacturing industries, sourced from the UN Monthly Bulletin of

Statistics and the International Labour Organization Yearbook of Labour Statistics, shows that since 1990, US industry raised its output by 17%; Canada by 10%; while France, Italy and the UK only managed 1-2%; but, for Japan and Germany, there were falls of 5% in output.

The image of the banking system was severely affected by accusations that well-respected Swiss banks had concealed extensive assets derived from Nazi Germany and its area of influence, and that Swiss banks had deliberately hidden the deposits of Holocaust and other victims. There were also questions beyond banking profits about the role of the 'neutral' nations in Europe during the World War II. Britain paid a high economic price for its non-neutral stance in both World Wars.

International Politics

The sovereignty of nation-states was increasingly diluted, regardless of the tendency towards fragmentation of larger nations to form smaller regional entities, by their inability to act without taking into account reactions from regional trading blocs, neighbours and the world community. Accordingly, international law reluctantly took on a strong resemblance to constitutional law as inter-state relations became increasingly judicialised and politicised. In the area of international adjudication, the workload of the International Court of Justice increased. Two new international tribunals began operations in 1996 - the World Trade Organization's (WTO) Appelate Body was formed to hear appeals against WTO panel reports, and the inaugural meeting took place of the International Tribunal for the Law of the Sea. The UK has yet to ratify the UN Convention on the Law of the Sea which will have implications for the UK economy in that, for example, uninhabited rocks without an economy (*e.g.* Rockall) cannot be used as a basis for territorial claims to fishing and mining rights.

Uncertainty about the future rôle of the UN extended into 1996. Given that unpaid contributions by members reached $2.8bn, $1.5bn of which were attributable to the USA, the organization was technically bankrupt. This led to a scaling-down of posts and activities reaching into all the programmes and associated agencies. Although the majority of nations supported the re-election of Boutros Boutros-Ghali to a second 5-year term as Secretary-General of the UN, the USA exercised its veto power in the Security Council. By the end of the year the Security Council and General Assembly had ratified the appointment of Kofi Annan of Ghana, the Under-Secretary-General for Peacekeeping Operations, to succeed Boutros Boutros-Ghali in January 1997.

The five recognised nuclear powers (China, France, Russia, UK and the USA) agreed in 1996 to ban nuclear explosions, some 51 years into the atomic era. Although India vetoed the draft Comprehensive Test Ban Treaty at the UN Conference on Disarmament, it was nonetheless approved by the General Assembly. By the beginning of 1997, 41 of the 44 nations recognised as possessing nuclear facilities and whose agreement is essential for the Treaty to come into force, had signed the Treaty together with 90 other nations. Even though neither Russia nor the USA had ratified the Chemical Weapons Convention, the fact that 65 other nations had done so meant that the Treaty entered into force in April 1997.

Conflicts were much in evidence in 1996. The NATO-led, 32-nation Implementation Force in Bosnia and Herzegovina enforced the peace accord negotiated in Dayton, Ohio, bringing about relative peace in the former Yugoslavia. On the other hand, war continued in the Russian republic of Chechnya, and civil wars still occurred in Central and South Asia. Military confrontation occurred in the Korean peninsula, and public order collapsed in Albania. The bloodiest conflicts were between the Hutu and Tutsi in Rwanda and Burundi, and between Rwanda and Zaire. Aggressive Chinese policy towards democratic elections brought about a confrontation with Taiwan, and there was also disagreement with Japan over ownership of the Senkaku islands in the China Sea. International terrorism and organised crime presented challenges to all nations.

A resurgence of tensions and armed conflicts in the Middle East was probably the most dangerous development at a regional level, with the potential to evolve quickly into full-scale international war. Abetted by those nations that wanted to gain re-entry into the Iraqi market, Iraq challenged the terms of the armistice following the Persian Gulf War, and the conflict overspilled into the Kurdistan region. Conflict also resurfaced between Israel and the Palestinian Arabs, leading to a serious deterioration in relationships between Israel and its Arab neighbours.

Poland and Hungary were admitted to membership of the OECD, the second and third former communist states to join the Paris-based research group

which studies economic conditions in industrialised countries.

Membership applications for the Association of Southeast Asian Nations (ASEAN) were accepted by the seven existing members (Brunei, Indonesia, Malaysia, the Phillippines, Singapore, Thailand, and Vietnam) in respect of Cambodia and Laos. Much to the chagrin of the EU and USA, the military dictatorship of Myanmar (Burma) was granted observer status. The ASEAN Regional Forum of ASEAN members, and 14 other nations with security interests in the Asian-Pacific region, discussed creating a Southeast Asia Nuclear-Weapon-Free Zone, opposing China's attempts to extend its jurisdiction in the Sea.

For the first time, a Euro-Asia summit to stimulate commercial relations and beneficial policies was held in Bangkok and involved the heads of state of the EU nations and 10 Asian countries.

The member nations of the Andean Group (Bolivia, Columbia, Ecuador, Peru and Venezuela) decided to rename the organisation the Andean Community, heralding the intention to move towards greater integration akin to that which occurred after the European Community was formed.

Populations

By mid-1996, the world population was estimated by the Population Reference Bureau to be 5.771bn, about 88 million greater than in 1995, with 98% of the increase taking place in the LDCs. The annual rate of increase was about 1.52% as birth rates declined in both LDCs and MDCs. At the present breeding and death rates, the world's population will double in 46 years. A population growth rate of 1.9% for LDCs compared with 2.2% for LDCs excluding China, would indicate a population doubling time of 32 years in the LDCs. Each day, the world population increased by about 240,000 people, the result of 383,000 births and 143,000 deaths. World-wide, 32% of the population was below 15 years of age; this figure was 38% of the population of LDCs excluding China, compared with 20% or less in the MDCs. Only 5% of the population was over the age of 65 in LDCs, compared with 14% or greater in the MDCs.

About 43% of the global population was urban, comprising 35% of the population of LDCs and 75% of MDCs; this accounts for the growing gulf between rural and urban perceptions of lifestyles and rôles. Life expectancy at birth was 64 for males and 68 in females for the LDCs, and 70 and 78 in the MDCs, respectively. One of the greatest challenges is the care of the increasing number of the elderly infirm.

The total fertility rate (average number of children a woman would bear in her life-time at the current rate) was 3.4 in the LDCs, down from 3.5 in 1995. Africa once again remained the region with the highest fertility, women having an average of 5.7 children, rising to 6.1 in urban sub-Saharan Africa. Africa also had the lowest life expectancy (53 for males and 56 for females), and the fastest population growth (2.8% *per annum*).

In the MDCs, Europe had a negative rate of population growth (birth rate minus death rate of -0.1%), primarily due to trends in the European republics of the former Soviet Union.

No massive new refugee influxes were reported during the year, and the world's refugee population was calculated to have declined from 14.5 million in 1995 to 13.2 million in 1996. Repatriation was regarded as the primary solution. The overall population of concern to the Office of the UN High Commissioner for Refugees (UNHCR) fell to 26.1 million, of whom 3.4 million were returnees, 4.6 million were displaced persons within their national boundaries, and 4.8 million were of humanitarian concern, mostly victims of conflict. Around 9 million people were thought to have moved within or between the countries of the Commonwealth of Independent States following the dismantling of the former Soviet Union.

Little change was reported in the humanitarian crisis afflicting the African Great Lakes region (Burundi, Rwanda, Tanzania and Zaire) where more than 2 million Rwandans and Burundians had fled into neighbouring countries. About 1.7 million refugees were repatriated voluntarily to Mozambique, but outbreaks of violence in Liberia postponed the repatriation of 750,000 refugees. Over 650,000 Malian, Ethiopian, Eritrean and Somalian refugees have returned in recent years. In Europe, approximately 250,000 displaced persons resettled in Bosnia and Herzegovina. The largest refugee caseload of concern to the UNHCR was the 2.2 million Afghan refugees in Iran and Pakistan.

Food Aid

Short-term food prospects for many low-income, food-deficient countries (LIFDCs) improved in

1996-1997. Food aid needs declined except for certain countries which experienced crop failures, natural disasters and continuing civil strife. FAO estimated that 40% of the population of Africa had been undernourished in recent years, and civil strife created special difficulties in the Great Lakes region. LDCs would need about 9-11 million metric tonnes (mmt) of food aid in the form of cereals during the 1996-1997 crop year, according to the results of an annual analysis by the US Department of Agriculture (USDA). Sub-Saharan Africa, Afghanistan, Bangladesh, Iraq, Laos, Tajikistan, Turkmenistan and North Korea were the main areas of aid needs. In contrast, food aid needs were reduced in most of the LDCs in Latin America and Asia in 1996, as a result of stronger economic growth and above-average agricultural production. On the basis of minimum commitments under the 1995 Food Aid Convention, the donor nations (Australia, Canada, EU members and organisations, Japan, Norway, Switzerland, USA, and various other contributors) were expected by the UN Food and Agriculture Organization (FAO) to supply 7.5mmt grain equivalents in the 1996-1997 crop year, up from 7.16mmt in 1995-1996, but down from 9.35mmt in 1994-1995, and an average of 13.88mmt during 1991-1992 to 1993-1994. The aid needs for each LDC were defined by the USDA as the difference between a target level of food consumption and what could be grown and commercially imported. The target was the average level *per capita* over the previous five years. The 9m-11mmt needed to meet that target in 1996-1997 would still fall short of supplying minimum nutritional standards.

Food aid is usually needed either in the short term to meet emergencies caused by natural or human disasters, or in the longer term to assist LDCs in their agricultural sectors to provide more food generally, as well as allow access to food and improved incomes for the large rural populations. Donors have provided food aid to LDCs as direct aid and as concessional sales at reduced prices or with low-interest loans. Because of the world cereal shortage in 1995-1996, cereal prices were at record levels and concessional sales were all but eliminated. Consequently, the LIFDCs increased their expenditures in cereal imports by 35% from 1994-1995, even though import volumes were down.

Cereal-aid shipments, mostly as wheat, were estimated by FAO to have been 7.2mmt, with 5.7mmt directed to the LIFDCs. The aid to LIFDCs was down by nearly 30% from the previous year, and down by nearly 40% from the average of the previous four years. Much of the decline in shipments was to sub-Saharan Africa, but there were declines in shipments to Latin-American and Caribbean countries. North Korea received more aid than hitherto. Food aid shipments to countries in Eastern Europe and the former Soviet Union (non-LIFDCs) were down by 30% from 1994-1995. Most of the decline in shipments was attributable to reductions by the EU and USA, both of whom combined still account for 75% of the global cereal food aid. In 1994-1995, over 30% of food aid went through multilateral channels such as the World Food Programme.

In November 1996, the FAO estimated that the 1996-1997 food aid shipments will have reached 7.5mmt, an increase of 4% over the previous year. Most of the modest increase was expected to have come from the EU and to have gone to the LIFDCs in Africa and Asia.

OECD figures show that, for 1996, MDCs reduced their state aid to LDCs, as a proportion of GNP, to the lowest level for 45 years, presumably because of 'compassion fatigue', public cynicism and downward pressure on public spending. Nevertheless, the decline was more than offset by increased private investment such that the total level of resources flowing into the LDCs reached record levels. Further analysis of the data shows that the private finance was predominantly restricted to a narrow band of fast-growing, high-income countries in Asia and Latin America. Direct aid by OECD countries to LDCs was $59bn in 1995, down 9% from 1994, and accounted for 0.27% of GNP of OECD members. Private investment increased to $159bn in 1995, taking total resource flows into the LDCs to $239bn. About 40% of aid went to Africa, 30% to Asia and 10% to Latin America.

The case for investment in agricultural R&D to assist LDCs is clear. Besides generating agricultural advances in the recipient LDCs, the MDCs reap further benefit from the international stability, economic growth and lucrative new export markets so derived. Refugee crises, costly emergency relief, and dangerous military interventions are avoided. The R&D investments can be based in part in the donor country and linked with the recipient region.

The World Food Summit was held in Rome in November 1996, under the auspices of FAO to dis-

cuss global food security, at a time when 14% of the world's population suffered from chronic undernutrition, and more than 80 nations were classified as LIFDCs. The world is facing a sharp decline in the supply of tillable land and fresh water *per capita*.

The Summit released a 'Declaration on World Food Security' that identified the causes and actions to correct food insecurity. The goal of the anachronistically lavishly entertained summit was 'reducing the number of undernourished people to half their present level no later than 2015'. Poverty was recognised as the primary cause of food insecurity, not simply a global shortage of food. It was ironic that the majority of the world's most hungry people lived in rural areas, and more investment in agriculture was a priority, as was improved mechanisms to deal with food aid crises.

A so-called 'plan of action' was adopted by the World Food Summit: no new international bureaucratic structures were erected; nations were not asked to make specific pledges of support; and nations, and non-governmental and international organisations were expected to decide their own courses of action. The FAO Committee on World Food Security will have responsibility for monitoring progress.

The World Food Summit took place more than two decades after the 1974 World Food Conference, and the Overseas Development Institute noted that there are numerous achievements worthy of declaration. The proportion of undernourished people has fallen from 38% in 1969/71 to 20% in 1990/1992. A combination of new technologies and market development has stimulated world food production, such that it has outpaced population growth, although *per capita* food production has not increased in most highly indebted, low-income countries, particularly in sub-Saharan Africa. In the early 1990s, there were about 850 million people with inadequate access to food, down from 900 million in the early 1970s, even though the population of the LDCs had increased by 1.5bn over those 20 years. Famine has been largely confined since 1974 to conflict situations; drought-related crises affecting pastoralists in marginal regions have been alleviated for the most part. FAO's Global Information and Early Warning System has met the needs of those donors lacking access to the highly competent USDA intelligence network. Presently, there is no widespread sense of urgency or deepening crisis. Yet, despite all those remarkable achievements, there are far too many bodies with overlapping mandates, responsibilities, remits and missions; an institutional incoherence world-wide has led to valuable resources being spread far too thinly. Recent funding trends lean towards nations supporting high-profile, short-term special initiatives, rather than steady, longer-term investment in, say, the work of the Consultative Group on International Agricultural Research (CGIAR) network of Research Centers, or appropriate National Agricultural Research stations (NARs).

Agriculture and Food Supplies

According to FAO (http://apps.fao.org), total agricultural production in both MDCs and LDCs rose in 1996, as did total food production and *per capita* food production. Three longer-term themes were becoming evident in the global agricultural scene. Firstly, world markets for food and feed became more sophisticated and integrated as domestic agricultural policies began to be aligned with the imperatives of the Uruguay round of the General Agreement on Tariffs and Trade (GATT), leading on to the WTO. Secondly, total demand for food increased, especially for fresh fruit, vegetables and meat. Lastly, there has been an overall decline in food production and consumption throughout the 1990s in the countries of Eastern Europe and the former Soviet Union.

Best estimates of global food prices indicate an overall decline by as much as 6% in 1996, with wheat and barley leading the steep fall in cereal prices. Sugar prices fell less sharply. Coffee and tea prices slumped. The 'Economist' non-food agricultural products index was largely unchanged, but individual commodities differed widely in their price performance, *e.g.* rubber fell by 20%, cotton by 8%, but timber rose by up to 50%.

Cereals

As a result of a static supply of grain since 1990, during a period of rapidly expanding demand, the world faced a grain shortage in early 1996. According to USDA forecasts for the production year 1996-1997, total cereal production (wheat, coarse grains and milled rice) would be 1842 mmt, with a total utilisation of 1808 mmt, exports of 220mmt, and total ending stocks (which includes reserves) of 278mmt, some 8% above the previous crop year and approximately 15% of utilisation, the second lowest figure on record.

In early 1996, depleted grain stocks from previous years, alongside poor weather in the USA and high

prices, led to a world-wide surge in cereal production, reversing a downward trend in area planted since 1981. In tandem with projected higher yields, the expected increase in grain production led inexorably to a decline in prices. The only major grain-growing areas of the world to show a decline in area planted and production were the countries of Eastern Europe and the former Soviet Union, where production by 1996 was down by over 30% since 1990.

The volume of world grain trade has changed little since the mid-1980s. Trade volume in 1996-1997, however, was expected to suffer a 6% decline, accounted for by above-average harvests in the major importing countries.

Wheat stocks were expected to remain at a low level again in 1997, according to the International Grain Council - which cut its production forecast for the 1997 crop year to reflect planting reductions in Canada, Russia and the USA. It predicted world production of wheat at 583mmt, up slightly from 579mmt in 1996; consumption was expected to rise 7mmt to 582mmt.

Overpayments to EU cereal farmers during the four-year period to June 1997 were estimated to be in the order of Ecu 17bn, because market prices rose instead of falling as expected. Producers of durum wheat, grown mainly for pasta, accounted for less than 5% of EU cereal production but received at least Ecu 5bn. Such payments amount to nearly half the annual CAP budget of Ecu 40bn.

Oilseeds

The USDA predicted that the world production of oilseeds in 1996-1997 (256.3mmt) would exceed the production level of 1995-1996 (255.4mmt), but fall short of the record set in 1994-1995 (260.7mmt). A further decline in the area devoted to oilseeds was anticipated as growers switched to more profitable cereal production. In order of production levels, the main oilseeds are soy(a)beans, cottonseed, peanuts/groundnuts, sunflower, rapeseed/canola, copra/coconut and palm kernal. Edible vegetable oil production amounted to 86.5mmt, which includes palm oil and olive oil, the industry not regarding these as seed oils. Oilseed ending stocks fell to the very low level of 21.3mmt, giving rise to competitive world markets for oilseed, meal and oil, especially in view of importation demands from China. The USDA projection differs somewhat from that in the Oil World Annual 1997, which indicated a production deficit in 1996-1997 that will lead to the reduction in oilseed stocks to historical lows by Autumn 1997.

Plans by the EU to bring about a radical change to the Ecu 2bn annual aid to the olive oil sector were impeded by the five producer countries (France, Greece, Italy, Portugal, and the largest producer - Spain, which accounts for 40% of output). EU production is around 75-80% of world production.

Sugar

About 70% of the world's sugar supply was derived from monocotyledonous sugar cane and the remainder from the dicotyledonous sugar beet. A production level of 125mmt centrifugal sugar in the crop year 1996-1997 represented a 2% increase over the 1995-1996 record harvest. The world's largest producer was India (17mmt), followed by Brazil (14.5mmt).

World sugar consumption was expected to increase to 122.9mmt, a record level, with most of the growth in the LDCs. Brazil continued with its policy of processing sugar cane for fuel. In the MDCs, consumer preference for sugar substitutes on dietary grounds depressed the demand for, and prices of sugar. World sugar carryover stocks, at 24.7mmt, increased to about 20% of consumption, and were expected to increase to 22% by the end of 1997.

Coffee

There were large increases in the production of green coffee in Brazil, Indonesia, Colombia and Côte d'Ivoire, leading to a recovery in world coffee production for the production year 1996-1997, to 99.1 million 60kg bags. Coffee prices fell. In the previous year, severe frost damage severely depleted coffee production in Brazil, a nation which, in recent years, accounted for 25% of world production. Washed arabica accounted for about half of world consumption; its increased price volatility was because it was in short supply, particularly from the main source, Colombia. Robustas and unwashed arabicas, produced in Brazil and elsewhere, substitute for washed arabica in coffee blending. The International Coffee Organisation's market review suggests a shortfall in coffee production in respect to consumption during 1997.

Cocoa

Sharply increasing its earlier estimate of the 1995-1996 world cocoa bean production to 2.88mmt, the USDA predicted that the 1996-1997 production level would fall to 2.66mmt. Côte d'Ivoire at

1.05mmt remains the single largest producing nation, despite a 12% decline in production. World consumption was expected to exceed production, leading to a substantial drop in carryover stocks.

Cotton

In 1995-1996, the USDA estimated that cotton production reached record levels of 91.5m 480lb bales. The few main producers were the USA, China, India and Pakistan, but only the USA showed an increase in production over 1994-1995 levels. Production in China, India and Pakistan was lower because of insect infestation and white-fly-transmitted cotton leafcurl geminivirus leaf virus, and a decrease in planted area. The 1996-1997 production level is predicted to be lower than the 1995-1996 level. World consumption of raw cotton was expected to rise by 1.1% from the previous year to 85.7m 480lb bales in 1996-1997, reversing the downward trend of recent years, but the cotton trade was still expected to decline as cotton stocks increase by 3-4%. More than 40% of the stocks are held by China. The major cotton exporters in recent years were the USA, Uzbekistan, French Africa and Australia.

For the first time, commercial cotton growers in Australia and the US planted genetically engineered (or genetically modified; GM) cotton, developed by Monsanto. The GM cotton contained the 'Bollgard' (Bt) gene derived from *Bacillus thuringiensis*, a soil-borne bacterium toxic to heliothis caterpillars. A new genetically engineered cotton, resistant to Buctril herbicide, was available in 1996. Mid-season reports from the Australian Cotton Research and Development Corporation confirmed that more than 30,000 hectares of GM (INGUARD™) cotton used 68% less pesticide spray.

Rubber

The International Natural Rubber Agreement was finally ratified by China and the USA, the last two signatories. The Agreement is designed to try to stabilise prices and supplies. Many manufacturers complained of shortages of this natural product that requires intensive labour inputs for harvesting. Synthetic products derived from polybutadiene and styrene butadiene offered tough competition, and rubber prices fell.

Tobacco

Against the efforts of anti-smoking organisations and bodies such as the World Health Organisation, the production and consumption of tobacco increased. Half the world's cigarettes were smoked in East Asia. The world production of raw tobacco, at 6.33mmt, was the largest total since 1993, with China, the USA, India and Brazil the major producers. Carryover stocks from previous harvests declined.

In the USA, regulations were approved to give the Food and Drug Administration (FDA) the authority to regulate the marketing and sale of tobacco products to young people. In 1997, following numerous attempts over many years to seek legal redress from tobacco manufacturers, US governmental coercion brought about agreement on multi-billion dollar payments by the manufacturers to recompense public-sector health costs and individual claims, as well as agreement to restrict tobacco advertising. The tobacco companies also agreed to pay fines should teenage smoking fail to decline. Retroactive confiscation of assets, whilst allowing production and sale of the offending product to continue and pay taxes, is unusual in a modern democracy. Health risks faced by the individual smoker are well known, as are risks for an extensive range of other hedonistic and essential items, some even more socially offensive and equally health-damaging than tobacco smoke. Does democratic power exculpate the individual from responsibility for his or her well-being, and the well-being of those around them?

Wood, Paper and Pulp

Heavy pressure remained on traditional wood supplies during 1996, but there was a drop in prices for many forest products, especially pulp, panels and non-structural lumber. Scarce raw materials have led to technology-driven improvements in the use of traditional and alternative sources of plant fibres, and as expansion of new manufacturing capacity accounted for the drop in prices.

Not surprisingly, given the reduced rates of extraction, tropical timber producers suffered shortages of raw materials, and low prices caused by increased international competition. Malaysia, in line with an international agreement among tropical producers to reduce harvests to 'sustainable' levels, announced it would cut its annual harvests by 19% to 30m m^3 by 2000. Japan's economic recovery strengthened demand in the region for wood products.

Oversupply occurred in Europe as high-producing Scandinavian countries joined the EU. The UK increased production from the coniferous forests planted after World War II. A quota system was

introduced by the USA to regulate imports of Canadian timber, and this accounted for rising prices by the end of 1996. Russia, with about 57% of the world's softwood reserves estimated by satellite surveys, has suffered a dramatic decline in lumber output from 80m m^3 to 22.3m m^3 by 1996. A combination of poor infrastructure and political instability impeded proper and balanced exploitation of its forest resources.

For paper, pulp and board, the trends in 1996 indicate that output might not exceed 1995 levels. If true, there would have been a plateau to the 13 years of increased output in world pulp, paper and board output. World production in 1995, the last year for which secure figures were available, rose to 277.8mmt, an increase of 3.4% over 1994. The USA remained the largest producer and consumer *per capita*, accounting for nearly 30% of the world's output. Pulp and recycled paper prices have been volatile for the past 3 years, particularly in the face of aggressive pricing by many producers to try and sustain market share in East Asia in the face of low-cost competition from Indonesia and the USA. Future trends for the forestry industry point inexorably to greater consolidation and integration of organisations that are currently competing, and improvements in environmental standards if the industry is to thrive. Interestingly, the Finnish Government privatised several state-owned companies in the pulp and paper, chemical and heavy metal industries to increase its R&D budget by 25% over the next 3 years. The windfall is estimated to be worth a total of $650 million.

Depletion of global forestry resources and the loss of essential biodiversity were the factors which initiated the innovative research programmes at SCRI on tree genetics and breeding, using coniferous as well as tropical and temperate broad-leaved species. Forestry demands long-term planning, where breeding cycles can be as long as 50 years. Our related programme on alternative plant fibres is crucial to relieve pressure on tree harvesting.

Food Processing, Retailing and Consumer Issues

Surveys of trends in retailing point to growth in processed ready-to-eat meals and convenience foods, breakfast cereals, non-bread bakery products, soft drinks, meat substitutes, and chilled, frozen and fresh fruit and vegetables. Consumer interest was noted in dietary fibre, 'functional foods', fish oils, ethnic food, food labelling, environmental issues, food contamination (especially provoked by the scares over BSE and *E.coli)*, and the use of genetically modified (or manipulated, enhanced or engineered) organisms (GMOs).

Food poisoning incidents did not decline in 1996. Over 9000 cases of food poisoning caused by *E.coli* O157:H7 were reported in Japan, and a large number of cases were reported in Scotland. Tamper-proof packaging became commonplace in retail outlets.

Substantial growth occurred in fruit juice sales. By way of illustration, about 85% of orange juice traded on world markets comes from Brazil, which produces about 46% of the world's total orange juice. Florida produces about 39% and most of the rest comes from Spain, Mexico, Morocco, Israel and California. Brazil and Florida specialise in oranges for processing into concentrate for shipping to bottlers for reconstitution. World consumption of processed orange juice expanded to 2.2mmt in 1996, recently averaging 5.5% growth per year in Europe, and 12.5% in Asia. SCRI has considerable interest in the potential for blackcurrant, raspberry, blackberry, hybrid-berry, strawberry and other juices, as well as their essences, flavourings and distillates.

The US Congress announced its intention to repeal the Delaney Clause which prohibits any trace in food of materials causing cancer in laboratory animals. In future, the regulatory authorities would have to prove food to be unsafe before it could be banned, rather than the reverse - the present law requires the processors to prove their products are safe. This proposed change in law runs contrary to the trends in the EU, but is in the spirit of the WTO initiative.

In the EU, restrictions were placed on many food flavourings and colourants, but anti-dumping duties were lifted on importations of the sweetener, aspartame, from Japan and the USA. Olestra, a fat substitute developed in the USA, was launched.

The beverages market was mixed. Many of the major beer brewers attempted to carve out a share in the rapidly expanding, low-volume craft beer market. A lag in the sales of spirits in the USA led to more aggressive advertising. In Europe and the USA, the wine vintage was claimed to be fairly good for the most part. Sales of northern and southern hemisphere wines grew strongly, and consumption patterns in the non-wine-producing countries of the western world became more international and experimental.

On the bases of value and volume, cola drinks remained the major soft-drink products, but several new products based on plant products such as guarana, coffee, caffeine, and fruit juices were launched successfully. Sales of carbonated and still mineral waters increased. In the UK, there was a massive expansion in sales of alcoholic soft drinks ('alcopops') coinciding with a decline in the sale of cider and provoking an outcry about encouraging under-age drinking.

Developments in food processing included improved automation, new packaging materials based on polyethylene terephthalate and polyolefin, modified atmospheric packaging to improve shelf-life of animal and plant products, improved low-temperature cabinet design, diagnostics for potential spoilage, and recyclable packaging.

Consumers International, a federation of 215 consumer organisations in more than 90 countries, issued a booklet entitled 'Safe Food For All' that discussed food concerns such as agricultural trade policies, advertising and scarcity. The booklet was promoted by the UN Environmental Programme to enlighten consumers on various aspects of food production and consumption and their impacts on the environment.

Food safety scares, exacerbated by the announcement in March 1996 that a possible link may exist between BSE and Creutzfeldt-Jacob disease, had dramatic effects in consumer behaviour, bringing about short-term changes in diet for much of the population (see article on p.127). Public and political attitudes to agriculture and science were severely affected. What were actually regulatory fiascos founded on ignorance, became justifications for giving media exposure to trenchant anti-technology voices.

As GM 'vegetarian' cheese, where recombinant rennin/chymosin replaces rennin extracted from calf stomachs, and tomato paste reached UK supermarket shelves and as a wide range of products using GM soybean and maize is starting to come on stream, consumer and environmental lobby groups campaigned for monitoring and labelling of GMOs and all products derived from them, and for major constraints on biotechnology. In October, the World Health Organisation and the FAO held an expert consultation on food safety and biotechnology in an attempt to determine basic policies on both those issues. In Europe, contrasting with North America and Australia, as much emphasis was put on the ethics of genetic modification as on verifying the safety of the product. Certain groups kept up pressure to call a moratorium on biotechnology, blissfully unaware of the many beneficial and indeed crucial aspects of biotechnology which currently underpin wealth creation, healthcare and other aspects of the quality of life world-wide. The fact that the gastrointestinal tract is designed to deal with foreign nucleic acids and proteins, and safeguards mankind from taking on the genetic attributes of fresh fruit and vegetables, or any accidently swallowed animal or microbial life, has not crossed the minds of many of those who protest. Environmental escapee genes would have to compete with natural selection systems. The current monitoring and regulatory systems in force are already more rigorous and protected than those applied to almost any other area of human activity including new drug discovery.

In Central and Eastern Europe, as well as in countries of the former Soviet Union, there was evidence of import dumping of poor-quality and mislabelled food, but local producers were still undermined by widespread attitudes that foreign food was better than the domestic equivalents.

Environment

Few appreciate that the services of ecosystems and the natural capital stocks that produce them, are of fundamental value to humanity and to the total economic value of Earth. An international team estimated that the current economic value of 17 ecosystem renewable services (habitats, biological or system properties and processes, food, waste assimilation, erosion control *etc.*) for 16 biomes was estimated to be in the range of \$16-54 trillion (10^{12}) per year, with an average of not less than \$33 trillion per year. Global gross national product is around \$18 trillion per year.

In its first Global Environment Outlook report, the UN Environment Programme called for cost-benefit analyses to promote changes in energy use, disseminate environmentally sound technologies, tackle water shortages, and improve environmental data gathering and analyses for decision-making. Poverty, population growth, inefficient resource use and wasteful consumption in the MDCs were seen to be the main factors in unsustainable global development, where natural resources are being consumed faster than they can be renewed. The real challenge is to reverse past and current trends in environmental degradation.

In September 1996, eight nations (Canada, Denmark - on behalf of Greenland, Finland, Iceland, Norway, Russia, Sweden and the USA) agreed to create a Joint Arctic Council, with the intention of protecting the fragile Arctic environment, while encouraging, somewhat oxymoronically, long-term development in the region. For several years, there have been opinions expressed about creating a group to safeguard Nordic interests in the EU, interests which include Scotland and other low-density-populated parts of Northern Britain, where land-use activities are environmentally constrained.

Climate change remained at the forefront of environmental discussions in 1996. The second meeting of signatories to the UK Framework Convention on Climate Change met in Geneva. There was agreement to the EU and USA proposal that the OECD member states should adopt legally binding limits to emissions of 'greenhouse gases', with targets and timetables for their reduction from the turn of the millennium. Australia, Russia and members of the Organization of the Petroleum Exporting Countries opposed the proposal, and most LDCs were concerned about the effects of these mandatory reductions on the growth of their economies.

The UK Meterological Office and University of East Anglia released preliminary figures indicating that 1995 had the highest annual average temperature ever recorded since relevant meterological statistics began in 1856. They calculated that the average global temperature in 1995 was 14.84^{o}C; the NASA-operated Goddard Institute came up with a slightly higher figure. While 1996 appeared to be cooler than 1995, initial calculations indicated it was the eighth warmest to date, continuing the warm trend evident throughout the 1980's and 1990's. Nonetheless, no definitive conclusions about global warming could be reached without studying data over a longer period.

An umbrella group of 60 industrialised concerns, the Global Climate Coalition (GCC), claimed that the wording of chapter 8 (dealing with human influences on climate) in 'Climate Change 1995', the latest report of the Intergovernmental Panel on Climate Change (IPCC), had been changed after peer review. The GCC charge was rebutted by the IPCC. The report claimed that global warming had been detected. After allowing for the cooling effects of aerosols, IPCC Working Group I predicted a temperature rise of between 1-3.5 centigrade degrees by 2100, and a rise in sea-level of 15-95cms, sufficient to threaten large tracts of low-lying land of agricultural and urban importance over the globe. Working Group II noted that warming at the higher end of the temperature range would shift climatic zones towards the poles by about 550km, with consequent effects on natural vegetation and the spread of pests and diseases.

In July, the World Energy Council, a non-governmental interaction group promoting sustainable energy sourcing, reported that global carbon dioxide emissions from burning fossil fuels rose by 12% between 1990 and 1995, mainly from the LDCs. Emissions increased by 4% in most OECD members. Large increases of 35% and 30% were noted in the Middle East and in the Asia-Pacific region (except for Australia, Japan and New Zealand), respectively. Levels in Africa rose by 12.5%. With the honorable exceptions of France, Germany and the UK, all the industrialised countries (the MDCs) were unlikely to meet their target of reducing the CO_2 emissions to 1990 levels by 2000. In Central and Eastern Europe, and the former Soviet Union, 1995 emissions were over 70% above 1990 levels.

Alternative energy sourcing, like organic farming, remains a Cinderella topic. A study in 1996 by the Paris-based International Energy Agency concluded that by 2010, fossil-based fuels would still account for about 90% of total energy demand. Non-hydroelectric renewable sources (biomass, wind, wave, solar, geothermal) would account for only about 1%. The World Energy Council estimated that renewable sources could provide, subject to R&D investment, 5-8% of the world's power requirement by 2020. At the end of 1995, the signatories to the Montreal Protocol set new limits on the release of ozone-depleting substances. Industrial countries agreed to phase out methyl bromide (a fumigant and soil sterilant to control nematode pests among other uses) by 2010, and LDCs planned to stabilise its use at an average of the 1995-1998 levels by 2002. Breeding for pest and disease resistance remains a high priority internationally, commercially and environmentally, reinforcing the research strategy of SCRI.

With regard to halogens, tropospheric concentrations of chlorine attributable to anthropogenic halocarbons peaked near the beginning of 1994, and by mid-1995 were decreasing at a rate of 20-30 parts *per* trillion *per annum*. Bromine concentrations, however, were still increasing. Stratospheric concentrations of chlorine and bromine were predicted to reach a maximum

between 1997 and 1999, and decrease thereafter, provided that the adjusted and amended limits set by the Montreal Protocol on Substances That Deplete the Ozone Layer were not exceeded.

Contrasting with perceptions shaped by the impacts of acid deposition on vegetation, a report by the European Forest Institute on 22 studies in 12 countries showed that tree growth in Europe had increased over the past few decades. The reasons for this remain unclear, because many of the forests in the study were relatively young. More in line with perceptions was a survey published by the European Commission (EC) on forest conditions in the EU; 20% of all trees at specific sites showed clear signs of foliar damage.

Following on from various reports in 1995 from the WHO, the UK Expert Panel on Air Quality Standards, and the UK Committee on the Medical Effects of Air Pollutants, realigned their views on the potentially hazardous rôle of PM10, a category of airborne particles less than 10μm in size. The nature of the hazard is receiving more attention in urban areas worldwide. Planners, however, have yet to appreciate fully the atmospheric 'scrubbing' capacity of various types of vegetation adjacent to roadways and factories.

China, by incorporating into its Law the 1989 Basel Convention on the Control of Hazardous Wastes and Their Disposal (a convention which requires all exported waste to have an export permit as well as import approval from the destination country), was able to reject a wide range of toxic imports for recycling. The international waste disposal and recycling industry will now have to take on-board new technologies, rather than rely on cheap labour and low-standards of disposal.

Continued interest in various chemicals, notably the phthalates and nonyl phenols, that can mimic the physiological feminising effects of estrogens when released into the environment, drew attention to those xenobiotics which are able to modify growth and differentiation at parts *per* billion level. Extraordinary efforts and costs are required to eliminate them from the environment.

The EC published a report in 1996 on bathing-water quality on beaches. Three thousand European beaches failed to meet the standards laid down in the 1986 EU directive; 11% of inspected British beaches failed. Globally, the huge capital and recurrent costs in water purification, and the political implications of water distribution and usage, are becoming major forces in land usage, investment patterns, urban development and international disputes. More than 1100 dams were under construction during 1996, and development continues on one of the largest water-transmission projects in the world, the Great Man-Made River in Libya, which uses Saharan fossil aquifer water conducted to the coastal regions for drinking water and irrigation.

In the UK, the Environment Agency took over responsibilities formally exercised by the National Rivers Authority, the Inspectorate of Pollution, and local authority waste inspectors. The new Agency promised to pressurise industry to invest in environmental protection, conduct a public-education campaign, and publish regular 'state-of-the-environment' reports on the Internet. Separate arrangements were made for Scotland (Scottish Environment Protection Agency). In October, the Landfill Tax came into force, intended to encourage recycling of waste, but landowners feared that tax-avoiding fly-tipping would become commonplace. A 1996 report from the Royal Commission on Environmental Pollution, entitled 'Sustainable Use of Soils', made 91 recommendations to ensure adequate soil protection, a topic neglected legislatively compared with the protection of air and water quality. The topic of soil-plant dynamics is an area of strength in the SCRI research portfolio.

Earlier in the year, the UK suffered its worst oil spill since the 'Torrey Canyon' incident, when the Liberian-registered single-hulled tanker 'Sea Empress' ran aground near the entrance to Milford, Haven, Dyfed. Around 70,000 tonnes of the 130,000 tonnes cargo of light crude oil were spilled, contaminating beaches over a wide area.

Ingenious and daring tactics by self-proclaimed 'eco-warriors' delayed construction of the Newbury Bypass. Extensive road construction in the UK has evoked strong protests because large swathes have been cut through areas of outstanding natural beauty, areas of specific scientific interest and wildlife habitats. In 1997, similar protests were in evidence during construction of the extension to Manchester Airport. A feature of the various protests in recent times is the high level of public sympathy for protecting the rural environment.

With regard to the world's natural flora and fauna, discussions took place during 1996 on coordinating

the responses to the Convention on Biological Diversity (CBD). An international working group was established at the third meeting of the Conference of the Parties to the CBD. Work was completed by Botanic Gardens Conservation International on a new version of the International Transfer Format for living plant records maintained by botanic gardens, facilitating the transfer of electronic data. New categories and criteria, developed by the International Union for Conservation of Nature and Natural Resources (IUCN - the World Conservation Union), were used to evaluate the status of the world's wild animal species. The results were published in the IUCN Red List of Threatened Animals at the World Conservation Congress in Montreal.

In India and China, the two most populous countries in the world, rising living standards led to massive expansion of domestic and commercial flower gardens and horticultural suppliers of ornamental plants. In Europe and the eastern side of the USA, the harsh winter of 1995-1996 caused die-back of many perennials, giving fresh opportunities to plant suppliers.

United Kingdom Perspectives

Initial estimates pointed towards a growth of 2.5% in the UK GDP during 1996. Improving consumer confidence was reflected in recovery of the housing market and in related areas of spending. For most of the year, industrial manufacturing output was weak but rebounded towards the end of the year. Overall investment expanded by 3%. At the end of the year, unemployment was about 7.2% of the workforce. Underlying inflation moved to 3.3%, very low by historical standards, but the financial markets expressed concern about the fuelling of inflationary pressures and potential effects on exports of a rise in the value of sterling. Holders of Ecu-denominated EU grants are beginning to suffer the effects of Ecu revaluations, making EU funding unattractive for research linkages.

The Public-Sector Borrowing Requirement remained well above target, severely restricting the scope for tax cuts and/or increases in public spend, even in the politically favoured areas of education, health, and law and order.

Britain sharply improved its competitiveness, according to the World Economic Forum's Global Competitive Report 1997, putting it in seventh place worldwide, below Singapore, Hong Kong, USA, Canada, New Zealand and Switzerland. Acquisitions of UK companies by foreign corporations in 1996 reached $38.5bn, up 8% from 1995. UK-based companies purchased overseas businesses to the value of $34.1bn. Britain received about 40% of the inward direct investment reported by EU members in 1996, almost double its share of a year earlier. In addition, the UK attracted bigger inflows than any industrialised country except the USA. UK inflows, which rose to $31.6bn, were more than double those into France, the next most popular country, according to the OECD. Inward investments into most other EU states fell. The OECD data indicated that Britain was the third-largest country for investment after the USA and China during the period 1991-1995.

A survey by Coopers & Lybrand considered that accounting for tax in line with the newly established global accounting code being developed by the International Accounting Standards Committee, rather than in line with the UK's unique system of partial provisioning, would add 10% to the gearing of the UK's top 90 companies. Gearing is a measure of the extent to which a company is financed by debt rather than equity. This effect on gearing would hit hardest the capital-intensive sector and may influence future UK investments. The UK actuarial approach on pensions also differs from the international current-market-valuation system.

The UK R&D Scoreboard 1997, produced by the Department of Trade and Industry, demonstrated the relatively poor performance of UK companies which continue to have the lowest ratio of R&D to sales of any G7 country. In 1996, company R&D as a percentage of sales was 2.3 in the UK and Italy, compared with 4.0 in France, 4.3 in the USA, 4.7 in Germany, 4.9 in Japan, 6.2 in Switzerland, and 7.4 in Sweden. On a sector-by-sector basis, the UK was, with few exceptions, consistently below the sectoral average. Civil expenditure on R&D has declined in the UK in real terms uniquely among the G7 nations since 1986. According to a survey by the Confederation of British Industry and NatWest Innovation, manufacturers cut 'innovation' spending (which includes market research and training as well as R&D) from 6.2% of turnover to 5.9% in 1996, but non-manufacturers increased their 'innovation'

spending from 10.6% to 11.8% of turnover. Clearly, the UK Foresight Programme needs to retain its evangelical mode to address the paucity of R & D investments by UK industry.

Adverse publicity generated by the BSE and *E.coli* crises placed UK agriculture under hostile scrutiny by the public, politicians, overseas customers and competitors. Food safety, quality and traceability; animal welfare and dignity; malcontentment with the costs and processes of the Common Agricultural Policy (CAP); and urban perceptions of agriculture posed special difficulties. Not only was the industry destabilised, but regulatory issues, the rôle of science, food preparation and marketing, and waste disposal systems were examined in great detail. Data for UK agricultural production are readily accessible from the Ministry of Agriculture, Fisheries and Food (MAFF) on http://www.maff.gov.uk/. Yields of wheat, barley, oilseed rape and sugar beet increased markedly in the decade 1986-1996. Wheat production in 1996 increased to 16mmt, barley declined to 7.8mmt, oilseed rape increased to 1.5mmt and sugar beet rose to 1.4mmt. Production data for potatoes, hops, apples, pears, cauliflowers and tomatoes were only available for 1994. Output valuations for 1996 indicate that cereals were £3bn, oilseed rape £419m, sugar beet £360m, beans and peas for stockfeed £140m, potatoes £564m, horticultural vegetables £1.1bn, fruit £260m, and ornamentals £675. Of the input costs in 1996, seeds amounted to £334m, fertilisers and lime £823m, pesticides £459m, and farm maintenance £404m.

The net worth of UK agriculture rose to £60bn but profitability in the context of farm income declined by 7% from 1995 values to less than £46bn. Output rose by £377m, but subsidies to farming (a 52% rise mainly in the form of BSE-related payments) exceeded income, and cost increases rose more rapidly than total output value. Much of the 'profitability' related to currency devaluations since leaving the ERM in 1992, and the improvement in net worth reflected increases in land value. Strengthening of sterling in 1997 will seriously affect the value of Ecu-based subsidies and will put pressure on market prices and competitiveness in export markets. Introduction of the single currency (Euro) would have a direct bearing on agricultural profitability.

Over the last ten years, the total farming labour force in the UK declined by 12.4%, although employment and population levels in the rural areas have grown. The effects of the 1992 MacSharry CAP reforms would indicate that there will be a continuing annual decline in direct employment to give a smaller workforce distinctly skewed to the older age groups.

Scottish farm incomes declined by 18.5% in 1996 to £443m, with the gross output down 3.5% at £2bn. The value of farm crops declined 10.2% to £526m, with potato output at £103m, nearly half the 1995 level. Cereals rose to £369m.

The draft UK Plant Varieties Bill, which seeks to update the 1964 Plant Varieties and Seeds Act in line with UPOV 1991, raised questions about (i) protection afforded to holders of rights to an initial variety (cultivar) compared with the 1994 EC Plant Variety Regulation, (ii) hybrids, (iii) intra-specific-use groups, (iv) alignment of penalties for non-compliance with those agreed under EU legislation, (v) transition arrangements to bring all protected varieties under the farm-saved seed provisions from July 2001, and (vi) GM crops.

According to a report from Strathclyde University, speciality salad products offer the best prospects for the European salads industry. Over-supply of round tomatoes, conventional lettuce, cucumbers and peppers have led to market saturation, forcing out inefficient producers. Diversification into new cultivars offers potential for higher margins. Vine-ripened and specialist tomatoes, specialist lettuce and other salad species, and pre-prepared salad packs are rapidly developing areas of investment. UK consumption of salads was the lowest in Europe at 12kg *per capita*, compared with 20-40kg in most European countries, and 107kg in Spain.

Within the wider scope of the Uruguay round of GATT (see previous Annual Reports), signed in 1994, the EU accepted reductions in the volume of food exports onto the world market in return for retaining its direct support to the agricultural industry, a powerful lobby but largely inefficient industry in continental Europe. Consequently, exports of cereals and animal products from the EU are set to stagnate or fall by 2001, whereas exports of these commodities from Australia, Canada, New Zealand and the USA are projected to increase substantially to meet the needs of expanding populations, particularly in the Far East and Pacific Rim.

Before the end of the six-year transition period for reducing subsidised exports, a new round of negotiations will start in 1999 under the auspices of the

WTO. This will have the express intention of making substantial and progressive reductions in agricultural support and protection, an aim that will force changes in the CAP and Fonds Europeen d'Orientation et Guidance Agricole expenditure. In any case, changes will be essential for eastward enlargement of the EU.

Well in advance of the WTO negotiations, agreement has to be reached by consensus of EU member nations on reshaping the CAP. Questions about the high costs of the CAP; the desirability of redirecting CAP funds to other aspects of the rural economy; intra-EU competition; CAP-related fraud, corruption and anomalies; introduction of technological innovation currently quenched by subsidy; rural employment; maintenance of and access to the rural environment and its resources; and restructuring to achieve development of the agricultural and horticultural industries, cannot remain unanswered for long. In the USA, the 1996 Federal Agricultural Improvement and Reform Act will over 7 years liberalise farm policy, reduce government intervention in production and cut subsidies, thereby aligning many sectors of US agriculture with the requirements of the WTO.

Exposure of UK farm commodities to world markets would bring about a marked decrease in production costs to match those of non-EU competitor nations. Yield efficiency and competitive innovation need to become paramount, as do longer-term relationships with end-users. In the interim, price volatility will provide problems for most growers, but opportunities for the few with foresight or good fortune.

Biotechnology

Biotechnology is the collective noun for the application of organisms, parts of organisms or sub-cellular entities, or biological processes, to manufacturing and service industries, including agriculture, horticulture and forestry, as well as environmental management, pharmaceuticals and diagnostics. The aims of the technology in respect of plants encompass: biomass production; production of chemicals and useful products; decomposition of wastes and recovery of valuable components; generation of new types of organism thereby extending the scope and precision of plant breeding; exploitation of fermentation; diagnosis, prevention and treatment of diseases; unravelling metabolic pathways; selecting parents for breeding lines; checking ownership and origins of cultivars; assessing biodiversity; and propagation of cells and whole organisms.

Recent technological and intellectual advances in molecular genetics, particularly sequencing of genes and proteins, isolation and insertion of genes into receptor organisms, development of marker genes, and gene amplification (notably those techniques based on the polymerase chain reaction) have, within a decade or so, given rise to a highly pervasive multidisciplinary subject - the so-called 'new biotechnology' - such that civilisation might be regarded as entering the age of the biotechnologist. Although technology is usually defined as applied science of commercial value, biotechnology is far more than a straightforward technology, for it can be employed at a basic level to study the most fundamental processes of life.

With the advent of a battery of techniques to overcome barriers to sexual reproduction, as well as to insert genes or sequences into the DNA of receptor organisms and sub-cellular entities containing nucleic acid (this therefore excludes BSE or scrapie-like bodies) to form transgenic organisms (GMOs), the inherent similarity of the genetic language in the major groups of organisms has been unequivocally demonstrated. Exploitation of these discoveries in ways that do not abuse responsibility to safeguard the natural world is a prime concern. Political, public and industrial pressures - especially if ill-informed, prejudiced or conniving - could bias or jeopardise proper scientific advances and consumer acceptance of the technology. Regardless of these pressures, though, biologists are coming to terms with a fundamental reappraisal of the concepts of taxonomy and systematics. Genotypes are assuming as great an importance as phenotypes, with the added realisation that genes as well as money can be banked.

Biotechnology is a major growth industry worldwide, particularly in the USA. The original Technology Foresight and the current Foresight Programmes of the UK (see previous Annual Reports) and those of other nations have highlighted the potential rôle of biotechnology for wealth creation, quality of life and UK competitiveness. SCRI is at the forefront of those areas of plant biotechnology relevant to its mission and aims. The new technology offers novel approaches for the goal of achieving environmentally sustainable development.

Agenda 21, the participatory plan of action jointly formulated and agreed upon by the world community at the Earth Summit in Brazil in June 1992, proposed a number of inter-related actions aimed at

environmentally sustainable development. The Inter-Agency Committee on Sustainable Development designated the United Nations Industrial Development Organisation (UNIDO) as the task manager for chapter 16 of Agenda 21, which deals with environmentally sound management of biotechnology.

In 1995, UNIDO reviewed the progress achieved on the implementation of this programme and noted that many of the issues discussed in chapter 16 were also reflected in at least seven chapters of Agenda 21. If properly managed, biotechnology can play an essential rôle in supporting the economic and social development of both MDCs and LDCs. Biotechnology development and applications continue to grow at a rapid rate, leading to an expanding range of products and processes across several sectors that began with pharmaceuticals and health care and now extends to agriculture and the environment.

With respect to biosafety, one provision of the Convention on Biological Diversity, Article 8(g), required contracting parties to: "*establish or maintain means to regulate, manage, or control the risks associated with the use and release of living modified organisms resulting from biotechnology which are likely to have adverse environmental impacts that could affect the conservation and sustainable use of biological diversity, taking also into account the risks to human health*." Another provision, Article 19(3), stipulates that: "*The Parties shall consider the need for, and modalities of, a protocol setting out appropriate procedures, including, in particular, advance informed agreement in the field of the safe transfer, handling, and use of any living modified organism resulting from biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity.*"

What of the potential threats to biodiversity from modern biotechnology products, particularly organisms with novel traits? While the term biotechnology is nowhere clearly defined, the way in which it is used suggests it is most commonly meant to refer to plants, animals, and microbes that have been modified with recombinant DNA techniques. The largest category of such organisms is new agricultural varieties of existing crop plants.

The single largest threat to biological diversity arises from the conversion of natural habitats or native lands to urban development or to agriculture, often with monocultures, or their ecological near-equivalents. After this threat comes the dangers from habitat degradation through pollution or unsustainable extractive practices such as clear-cut logging, overfishing and mineral extraction. Against this background, the threat to biological diversity from the products of modern biotechnology is infinitesimal. But what if all the products presently in the R&D pipeline were now on the market?

Nearly all the new crop cultivars being produced with the techniques of modern biotechnology have been modified or selected using biotechnology to sustain or increase yields, whether through imparting to them resistance to pests or diseases or through increasing their ability to withstand competitive pressures (or to eliminate such pressures) from, for example, weeds or other biotic or abiotic stresses. It has been argued that if the genes added to existing cultivars to impart such characteristics were to flow (generally by sexual recombination) into wild or weedy relatives, weed problems could be exacerbated or wild, pristine gene pools could become contaminated. In the vast majority of cases, however, the pests or diseases detrimental to agricultural yields are not the limiting environmental constraints on the wild relative being receptive to out-breeding from the domesticated cultivars. Experience shows that selection pressures found in nature do not favour such gene flow from modified crops to wild relatives.

A far more likely path through which potential characteristics or traits from genetically modified crops could have an impact on biodiversity is, in the absence of constraining population growth, by decreasing the rate of habitat loss through increased yields. The most likely impact on biodiversity from novel crop varieties, therefore, is to alleviate the main threat.

By 1996, the future of biotechnology in the European Union was fast becoming a victim of the very debates surrounding it. For the most part, food and environmental safety of GMOs, in tandem with commercial development, occupy the thoughts of the regulatory authorities and the public in the USA, Canada, Japan, Argentina, *etc*. Conversly, the EU is engaged extensively and exhaustively in ethical and moral discussions of the processes of biotechnology, operating with an incomplete and inconsistent legal framework, an unclear patent position, indecision over labelling of GMOs and their products, an inadequate investment climate, risk-averse entrepreneurs, and poor public acceptance aided by aggressive antibiotechnology organisations and insensitive biotechnology industries. In appealing for action

rather than endless discussion, Sir William D P Stewart, President of the UK BioIndustry Association and former Chief Scientific Adviser to the Government, whose landmark 1993 Science White Paper helped revolutionise and re-focus UK science, forecast the value of the global biotech industry to reach $90bn by the turn of the century. Ernst and Young estimate that by this time the world agricultural and food biotechnology market could reach $46bn.

Concerns about biotechnology relate to nine main areas.

1. Even though genetic modification was the first technology to have a raft of controlling legislation in place to ensure human health, and environmental safety, well before the first products reached the market place, environmentalists have expressed concern at the lack of statutory post-approval monitoring when GM crops move from field trials to commercial production. Others have reservations over the lack of uniform and comprehensive international standards for GM regulation, approval and labelling.

2. The widespread use of genetic modification might lead to the erosion of biodiversity, with increased dependence on a narrowing range of agricultural and horticultural crops. Biotechnology, however, provides the tools to measure and extend biodiversity, and improved crop performance reduces pressure on fragile habitats.

3. Not enough is known about how ecosystems work, and the release of GM crops could have disastrous consequences. GM crop release could disrupt the environment through the undesired spread of a modified crop, by transfer of chemical or pathogen resistance to weedy relatives, or the unexpected production of harmful toxins. World-wide, GM crop releases to date prove otherwise, but monitoring is essential.

4. Herbicide-tolerant crops may tie farmers in to seed-agrochemical packages, possibly leading to increased herbicide use and the risk of herbicide-tolerant crops and their genes spreading into the wild environment. Of course, herbicide tolerance exists in conventionally bred cultivars, and experience of herbicide-tolerant GM crops, albeit only over a short time scale, shows major environmental benefits in reduced herbicide usage. US farmers report a 5% yield rise and 33% less herbicide use in Herb® GM soyabean crops.

5. Using biotechnology to develop pest- and disease-resistant crop varieties could possibly lead to the creation of new pests or diseases, or the transfer of resistance to wild relatives. This has not been shown to be valid to date, but monitoring is required.

6. Herbicide- and antibiotic-resistance genes used as markers in the GM process might be transferred into the environment or human food chain. Many of the marker genes were only required in the early steps of breeding selection and have been superseded; to date, there are no unacceptable risks.

7. Biotechnology could inadvertently result in higher levels of human toxins, a reduction in beneficial nutrients, unexpected allergic reactions, or even the inducement of long-term metabolic consequences. This is an area for the regulatory authorities.

8. For some religious and other groups and individuals, biotechnology is 'unnatural', 'ungodly', and unacceptable ethically and morally. Strongly held views are difficult to change and democratic rights must be expressed to ensure safeguards. A UN code of bioethics is expected in 1997. It is really the threat of misuse of genetic engineering that raises questions of ethics.

9. Some patent protection relates to discovery rather than invention, and some patents are unacceptably broad-spectrum, oppressing releases of competing products or organisms. In their decision in the Biogen v Medeva biotechnology case, the House of Lords in 1996 considered priority dates, the need for the specification to contain an 'enabling disclosure' to allow the invention to be performed over the full width of the claims, the date at which the specification would be sufficient, and obviousness. Broad claims in future will be difficult to sustain legally without adequate enabling disclosure.

In February 1997, the team led by Ian Wilmut at the Roslin Institute in Edinburgh and PPL Therapeutics gained world-wide publicity surrounding the birth of a live lamb, 'Dolly', developed from a single cell originating from a mammary-gland cell line taken from an adult sheep. Dolly, whose picture graced front pages and covers of newspapers and journals, was the first mammal to be asexually cloned by transferring the nucleus from a donor sheep cell, cultured *in vitro*, to an unfertilised sheep oocyte from which the nucleus had been removed. As a consequence, Dolly was (with due allowance for cytoplasmic effects) genetically identical to the sheep from which the donor nucleus had been taken. This excellent research was disgracefully and inaccurately projected

by certain sections of the media and various zealots. Hastily drafted legislation to ban human cloning, rather than introduce a brief moratorium, was effected in many countries, obstructing highly desirable aspects of human cloning, such as skin grafts, drug production and 'spare-part' organs. As an editorial in *Nature* points out, the history of science suggests that efforts to block its development are misguided and futile. The quest for knowledge is inevitable; the responsible deployment of knowledge, however, presents the greatest challenge to modern society, and to those who cannot comprehend, adapt to, or benefit from change.

It should be pointed out that for thousands of years, plants have been asexually cloned from cuttings, offshoots, corms, bulbs, rhizomes, tubers, stolons and buds. Modern biotechnology, in tandem with tissue, organ and single-cell culture systems, make plant cloning more efficient, more predictable, and invaluable for phytosanitary, mass propagation and phytochemical purposes. Selective nuclear and organelle additions and subtractions are revealing the rÛles of the various cellular components. By introducing a dedifferentiation phase, it is possible to create valuable and fascinating somaclonal variation.

New forms of legislation, regulation, product labelling, industry-wide codes of practice *(e.g.* that operated by the British Society of Plant Breeders, the National Farmers Union and United Kingdom Agricultural Supply Trade Association), monitoring systems and the like have been or are being implemented within the EU. Some are fully justified, but others meet poorly informed, often paternalistic political and pressure-group concerns. Resources that could be spent on R&D are being diverted to overbearing regulation and resource-sapping bureaucracy in a zealous application of the precautionary risk principle. Professor John Marsh of Reading University stated that *"there is a serious problem in striking a socially responsible balance between the influence of the articulate and the evidence of the informed"*.

Within the EU, labelling will only be voluntary for transgenic maize, soybean and 11 other products awaiting approval. Thereafter, all transgenic seed and products deemed to be 'live' must be labelled to allow users further down the food chain to identify them. Labelling will not be necessary for derived ingredients and products from transgenic crops which are chemically identical to conventional foodstuffs. Scientists are fully in favour of all information being provided to consumers, *via* labelling if necessary. The labelling must be complete, equitable, non-pejorative and cover conventional and transgenic organisms.

In North America and Europe there are no food shortages and there is growing awareness of food safety and environmental issues. Biotechnologically derived medicines or non-food items (*e.g.* chemical feedstocks, fibres, environmental clean-up) and processes do not provoke such adverse reactions as GM foods. Nonetheless, in 1996, large quantities of genetically engineered maize, soybeans, cotton and potatoes were planted in the USA and much larger areas were scheduled for 1997. Thus, in 1996, there were 200,000 hectares of Bt maize, representing 0.6% of the total maize crop, with the projection of a ten-fold increase in 1997. For Herb® soyabeans, the 400,000 hectare planting in 1996 was expected to increase to 3-4 million hectares in 1997, with an additional 100,000 hectares in Argentina. By 2000, the USDA expects 40-50% of US crops to be GMOs. Transgenic crops were also grown extensively in China, Australia and elsewhere. Enzymes and other metabolites that influence the texture, appearance, preservation, flavour and nutritional quality of food are under biotechnological development.

In September 1996, the Senior Advisory Group Biotechnology and the European Secretariat of National BioIndustry Associations, which includes the UK BioIndustry Association, united to form EuropaBio, the new European Association for Bioindustries. EuropaBio will represent the interests of more than 500 companies and 8 national associations in Europe involved in the R&D, testing, manufacture, sales and distribution of biotechnology products. The industry has already created more than 180,000 jobs in Europe.

According to Keith Binding of Arthur Andersen, in 1996 in the UK there were 219 'biotech' companies (31 'agbio', 76 'biopharm', 50 diagnostic and 62 suppliers), employing more than 10,500 staff, with a revenue of £702m and an R&D spend of £190m. By the end of 1998, it is estimated that there would be 265 companies (45 'agbio', 125 'biopharm', 45 diagnostics and 50 suppliers) employing over 13,750 staff, with forecast revenue of the current companies alone in excess of £1.5bn and R&D spend of £319m. Very little venture capital is invested in non-health biotechnology at present, but never before have the R&D advances, discoveries and inventions been so exciting.

Most investments in molecular genetics relate to human genomics. The pace of the Human Genome Project (HGP) quickened in 1996. Scientists from the USA, Canada, Europe and Japan published the most complete map to date, detailing the sequence and location of more than 16,000 of the estimated 50,000-100,000 human genes. The new map, available on the Internet through the US National Library of Medicine (http://www.ncbi.nlm.nih.gov/science96/), is a valuable source of information. The range of agricultural, horticultural and forestry crops, their economic and environmental value, genetic complexity and genome size, will eventually mean that a future redistribution of effort and resource from the HGP to crop genomics will have to take place. The high-profile genomics research projects at SCRI are especially productive and influential; their potential impacts on agriculture, horticulture, forestry and the natural environment are beginning to be appreciated outwith the scientific community.

Research Assessment in the UK

Public-sector research in the financial year 1996-1997 contracted in financial terms and in the numbers of scientists and support workers employed. The contraction is set to continue. The trends and the various reviews, initiatives and constraints are described in previous Annual Reports. One conspicuous feature of British science has been the virtual demise of pure and applied botany in academia, to leave but relatively few specialist university departments and individuals to link with the few Public Sector Research Institutes concerned with the plant sciences. There is a real shortage of UK-based, qualified botanists to review scientific manuscripts and grant applications, and many of the existing staff will retire within the next 10 - 15 years. In contrast to the squeeze on UK science funding, however, the rate of global scientific progress has accelerated and links with industry have become increasingly productive. Attempts have been made to measure the quality, productivity and impact of the research funded by the public sector. Considerable emphasis seems to be placed on bibliometric data, particularly on citation analysis, which is claimed to measure the international impact of the research (*i.e.* amount of attention given to a piece of work) with a large measure of impartiality said to contrast with peer review.

Citation indices do have drawbacks *e.g.* (i) there is a varying time lag between publication and citation, depending on the journal and the field of study. The citation window of three years used by the major providers of data has only empirical support and will vary from subject to subject, particularly where long-term multidisciplinary research is involved. (ii) Publications covered by the main US provider of data do not include books, a large number of specialist journals, and conference reports. (iii) A strong bias exists towards US journals, disfavouring non-US journals, and thereby prompting scientists to publish in expensive journals and 'bandwagon' journals. On-line journals such as *Molecular Plant Pathology On-Line*, pioneered by Adrian Newton at SCRI, are not presently incorporated in citation analyses. (iv) Citation can be positive or negative, and although there are attempts to eliminate self-citation, there is a modern trend to brevity by citing reviews, effectively eliminating citation of originators, discoverers and pioneers. (v) Citation cartels can build up, as groups collaborate and for funding reasons wish to reduce recognition of competitors. (vi) There is a strong techniques bias and bandwagonning into high-impact topics to the detriment of specialist, but nonetheless crucial areas of science. Those in unique and highly specialist, sub-discipline-related areas are particularly disadvantaged. Applied and strategic areas of work and the relevant journals are diminished in stature, even though applied and strategic areas of science can initiate areas of basic science. Citation analysis works best for basic or fundamental research driven by curiosity at an individual level, but is not always a measure of innovation. (vii) Although widely carried out, cross-field comparisons are invalid; research communities differ greatly in their size, nature and duration of their work, and their methods of communication. (viii) Citation analyses reflect history - so-called fast-track fossil records - potentially supporting the declining rather than recognising the rising individual group or institution. (ix) Problems exist over the classification of articles, their titles, key words, names and initials of authors, their addresses, other index terms *etc*.

Alternatives to citation analyses are not hard to find with the necessary attributes of impartiality and international impact. At SCRI, our mission and remit are related to, and coordinated with those of the other Scottish Agricultural and Biological Research Institutes and our sister institutes of the Biotechnology and Biological Sciences Research Council, and Horticulture Research International; namely, we are driven by a quest to solve difficult, long-term research problems that will lead to wealth creation and improved competitiveness for our related industries, and we aim to contribute to the

understanding and quality of life. The research is essentially but not exclusively strategic in nature; sustaining, characterising and exploiting international-grade genebanks and germplasm collections; advancing plant breeding, genetics, pathology and physiology; taking forward biotechnology; pioneering predictive modelling; and fostering many other areas highlighted in the UK Foresight Programme. We also sustain underpinning innovative research.

The research programme in each of these various institutions, although not based simply on the curiosity of an individual, is dependent on talented and special individuals. Our programmes are multidisciplinary, high-quality, and demonstrably high-impact simply by virtue of their enormous beneficial effects on agriculture, horticulture, biotechnology and veterinary studies throughout the world over many years. Thus, such measurements as numbers of relevant publications (refereed and non-refereed), peer-reviewed books and chapters, full economic costs *per* publication, and *per* scientist, invited addresses to conferences, refereed conference proceedings, patents, cultivar releases, and their market share, competitive grants and contracts awarded, peer reviews, industry links *etc.* collectively have long been deployed by senior staff in the UK institutes to measure quality, productivity and impact. As industrial funding, exclusivity and market impact become more important, the relevance of citation indices to mission-driven organisations will diminish.

In an age of ferociously tight budgeting, forensic monitoring and perpetual review, cross-comparisons between individuals, groups, institutions and nations are in danger of attaining new heights of intellectual sterility. Scientific output needs sophisticated measures and patience, without which publicly funded science will become very short-term and 'clubby'. Science is also fast becoming a career to be avoided by talented young people, who do not want an unstable and poorly rewarded vocation that depends on a long apprenticeship learning and exploiting the vocabulary, concepts and technologies of science. SCRI, pleasingly, provides a productive and quality environment for research, in a beautiful setting by the River Tay. We plan for the future.

People & events

Tim Heilbronn

Senior appointments Dr Peter Palukaitis was appointed Head of Virology in January. He has been a major contributor to research in plant virology for nearly 20 years, including the last 11 years when he ran a leading research laboratory as a Professor in the Department of Plant Pathology at Cornell University, Ithaca, NY, USA. Within the scientific community, his current research is considered to be at 'the cutting edge' of plant virology, and he is Senior Editor of the international journal *Virology*.

Dr Palukaitis was born in Adelaide, South Australia, and completed his B.Sc. (Hons) Degree and Ph.D. in biochemistry at the University of Adelaide, before joining Cornell University as a Postdoctoral Fellow in 1980. He is a recipient of numerous awards, including the prestigious Alexander von Humboldt Foundation Award, and the McKnight Foundation Award for Individual Research Projects in Plant Biology. He is a prolific writer, with nearly 150 publications to his name, including reviews, papers in learned journals, and proceedings of Conferences.

Virology is a dynamic scientific subject with major implications for biotechnology, pathology and biochemistry. A Department Head with the international credentials of a scientist such as Dr Palukaitis, is considered a major coup for the Institute.

Retirements We said farewell to a number of colleagues, including Mr Ron Clark, Head of the Data Processing Unit, who retired in May after exactly 25 years at the Institute.

Honours Dr Derek Brown has received a number of international awards for his scientific research into nematode transmission of plant viruses. During 1996, he was elected a fellow of the American Society of Nematologists, the youngest scientist to hold this award; he received a Distinguished Service Award from the European Society of Nematologists, the first award of its kind to be made by this, the oldest Nematology Society; and he also had the distinction of being the first western scientist to be made a Fellow of the Russian Society of Nematologists. In November, he was awarded the prestigious Skrjabin Commemorative Medal for 1996 from the Russian Society of Parasitologists, The Russian Academy of Science. Dr Brown is the youngest scientist to receive this award, the first from the west, and one of a very small number of plant nematologists to receive the medal, as the award is more usually given to scientists investigating human and animal parasites.

Dr D. Brown

The Peter Massalski Prize for the most meritorious research by a scientist under the age of 36 at the Scottish Crop Research Institute, was awarded to Dr Craig Simpson of the Cell and Molecular Genetics Department. This represents a hat-trick for the Department, who have been outright, or joint-winners of the prize, on three of the four occasions of its award.

Dr P. Palukaitis

Dr Simpson's main area of interest is in plant 'RNA processing'. His breadth of expertise has underpinned the success of the RNA Processing Group at SCRI, which is unique in the UK, and one of only five laboratories world-wide. He has further interests in plant disease/insect resistance mechanisms, and has international collaborations, as well as linkings with scientists from departments throughout the Institute. A key achievement was establishing the genetic sequence of a protein affording resistance to mould

Dr C. Simpson (l) being presented with the Peter Massalski prize by J.L. Millar.

Perth & Kinross Councillors.

attack on raspberries and kiwifruit. The latter gene was cloned in New Zealand by Dr Simpson following an award made to him in 1994 under the Waitangi Fellowship Scheme, funded by the Bank of Scotland, in conjunction with Countrywide Bank of New Zealand.

The Peter Massalski Prize was established by Professor T.B. Massalski in memory of his son, Peter, who worked at SCRI until his untimely death. It has been awarded biennially since 1990.

Visitors We were pleased to welcome the Earl of Lindsay, Minister of State for Agriculture, Forestry and the Environment in Scotland, who spent half a day at the Institute in January. Other distinguished visitors included a number of political figures, notably the retiring Lord Provost of Dundee, Mr Norman McGowan JP, who selected SCRI as one of a small number of key organisations to visit in February; the Perth & Kinross Councillors who visited in March; and Ms Roseanna Cunningham, MP for Perth and Kinross, and Mr Andrew Welsh, MP for Angus East, who visited SCRI in April. We also received a visit by members of *Lindum Agricolae* (an influential agricultural group) accompanied by Mr Jim Godfrey (Governing Body) in April.

Lord Provost of Dundee, Mr N. McGowan JP with Dr J. Bradshaw.

A short course, on Lipid Chemistry and Analysis, was organised by Dr Bill Christie, Professor Frank Gunstone, Dr Charles Scrimgeour and Dr Gary Dobson, together with MRS Ltd. It was held at SCRI and attracted an international audience. Dr Lesley Torrance was the local organiser for the Third Symposium of the International Working Group on Plant Viruses with Fungal Vectors, which brought 70 leading scientists from 14 countries to

Dr W. Christie (r) with delegates attending the Lipid Course.

Ms Roseanna Cunningham, MP for Perth and Kinross, Prof. J.R. Hillman and Mr Andrew Welsh, MP for Angus East.

Professor J.R. Hillman, Professor T.M.A. Wilson, Dr I. Cubitt (Axis Genetics Ltd), Dr N.W. Kerby.

Dundee for a 3-day meeting in August. Dr Iain Young and Dr Brian Griffiths assisted with the organisation of the annual conference of the British Society of Soil Science, in conjunction with Dundee University. More than 130 experts attended the 4-day meeting in September, including the Australian Government's chief soil scientist, Professor Roger Swift. Dundee is featuring increasingly as a venue for conferences, with Institute staff again playing a major rôle in the organisation of the triennial North of Britain Crop Protection Conference, held in March and now termed 'The Dundee Conference' in deference to its long standing association with the city.

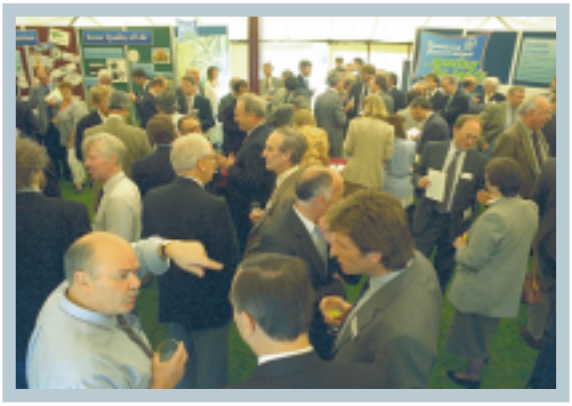
A Press Conference was held at the beginning of the year to announce an agreement between MRS Ltd and Axis Genetics Ltd, to develop pioneering research which harnesses plant viruses, programming them to produce commercially-viable quantities of large proteins on their surfaces. This 'OVERCOAT™ protein' story generated considerable interest from the National and International media, with film crews from Grampian TV, Sky TV, Landward, Country File and Belgium TV visiting SCRI.

The Beechgrove Garden team visited with Mr Jim McColl M.B.E., the well known Horticultural Consultant and broadcaster, to film a feature on strawberries with transgenic resistance to vine weevils, and chips made from *Solanum phureja* potatoes, for their programme broadcast in July. We were delighted when Mr McColl accepted an invitation to become an Honorary Research Fellow of the Institute, in recognition of his long-standing contribution to agriculture and horticulture. He was awarded the M.B.E. for Services to Horticulture in the 1996 New Year's Honours List, and was awarded the Pearson Memorial Medal from the Horticultural Trades Association in 1995. He has demonstrated over many years his outstanding knowledge of horticulture and his commitment to advanced plant science in Scotland. His infectious enthusiasm and professionalism will be of great benefit to the Institute. Links between the Beechgrove Garden and SCRI have recently been strengthened following plans to stock the new fruit garden with soft fruit varieties bred by the Institute, and also to include new swede and potato varieties in their plots.

(l to r) E. Ross MP; A. Welsh MP; Professor J.R. Hillman; Councillor Patricia Barr, Deputy Lord Provost, Dundee; Professor T.M.A. Wilson; J. McAllion MP.

Open Day The Institute opened its doors to special guests on Friday 7 June, and members of the public on Saturday 8 June. For the first time, we included a special Schools' Preview, with an opportunity for selected schools to tour the exhibits on the Friday morning. From comments received, it would seem that the Open Days were judged to be an outstanding success by the c. 1700 visitors who attended over the two days.

The Director welcomed the special guests at an important phase in our development. "There has been massive investment in the site over the last 15 years, building on the enormous achievements, often unsung, of the staff in our founding institutes, SHRI



and SPBS. Our science has widened to encompass new technologies, new scientific disciplines, new concepts and new challenges. Every year we make a point of taking onto the staff internationally outstanding young scientists, new blood, to reinforce our growing international stature."

Professor Hillman told the visitors that it was no coincidence that SCRI is unrivalled in the UK for its scientific productivity per scientist, cost per employee, and cost per scientific paper, without compromising on quality or innovation.

Turning to the impact of the withdrawal of Scottish Office funding for near-market research at the end of the 1980s, he described how Mylnefield Research Services Ltd has grown and thrived in a highly competitive commercial market. "We now have marvellous links with a whole range of industries from agriculture to food and drink, from biotechnology to forestry, and from local industry to multinational companies. Over 200 organisations now sponsor our work." His view was that science has never been so exciting as it is now, and the commercial opportunities have never been so great. On the other hand, it is getting more difficult to obtain taxpayers' money. The Director spoke about the 'Scrutiny' exercise, followed by the 'Prior Options' analysis. He wholeheartedly supported the conclusions of this thorough and rigorous review, and welcomed the enormous support offered to SCRI by SOAEFD, our primary sponsor, and all our other sponsors and sister organisations. He welcomed any move which allows us to compete openly and fairly for funding, any opportunities for a reduction in micromanagement interference, and the removal of many of the limitations inhibiting the setting up of satellite companies.

He concluded by saying that it was clear to the Governing Body and SCRI staff that regardless of all our previous achievements, all of the developments currently in the SCRI and MRS Ltd pipelines mean that the best has yet to come.

CHABOS - Committee of Heads of Agricultural and Biological Organisations in Scotland

T.J.W. Alphey

The Director of SCRI is a member of the Committee of Heads of Agricultural and Biological Organisations in Scotland (CHABOS). CHABOS was formed in 1994 to promote an integrated approach to agricultural, biological and environmental research and development, technology transfer, and policy support. Member organisations embody intellectual enquiry, education and public appreciation of science.

Organisations presently represented on CHABOS include:

Fisheries Research Services

Forestry Commission Research Division

Hannah Research Institute

Macaulay Land Use Research Institute

Moredun Research Institute

Rowett Research Institute

Royal Botanic Garden Edinburgh

Scottish Agricultural College

Scottish Agricultural Science Agency

Scottish Crop Research Institute

Between them, the organisations offer a wide and unique range of skills. The ability of CHABOS to progress scientific discovery and to apply the findings to Industry strengthens the competitive position of the United Kingdom in the international market place. Wealth creation, improving the quality of life, and enhancing the competitiveness of UK companies, are key components of Government policy on science and technology.

The mission of CHABOS is -

'To use the unique scientific and technological strengths within CHABOS to enhance our understanding of the natural world and to promote the utilisation of natural resources for wealth creation and the benefit and well-being of society.'

To this end, a number of objectives have been identified and are listed in the 'CHABOS Science Strategy 1997-2000' as follows:

(a) To develop coordinated and internationally-competitive programmes of research in biological and environmental sciences.

(b) To capitalise on the scientific expertise within the member organisations, to nurture their creative and innovative talents, and to carry out research of the highest international quality.

(c) To promote technology transfer and contribute to technology foresight through continued interaction with industry and Government departments.

(d) To provide advanced education and training in biological, environmental and agricultural sciences and cognate subjects.

(e) To advise and represent Government in Science, Engineering and Technology (SET) policy matters relating to agriculture, the environment and fisheries.

(f) To maintain monitoring programmes on disease prevalence in crops and animals and on environmental pollution.

(g) To promote the public appreciation of science and its contribution to national prosperity, health and well-being, and protection of the environment.

(h) To manage plant collections, and promote their use in plant systematics.

CHABOS facilitates the formal coordination of programmes between member organisations and with scientists from other organisations and universities in Scotland and elsewhere. The integrated programmes provide the critical mass needed to progress science of ever increasing complexity. The multidisciplinary nature of CHABOS enables it to take an holistic approach to a range of problems from molecular and cellular levels to whole organisms, from laboratory studies to ecosystems, and from aquatic systems through soils, plants, animals to man and his environment.

The research programmes fall into four broad categories: Biotechnology; Food, Nutrition and Health; Animal Production, Health and Welfare; and Biodiversity, Sustainable Systems and Land-Use. Within these categories, several noteworthy coordinated programmes have been established: Soil-Plant-Microbe Interactions (MICRONET); Animal Welfare; Fetal and Post-natal Development; Plant Health; Control of Helminths; and Zoonotic Organisms in the Food Chain. The programmes are continually reviewed and new areas considered. For the coming year, four new coordinated programmes are under discussion: Peptides for Pharmaceutical and Related Uses; Control of Food Intake; Environmental Pollution and Biological Remediation Soil Conservation; and Molecular Tools for the Genetic Characterisation of Plants and Animals and as Diagnostic Aids.

The CHABOS programmes align well with Government policy and with the aims of the Foresight (formerly Technology Foresight) Programme. The programmes are timely and pervasive, meeting the present needs in SET for enhancement of national prosperity and quality of life. The size, resources and adaptability of CHABOS are such that its research can be readily refocused to address new priorities.

Much of CHABOS research is funded by SOAEFD as an integral component of the UK science programme. In addition, CHABOS also carries out research on behalf of other Government departments and agencies including DoE, DTI, MAFF, ODA and the Research Councils. Particular attention is paid to the needs of Industry through Governing Bodies, Management Boards, local Consultative Panels, participation in Foresight activities and subsidiary companies such as Mylnefield Research Services Ltd.

Although each member organisation has established successful mechanisms for technology transfer, quality control and quality assurance, education, training and advocacy of science, information exchange through CHABOS ensures that organisations benefit mutually from the experience of others.

Plant genetics

George R. Mackay

The breeding of new, improved, cultivars takes time. In a complex heterozygous tetraploid, such as the cultivated potato, Solanum tuberosum ssp. tuberosum, the probability of combining, in a single cultivar, all the desirable attributes of an 'ideal potato' is remote. In circumstances where the breeding of cultivars per se is perceived as a 'near market' activity, no longer worthy of public sector support, it is extraordinarily difficult to attract financial support from the private sector to fund the breeding of new cultivars. However, without that support, it will be impossible to exploit or objectively validate the findings of more fundamental research into genetics and breeding methodology, that is designed to underpin the production and release of new improved cultivars, needed to ensure the sustainable production of a basic human foodstuff into the 21st century.

An understanding of the genetic architecture of economically important traits, the development of molecular marker aided selection and 'genetic engineering' in its widest sense, may provide information and technologies to aid the breeder. Nevertheless, however genetic variation is created, the means to identify clones with combinations of disease and pest resistance, quality for table or processing use and the yield demanded of a modern cultivar, will continue to be required if the products of this strategic research are to reach the market place.

Research into selection methods, whilst less academically exciting than research into cell and molecular genetics, is capable of developing the means to produce new improved cultivars more effectively and efficiently than traditional, empirically based selection methods employed hitherto. In 1996, three clones were submitted to National List Trials as potential cultivars within six years of sowing seedlings. The targeted accelerated breeding of new cultivars is a reality if the germplasm resources and skills of researchers at the SCRI can continue to receive the public sector support needed for their research, and private sector support for the production of cultivars. A marriage of public sector commitment and private sector investment can be of mutual benefit to the industry and the UK economy.

In the core-funded tetraploid potato breeding research programme, progeny tests are being developed and used to replace traditional, empirical, phenotypic recurrent selection. The second cycle of the multi-trait recurrent selection programme (Ann. Report 1994, p.39) started in 1994. From the 428 crosses attempted, 178 were successful. Of these, 137 crosses produced sufficient seed to be sown and assessed in glasshouse progeny tests for resistance to *Globodera pallida*, the white cyst nematode, and to late blight, as well as for their agronomic/commercial potential by

visual appraisal of their tubers[1]. A selection index, in which these three traits were weighted by their heritabilities, was then used to select the best 36 progenies for further testing and trialling in 1996, as tuber progenies at Blythbank, the Institute's high grade seed site. Seven progenies subsequently were eliminated as too susceptible to tuber blight. At harvest, the six most attractive looking clones from each of the 29 progenies were selected. Two tubers were retained for use as parents in 1997, four as seed for replanting at Blythbank, and six chats for testing for their resistance to both species of potato cyst nematode. The 108 most resistant clones, so identified, will be used in the next crossing cycle in 1997. In contrast to the commercially-targeted breeding programmes, this strategic research will lead to a greater understanding of the genetic architecture of late blight and PCN resistance; the production of enhanced germplasm for use as parents, or as potential cultivars; and characterised, segregating populations, essential for the development of molecular markers linked to important disease resistance quantitative trait loci, that may one day further facilitate genotypic selection.

The disease skinspot, *Polyscytalum pustulans*, has never been a particularly high priority for R&D at SCRI. However, it is now becoming of some consequence to processors where it can cause substantially increased peeling losses and, as a skin blemish disease, can markedly reduce the value of ware potatoes, where skin finish or eye appeal now overrides more economically important diseases such as late blight. However, before any breeding effort can be directed against such a disease, we need the means to access and identify sources of heritable variation for resistance. Recent research in Crop Genetics has developed and validated a progeny test for resistance to skinspot. This will facilitate studies on the inheritance of resistance, and possibly the means to apply selection pressure for resistance.

Research into, and development of, parental clones with improved resistances to the common viruses continues. Several clones with multiple copies of genes conferring resistance to PVY and PVX, as well as good field resistance to PLRV, have been bred. Of the recent National List submissions, two have resistance to PLRV and three resistance to PVY, similar to those for cv. Pentland Crown, a tribute to this strategic approach to breeding for virus resistance.

Our research into the potential of the diploid cultivated species, *Solanum phureja*, for the production of value added foods, such as par-fried chilled or frozen French fries, rostis and other products, has been greatly aided by the appointent of a food scientist in MRS Ltd. In collaboration with the Management and Consumer Studies Department of Duncan of Jordanstone College, University of Dundee, and the Hannah Research Institute, dehydrated powder of *S. phureja* has been produced and shown to reconstitute into a very tasty, instant mash that is being used in further product development. Moreover, potentially useful, high levels of resistance to *Erwinia* have been found in SCRI's long day adapted *phureja*. This could prove both a useful source of genetic variation for incorporation into cultivated tetraploids and/or for more basic research into the molecular basis of resistance.

The identification and use of rare male fertile dihaploids, amongst SCRI's extensive collection of *tuberosum* dihaploids, has also substantially enhanced the potential of this material for research into genetics, which is extremely difficult at the tetraploid level.

In 1996, the first field trials were grown of genetically modified clones from cross-departmental collaboration into the modification of starch-sugar biochemistry of potatoes. These may result in superior modified variants of existing processing cultivars. The tubers have been harvested and stored for analyses of their sugars and fry colours.

Somatic fusion products from a PMB-funded PhD project were also field-grown, and early indications are

that, whilst these are phenotypically similar to the recipient cultivar, some possess the resistance to potato cyst nematode of their wild species donors. An S.E.T. project, funded via MRS Ltd, has carried this research further, and several hundred fusion products of a number of cultivars and wild species have been produced. Further research is in progress to ascertain the precise genetic constitution of these hybrids and their potential as new improved cultivars or parental material.

The appointment during 1996 of two highly qualified molecular biologists to Crop Genetics has greatly strengthened our efforts into the exploration and exploitation of research at the cell and molecular level. The application of techniques such as bulk segregation analysis to traits such as the PLRV low titre character[2] may greatly aid selection for this trait, where classical methods will be too time-consuming and expensive. A greater understanding of general relationships between the cultivated potato and its wild tuber bearing relatives of the genus *Solanum*, will also greatly aid the transfer of disease and pest resistances from the Commonwealth Potato Collection than has been possible hitherto.

This amalgamation of classical breeding approaches to potato crop improvement, with modern cell and molecular based techniques, must bode well for the future. Breeders can provide the characterised populations which are essential for more fundamental research; hopefully the fundamental researcher can provide the genes and the means for the breeders to produce the products that industry and the world needs in the 21st century.

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The targeted and accelerated breeding of potatoes

G.R. Mackay, D. Todd, J.E. Bradshaw & M.F.B. Dale

Historically, the state-supported potato breeding programmes of the Scottish Plant Breeding Station, and subsequently SCRI, were not specifically targeted at any particular end user. Potatoes in the UK have multiple end uses, the main being table use, processing and export as seed (Fig. 1). However, each of these major uses can be further divided into more specific areas, each with specific requirements. For example, table cultivars may be destined for boiling, baking, or salad use, and 'new' (immature) potatoes have a special niche in the UK market. Whilst there are common needs for cultivars for processing into french fries or crisps, the processors prefer specific cultivars for one or other use. Seed potatoes for export may be for re-importation into the UK as ware, or for local consumption.

The potato is also subject to attack by many diseases and pests: fungi, bacteria, viruses

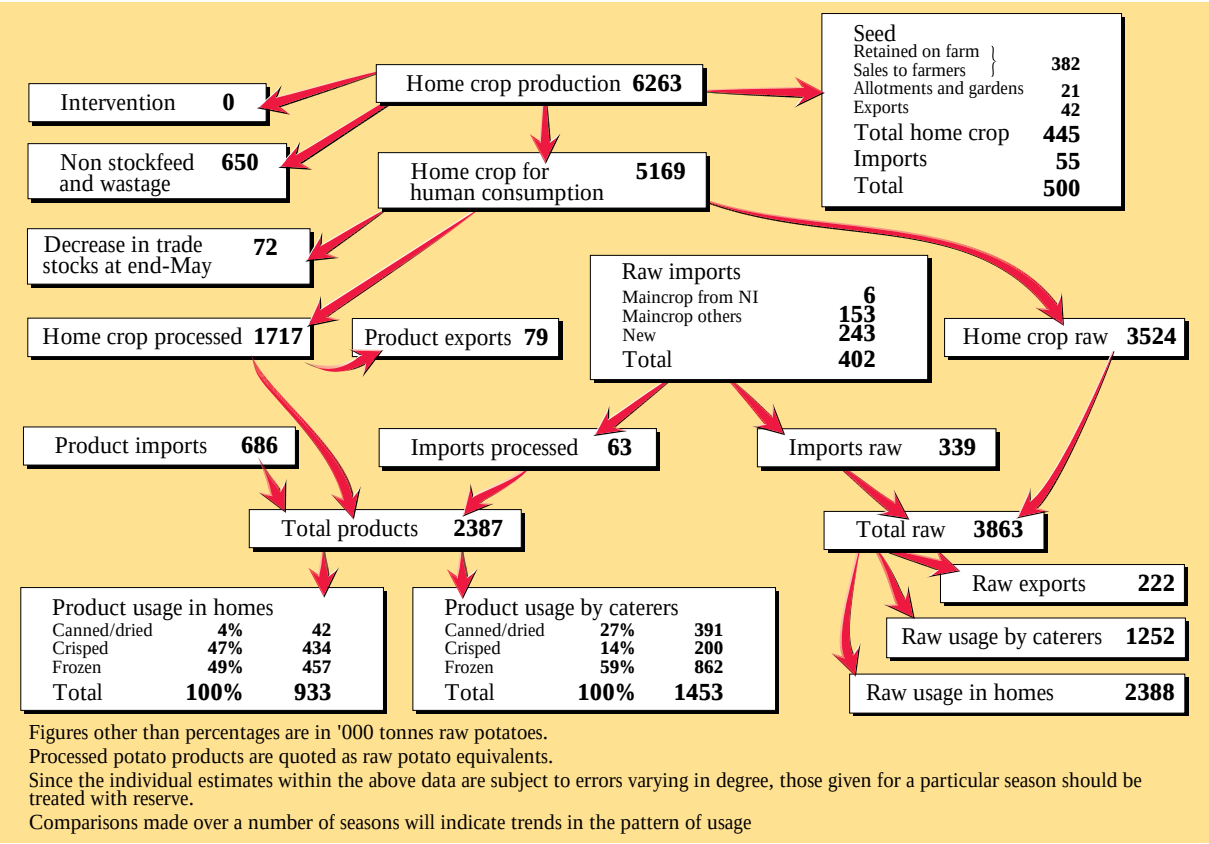

Figure 1 Flow chart for potatoes in Great Britain: June 1994 - May 1995 (Based on Potato Marketing Board figures).

and nematodes (Fig. 2). Since sustainable agricultural production ultimately depends on cultivars with durable resistance to diseases and pests, the SCRI breeding programmes were strategically orientated towards breeding for resistance, rather than the more immediate perceived needs of the market place, for which disease susceptible cultivars continue to be grown, protected by liberal use of agrochemicals.

Faced with such a proliferation of targets, it is remarkable that the state breeding programme managed to combine its strategic objectives of enhancing disease and pest resistance in cultivars which also possess the yield, quality and agronomic traits demanded by the market.

The probability of combining all desirable traits into a single, ideal, cultivar is so unlikely as to be regarded as impossible.

SCRI's research into conventional breeding methods, therefore, focuses on increasing the efficiency of selection for the most important disease resistant traits, such as late blight, PCN and viruses, whilst continuing to select for clones not too susceptible to relatively less important diseases, and for clones whose yield potential and quality were as good as or better than existing cultivars, as well as for all possible end uses (Fig. 3).

With the withdrawal of state support for the breeding of finished cultivars, the research into improved breeding methods and studies on the genetic architecture of important traits continues (SCRI Ann. Rep. for 1994). However, SCRI breeders firmly believe that, with their improved selection methods (and access to the enhanced germplasm which their research has produced as parental material), the lengthy period of selection from cross to submission of a potential cultivar to National List Trials can be substantially shortened, provided the breeding objectives are more precisely targeted to an end user's needs.

A major problem facing potato processors in the UK, and elsewhere in the world, is the inability to store tubers for extended periods at low temperatures to keep them in good condition without them sugaring. Low temperature sweetening is probably the single most important problem facing the manufacturers of

Yield	Tuber number, tuber size, bulking rate, drought resistance, storageability (marketable vs. total yield)
Conformity	Tuber shape, regularity and uniformity
Absence of growth defects	Secondary growths, hollow heart, growth cracks
Quality	Table and processing: enzymic browning, after-cooking blackening, sloughing, texture, dry-matter content, sugars (crisp colour), storage characteristics (dormancy)
Resistance to mechanical damage	External: shatter cracks, scuffing Internal: bruising (black spot)
Eye appeal	Consumer preferences: skin colour, flesh colour
Miscellaneous disorders	Internal rust spot, wind damage, sensitivity to herbicides
Disease and pest resistance	Late blight - tuber and foliage Common viruses, PVX, PVY, PLRV Cyst nematodes, *rostochiensis* & *pallida* Common scab Gangrene Wart Skinspot Powdery scab Spraing (tobacco rattle virus) Soft rot Dry rot

Figure 2 Some of the traits which potato breeders have to consider in selecting cultivars for multiple end use.

French fries and crisps and, indeed, many other fried potato products. SCRI research has identified sources of low temperature, sugar stability amongst its diverse *Solanum tuberosum* genepool[1] (Fig. 4a). Crosses between these clones and other 'normal' breeding clones and cultivars have shown that this trait has a heritable component and, moreover, that progenies (= families) with superior low temperature storage characteristics, can be identified very early in the selection process (Fig. 4b). By determining the sugar stability of tuber progenies during storage at 4°C, in their first field-grown clonal year, it is possible to select those progenies which have the greatest likelihood of containing superior clones within 2 years of sowing true seedlings in the glasshouse. By concentrating selection for superior clones within these progenies, SCRI research has shown that potential cultivars for processing can be identified much more efficiently than by selection of clones with some processing potential in a more general breeding programme.

Year			
0	Decide objectives, choose parents, make crosses		
	Glasshouse		
1	*c.* 200 pair crosses - progeny tests where applicable		
2	*c.* 20,000 seedlings in pots - no selection		
	Seed site		
3	20,000 single plants (first clonal year) - visual selection at harvest		
4	4,000 4-plant pots - visual selection at harvest		
	Assessment for yield and quality and special disease tests		
	Seed site number of plants	Clones number	Ware site number of plots and plants per plot
5	6	1000	2 x 5
6	20	360	2 x 10
7	100	120	2 harvests of 2 x 10
8	300	40	Ware trials at a number of sites
9	700	20	Ware trials at a number of sites
10	2000	4	Official trials
11	2000	2	Official trials

Figure 3 An outline of the SCRI potato breeding programme - for cultivars for all possible uses.

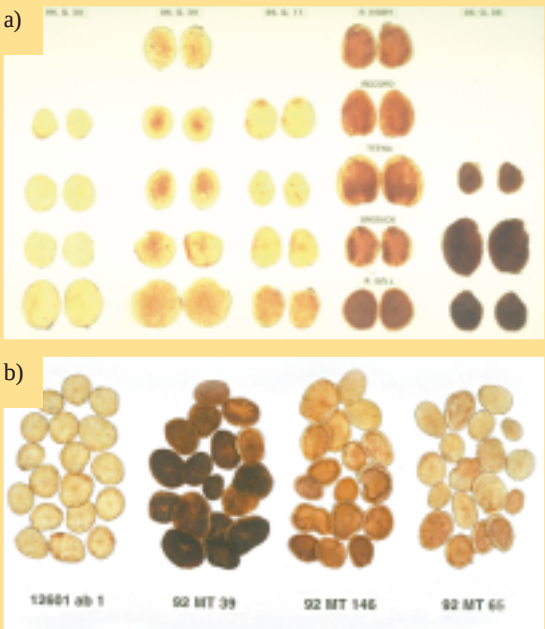

Figure 4 a) Fry colours of crisps of some SCRI low temperature sugar-stable clones compared with cultivars after 8 months storage at 4°C.
b) Fry colours of crisps of three tuber progenies compared with an SCRI sugar-stable clone (12601ab1) after storage at 4°C.

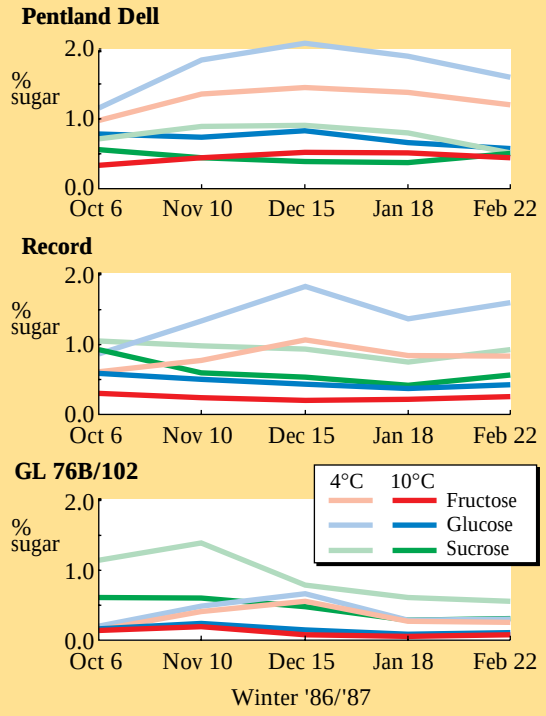

Figure 5 Comparative data between two of the most popular processing varieties in the UK and an SCRI breeding clone showing changes in tuber sugars over time when stored at 10°C and 4°C.

In 1991, Mylnefield Research Services (MRS) Ltd entered into an agreement with Messrs McCain (UK) Ltd and Golden Wonder Ltd, whereby the two companies agreed to fund a breeding programme targeted at their specific needs, based on SCRI's research findings.

In 1991, 43 progenies, produced by hybridisation between SCRI clones with phenotypically superior storage characteristics, and cultivars such as Record and Pentland Dell, currently favoured by the industry but requiring improvement (Fig. 5), were sown in the SCRI glasshouses. At harvest, a single tuber from each seedling was retained. No selection was attempted at this stage, as SCRI research has been unable to demonstrate that fry colour and sugaring characteristics, after storage, of glasshouse-grown seedling tubers are sufficiently correlated to subsequent performance of the same clones grown in the field, to merit selection being applied. The tuber progenies were grown as single plants at SCRI's high grade seed site, Blythbank Farm near Peebles, in 1992, using a randomised block design wherein each tuber progeny was represented by four replicate plots of 15 clones (60 per progeny in total). At harvest, four tubers of each clone were retained for seed, and two for storage - one at 4°C the other at 10°C.

After 4 months in store, the tubers were fry tested and fry colours scored on a 1 to 9 scale (1 being very dark, 9 being very pale). Samples were also taken for chemical analyses of sugars as part of SCRI's core research into the genetics of low temperature sweetening, but these results will be reported elsewhere.

Analysis of variance of the fry colour data showed significant variation between the progenies and some were clearly superior to others ex 4°C storage. Following discussions with our commercial sponsors, 17 progenies were identified as meriting further trialling in order to select superior clones from within them. These were then grown at Blythbank in 1993 as four plant plots, using the seed tubers from the 1992 singles, again as replicated blocks. At harvest, the produce was assessed visually in the field for yield, tuber shape and size, absence of defects and given an overall preference score by the breeders. Clones were also given a numerical score which reflected their tuber shape in terms of length x breadth ratio. Long ovals are preferred for French fry manufacture and short ovals or round tubers for crisps. Two samples of three tubers were also taken for storage at 4°C and 10°C. These samples were subsequently fry tested and fry colours ascertained. Analyses of these data identified clones within progenies with superior storage characteristics (paler fry colours) and acceptable agronomic characteristics which enabled us to partition the population into those clones more suitable for French fry production or those for crisp production.

In 1994, the selected clones were grown in replicated trials at sites in England provided by the companies on farms where ware production for processing is normal practice. This was an important component of this programme as, usually, clones undergoing selection would be trialled and selected for at least three more clonal generations only at Mylnefield. Breeders are aware that genotype x environment interactions can influence agronomic performance and quality traits, causing difficulties if selection is practised in one environment for cultivars to be produced in another. Details will be published elsewhere, but analyses of the data from these trials, including fry tests post-storage, identified several clones superior to the rest of the material and to the standard control cultivars. Trials of selected clones continued in 1995 and the best clones were also rapidly multiplied by

1991 Glasshouse
43 progenies 4 replicates of 18 seedlings circa 3100 seedlings No selection

1992 Blythbank
43 progenies 4 replicates of 15 clones circa 2500 clones 1 plant per clone
Fry 4, fry 10 (Progeny selection)

Between 1991 and 1993 all material was held in common. Clones reaching the stage requiring ware trialling were divided by shape into crispers (G.W.) and chippers (McCain)

1993 Blythbank
17 progenies 960 clones & controls 4 plant plots, 1 replicate Fry 4, fry 10 Visual agronomy

1994 England & Mylnefield
136 clones & controls 5 or 10 plant plots, 1 replicate per trial site Fry 4, fry 10, percent dry matter
Visual agronomy, internal defects, tuber size grading

1995 England & Mylnefield
28 clones & controls 10 plant plots, 2 replicates per trial site Fry 4, fry 10, percent dry matter
Visual agronomy, tuber size grading Internal defects Screening for disease resistance

Glasshouse
Micropropagation of best clones

1996 England & Mylnefield
16 clones & controls Replicated trials Fry 4, fry 10, percent dry matter Visual agronomy,
tuber size grading, Internal defects, Screening for disease resistance

Blythbank
Approved stocks **NLT submissions** of 87.Q.2 A 27, 90.Q.2 A 9 and 87.Q.1 A 20

Trial sites
Blythbank High grade seed farm. Peebleshire, Scotland
Mylnefield Ware site. Dundee, Scotland
English sites Yorkshire and Lancashire, England

Fry 4 and fry 10 Crisp colour after storage at 4 and 10 degree C.
Visual agronomy A visual assessment of yield, size, shape and growth defects

Figure 6 The accelerated breeding programme targeted specifically at cultivars for processing into crisps and French fries.

micropropagation in SCRI glasshouses. In 1996, it was possible to grow Approved Stocks from the micropropagated minitubers whilst trialling continued. In October, three clones were submitted for National List Trials as potential cultivars (Fig. 6). A fourth clone identified as more suitable for French fries, produced too few seed tubers for Approved Stock purposes in 1996, a probable consequence of its tendency to produce rather few, large tubers per plant. It will be multiplied in 1997 and hopefully submitted in the autumn. These four new cultivars have been rapidly identified from a relatively small, but targeted, initial population of *c.* 4,000 seedlings.

Figure 7 Anya.

The most advanced clones have undergone some limited tests for disease and pest resistance. Two have been confirmed as having resistance to the golden cyst nematode and all are equal or superior to current preferred processing cultivars in terms of their resistances to those diseases against which they have been assessed so far.

This practical demonstration of the application of scientific method to potato breeding, confirms the power of SCRI's improved conventional breeding methods. Successful, targeted and accelerated breeding requires access to superior, preferably progeny-tested parental germplasm, well-defined albeit limited objectives, and the means to apply selection pressure reli-

ably as early as possible in the environment in which ware production is planned.

This technology need not be restricted to breeding cultivars for processing. In 1986, breeders at SCRI made some speculative crosses between the old cultivar Pink Fir Apple and other cultivars, anticipating a potential niche market for the products of such crosses. However, with withdrawal of funding for this 'near market' research, the project was abandoned. With financial support and collaboration from Messrs Whitworth of Chatteris, some of the clones from these crosses were resurrected and, in 1996, the cultivar Anya was being retailed off a major supermarket's shelves (Fig. 7). Given similar specific objectives, SCRI has both the germplasm and the technical know-how to produce varieties for the 21st century if the industry wills it.

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Breeding swede, forage rape and kale cultivars with resistance to clubroot (*Plasmodiophora brassicae*)

J.E. Bradshaw & W.H. Macfarlane Smith

Clubroot disease is almost synonymous with brassicas and is caused by a soil-borne fungus, *Plasmodiophora brassicae* Woronin, whose spores can remain viable in the soil for many years. As a consequence, it has proved difficult to control by either chemical or cultural means. The need for resistant cultivars was recognised by Davey at the Scottish Plant Breeding Station (SPBS) as early as 1926, but no real progress had been made by 1960. It had proved difficult to distinguish between actual resistance and escape from attack due to an unevenness of infection in both field plots and boxes of infected soil, and no sources of immunity had been found.

Figure 1 Seedling test for clubroot resistance.

Breeding for resistance resumed at SPBS in 1975, by which time selection for resistance could be done reliably by inoculating seedlings grown in a heated glasshouse and scoring for severity of galling 5 to 6 weeks after inoculation (Fig. 1). Furthermore, 15 European Clubroot Differential (ECD) hosts had been agreed for use in clubroot population surveys[1]. They comprised five hosts from each of *Brassica rapa* (turnip and Chinese cabbage), *B. napus* (swede and forage rape), and *B. oleracea* (kale and cabbage). *B. napus*, which can be regarded as an inbreeding species, is the allotetraploid of *B. rapa* and *B. oleracea*, both true outbreeders (Fig. 2).

Clubroot population surveys One of the clubroot population surveys was done at SPBS during 1975 with 10 populations from widespread geographical localities in the UK. It revealed that races capable of attacking the five *B. napus* differentials, and also swede cultivars with reputed resistance, were common

Figure 2 Swedes (centre) belong to the species *B.napus* which is the allotetraploid of *B. rapa* (turnip, left) and B. oleracea (kale, right). In other words, swedes contain the two sets of chromosomes from the turnip group (aa) together with the two sets from the kale group (cc).

in the UK. In contrast, two of the five *B. rapa* differentials, ECD02 and ECD04, showed a very high degree of resistance to these otherwise extremely virulent populations. Resistance was thought to be under the control of three dominant genes which occurred together in line ECD04[2]. It was therefore decided to transfer these resistance genes to swedes and forage rape, or at least those necessary for resistance to the most pathogenic UK population of clubroot available, namely C56 from a collection made in S.E. Scotland by Lewis[3].

Although kale has traditionally been regarded as more tolerant of clubroot than swedes and forage rape, seedlings of cultivars grown in the UK were susceptible in clubroot tests at SPBS. Therefore, in 1980, a more extensive screening of 48 kale cultivars of diverse geographical origin was done using two populations of *P. brassicae* (C38 and C56). Included in the screening were 48 cultivars of other botanical varieties of *B. oleracea*. There were no clubroot population x cultivar interactions and the most resistant cultivars were kales, although there was a wide range between the most resistant and most susceptible ones. It was, therefore, decided to use 120 of the most resistant plants from the 16 most resistant marrowstem kale cultivars to start a selection programme for clubroot resistance. Since then, extensive surveys done elsewhere have confirmed that kale is the most resistant botanical variety of *B. oleracea* but with significant variation both within and between the morphological types of kale. High levels of resistance have also been found in some landraces of cabbage, of which the German landrace Bohmerwaldkohl was the most resistant[4].

The breeding of swede cv. Invitation An autotetraploid form of ECD04 was produced by colchicine treatment and crossed with tetraploid kale using the latter as female parent. Five hybrids were produced in 1976, following the culture of immature embryos under aseptic conditions on a solid agar medium (Fig. 3). One of the two hybrids with a complete set of 38 chromosomes, was crossed to the swede cultivars Marian and Ruta Øtofte to confirm its fertility, but it certainly did not resemble a commercially acceptable swede. Therefore, three further backcrosses of clubroot-resistant plants to lines derived from modern swede cultivars were made over the period 1980 to 1982. However, the aim of the programme was not simply to introgress clubroot resistant into existing cultivars, but rather to breed a new cultivar.

Hence, selfing of resistant plants began in 1983 to produce 'F_2' populations for a pedigree inbreeding

Figure 3 Culture of immature embryos under aseptic conditions on solid agar medium.

Figure 4 Yield trial of swede F3 families in 1986.

programme. These were handled in different ways depending on the quantities of seed secured; either resistant seedlings were saved from a clubroot test or mature plants were visually selected from the field. Then from F_3 (135 families assessed) to F_5 (127 families assessed), families were assessed in yield trials (Fig. 4) and clubroot tests so that family selection could be practised for yield and agronomic characters, as well as single plant selection for clubroot resistance.

In 1991, the six most promising F_5 families were multiplied for subsequent evaluation in replicated yield trials. Interestingly, four came from the same F_1 plant, two via one set of F_2's and two via the other set. Hence, ultimately both methods of handling the F_2 generation gave similar results, although further research is required as in one method whole F_3 families were discarded for morphological defects and in the other as susceptible to clubroot. The best F_5 family was entered into official National List Trials in 1993. Its dry matter yield at 11.98 t/ha was on a par with that of the controls (cultivars Angela, Magres, Marian, Melfort, Ruby and Ruta Øtofte) whose mean was 11.79 t/ha, and its dry matter content was high, between Magres and Melfort. Its powdery mildew resistance was as good as that of the resistant controls, Magres and Ruby, and its neck length and shape were acceptable. Furthermore, in more extensive clubroot tests with five populations from S.E. Scotland, it was confirmed resistant, with low or zero disease indices. In 1995, it was added to the National List and granted Plant Breeders' Rights as the cultivar Invitation. The first certified seed was sold in 1996, 20 years after the original synthetic *B. napus* was produced. The breeding of Invitation is summarised in Figure 5.

The breeding of forage rape cultivars Arran and Bonar The breeding approach followed with forage rape was similar to that used in the production of the

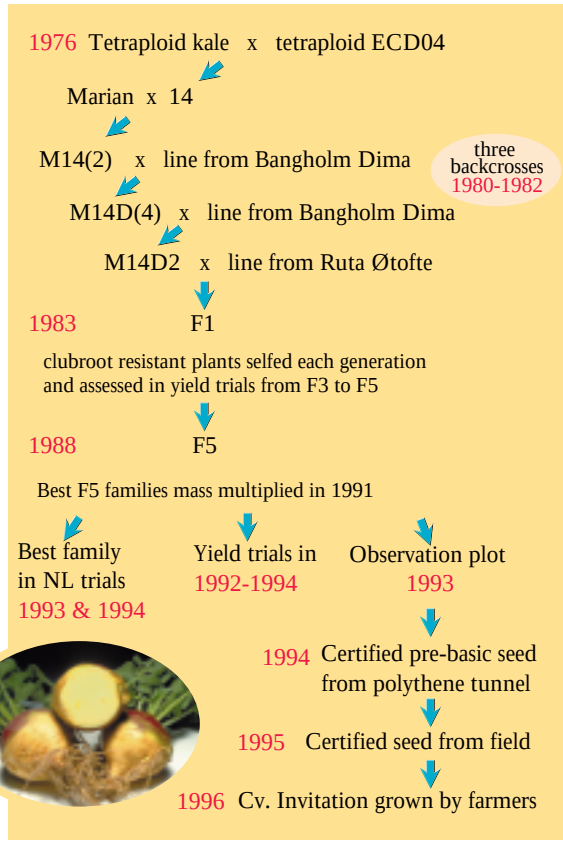

Figure 5 Pedigree of swede Invitation.

swede Invitation, with crosses between tetraploid *B. oleracea* lines of kale, cabbage or kale/cabbage hybrids and tetraploid *B. rapa*, but included oriental salad vegetables as well as ECD 02 and ECD 04. The resulting, re-synthesised, *B. napus* lines were generally forage rape-like in appearance, though some had a swollen hypocotyl region more typical of a swede. The majority of those lines had poor fertility and produced little or no seed on self-pollination. In an effort to improve fertility, to improve clubroot resistance even more, and to produce forage rapes with the required commercial attributes, further crosses were made between these lines and the forage rape cv. Nevin (ECD 06). This cultivar came from a cross made at the Welsh Plant Breeding Station between New Zealand clubroot resistant rape (ECD 09) and Wilhelmsburger swede (ECD 10), and has resistance to some races of *P. brassicae*. Initially, the progeny were tested for resistance to clubroot in a field known to contain the disease. The exact clubroot population present at that site was not determined. Accordingly, the lines which were resistant could not be determined as having the ECD 02 or 04 forms. Subsequently, more exact tests showed that none of the lines pos-

sessed the full ECD 02 or 04 types of clubroot resistance and had only similar field resistance to Nevin. However, towards the end of this particular breeding programme, an opportunity arose to test a selection of these lines in New Zealand. Six of the lines showed good resistance to the major clubroot race (ECD 21/31/31) in that country and one line combined especially good resistance with good resistance to powdery mildew (*Erysiphe cruciferarum*), *Alternaria* spp. and attack by aphids, as well as having a high fresh weight yield and high yield of digestible organic matter. This cross, [(Thousand head kale x curly kale)4x x (B. rapa ssp nipposinica)4x] x Nevin, was subsequently named cv. Arran and marketed successfully in New Zealand by Wrightson Seeds (formerly Dalgety).

The next stage in the programme was to make further crosses between the Arran-type hybrids and other existing cultivars. The aim was not only to improve clubroot resistance, but to seek better yield, higher levels of digestible organic matter, lower levels of s-methyl cysteine sulphoxide (an antimetabolite in animal feeding) and increased protein content. Crosses with forage rape cv. Samo proved to be particularly successful. One line, subsequently named cv. Bonar, gave excellent results in official trials, outyielding the control cv. Emerald by up to 15% (fresh weight yield). Bonar also had good resistance to powdery mildew and better resistance to clubroot than Nevin. Ownership of this cultivar passed to Unilever, following the sale of the National Seed Development Organisation. It is being marketed in New Zealand by Wrightson Seeds, but a decision on marketing in the UK has been deferred. In any case, material now coming forward from private sector collaborations at SCRI may well have superior yield performance.

The final stage in the breeding programme was virtually identical to that used in the swede breeding programme, with lines of *B. rapa*, known to possess the ECD 04 type of resistance, crossed to *B. oleracea* at the tetraploid level. The progeny were tested against UK clubroot populations and shown to have one, two or even three of the major genes postulated for resistance. Further crosses with *B. napus*, synthesised from other clubroot-resistant lines of *B. rapa* and *B. oleracea*, with non-differential resistance, were planned. However, at this stage, public sector support for forage rape breeding was withdrawn.

Much of the breeding material was discarded, following screening solely for yield, and only small amounts of seed kept of the relatively few lines which were likely to combine yield and good clubroot resistance. Most of the latter material is of very doubtful seed viability today and almost certainly further progress would necessitate repeating the crossing, selection and clubroot testing process. This will only happen if private sector collaborators decide that good clubroot resistance would give a financial return commensurate with their investment. However, there is no doubt that forage rape with good clubroot resistance can be produced. This could be especially relevant in an era when farmers and consumers alike, are taking a fresh look at the nature and source of animal feed.

The breeding of kale cv. Caledonian The starting material was 120 of the most clubroot resistant plants from the 16 most resistant marrow-stem cultivars assessed in 1980. As kale is an outbreeding crop species, improvement of this foundation population by a number of generations of recurrent selection, before cultivar production, was a logical breeding method. In order to determine the amount of heritable variation present for clubroot resistance, and the level of resistance that could be quickly achieved, a sub-population was selected solely for clubroot resistance, whilst the main population was selected for a number of traits.

Four generations of selection, involving single plants, half-sib and full-sib families, resulted in 168 disease-free plants from 24 disease-free families in a clubroot test where 65.1% was the mean infection of five kale controls (cultivars Bittern, Canson, Condor, Kestrel and Merlin). Thirty-six full-sib families were produced from the disease-free plants in 1988. In a yield trial in 1989, a bulk of these families had a dry matter yield of 11.39 t/ha compared with 9.90 t/ha for the mean of the five kale controls and, in fact, outyielded them all, thus confirming that a simple open-pollinated cultivar can outyield complex hybrids such as Bittern, Condor, Kestrel and Merlin. It was, therefore, decided to mass multiply the 36 families in a polythene tunnel in 1991 to provide seed of an open-pollinated cultivar for National List submission in January 1993, after further encouraging trial results in 1992.

In two out of three years of National List testing, cv. Caledonian, as it became known, was distinct from all entries except Condor, so a special clubroot test was done which showed that Caledonian was more resistant and hence distinct from Condor. Caledonian was added to the UK National List and granted Plant Breeders' Rights on 20 May 1996, and pre-basic seed

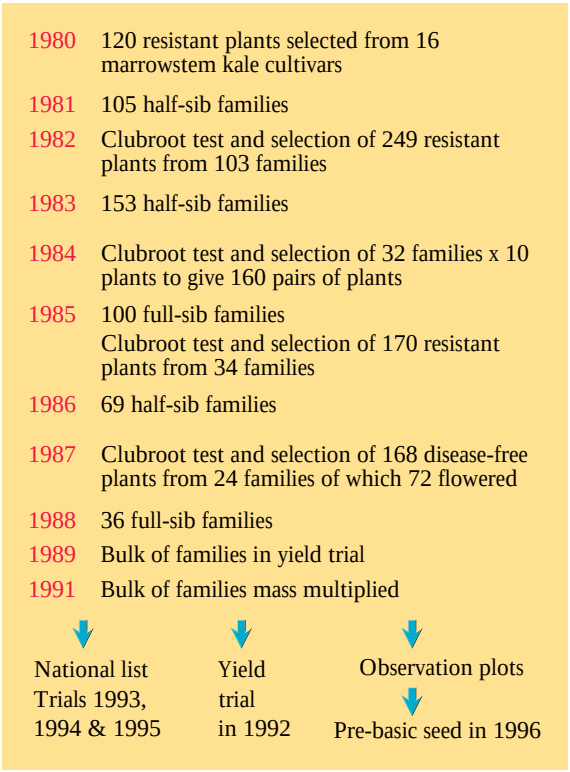

Figure 6 Pedigree of kale Caledonian.

was produced for multiplication in 1997. The breeding of Caledonian is summarised in Figure 6.

The research project was completed by comparing a mass multiplication of the 20 most resistant full-sib families assessed in 1985, with a re-constituted foundation population comprising the same number of plants of each cultivar used in the original population but not selected for clubroot resistance[5]. The comparison revealed that a disease index averaged over six clubroot populations had been reduced from 41.2 to 12.5. This was lower than the most resistant cultivar in the original population, cv. Mixti at 28.8, and as good as Bohmerwaldkohl cabbage at 10.5. In comparison, the mean of the five kale controls was 61.1 and the value for the most susceptible control, cabbage cv. Septa, was 89.3. The re-constituted foundation population was also compared with the selected kale population in a yield trial with inoculated subplots and uninoculated controls. The foundation population suffered a 34% loss of DOM yield compared with 5% for the selected population.

Inheritance and durability of resistance The improved level of kale resistance was achieved by a number of cycles of recurrent selection, and hence exploited statistically additive genetical variation. However, extensive genetical studies would be required to determine the number of genes involved, their nature, and their relationship, if any, with the genes for pathogenicity and aggressiveness in the clubroot populations. The lack of clubroot population x *B. oleracea* genotype interactions in the final assessments and in the initial assessment of cultivars does, however, suggest that the resistance is non-differential in nature and hence may prove durable.

The swede breeding programme provided evidence for only one of the three postulated dominant genes in ECD04 being required for resistance to C56, and also good evidence of differential resistance when F_2 populations, F_4 and F_5 families, and controls were tested with other clubroot populations. Hence, whilst the resistance in cv. Invitation should prove useful in the UK in the immediate future, it may not be durable in the longer term, as happened with differential major gene resistance in stubble turnips in Holland. The next goal should, therefore, be to introduce high levels of non-differential resistance from *B. oleracea* where cabbages such as Bohmerwaldkohl, and kales such as Caledonian, look attractive sources of such resistance. However, a major breeding effort would be required to produce a commercially acceptable swede from a synthetic *B. napus* involving a clubroot-resistant kale or cabbage.

Conclusion Caledonian kale and Invitation swede were the products of plant breeding research funded by the Scottish Office, as were the forage rapes, Arran and Bonar. In order to make them available to farmers and growers, however, SCRI required commercial partners. In the case of Caledonian and Invitation, the Institute is pleased to say that this was provided by Sharpes International Seeds Limited who are now marketing these cultivars.

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Non-transgenic applications of plant tissue culture in potato

S. Millam, P. Davie, K. Harding, M. Grant & M.F.B. Dale

Plant tissue culture techniques have proved invaluable in basic studies of plant growth and development, and a number of methodologies have made significant commercial impact with the potential of further developments in the near future. Within the Crop Genetics Department, a number of key techniques are available which not only contribute to other research programmes but have resulted in the creation of valuable material for commercial exploitation.

Micropropagation Basic micropropagation techniques for application to potato have been long established. As over 1.25 million plants could be regenerated from a single shoot in the space of twelve months, the benefits of programmable, rapid clonal propagation are manifest. It is indicative of the uptake and utility of such technology that over 65% of the Scottish seed potato crop originates via a tissue culture stage.

The utilisation of micropropagation technology in our research programmes has a number of aspects:

- cost-effective germplasm maintenance and multiplication;
- all-year-round provision of clonal material for use in a range of *in vitro* studies;
- a crucial role in generating material for the targeted breeding programme (see p. 40);
- the multiplication of material for National List Trials and for explants used as controls in breeding programmes, notably within commercial, targeted, breeding contracts within the department.

Microtuberisation Scientists can also mimic the process of tuberisation in the laboratory. Such techniques have great scope for studies on the physiology and biochemistry of the tuberisation process but are unlikely to be used for commercial application due to uncertain dormancy.

We have developed a widely applicable method for the production of microtubers *in vitro*. The process involves:

- the culture of single nodes on a tuberisation medium containing cytokinin, chloroethyl-trimethylammonium chloride and high levels of sucrose;
- maintenance of cultures under short day conditions at 16°C for seven days;
- then, the placing of plates in total darkness at the same temperature for a further 28-42 days.

This method has successfully generated microtubers in over 30 potato cultivars (Fig. 1), twenty dihaploids and ten wild species of varying chromosome levels (from 2n=24 to 2n=72) and appears to be relatively, non-genotype specific.

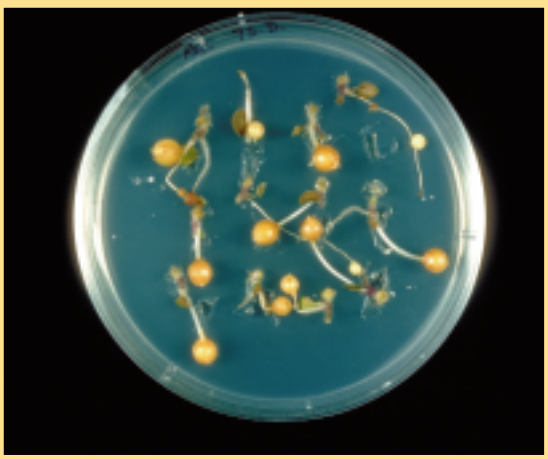

Figure 1 Microtubers arising from single nodes of cv. Maris Piper after 28 days culture.

Cryopreservation This technology, involving the long-term storage of tissue at ultra-low temperatures, has an important and often undervalued role in the conservation of plant genetic resources. It is of particular value in vegetatively-propagated species such as potato. The recovery and survival of material from extreme, low temperature conditions is dependent on many factors including genotype, cryoprotective agents, and freezing and thawing regimes.

In collaborative work with the University of Abertay, Dundee, techniques for the successful cryopreservation and, importantly, the assessment of genetic stability of the plants post-freezing, have been investigated.

A method involving encapsulation/dehydration, was applied to apical shoot-tips derived from diploid (*Solanum phureja*), tetraploid (*S. tuberosum, S. acaule*) and hexaploid (*S. brachycarpum, S. guerreroense* and *S. iopetalum*) germplasm. All species survived cryopreservation and plants exhibited normal developmental patterns with regard to morphology, flowering, berry set and tuber formation. Cytological studies revealed that the ploidy status was maintained and no chromosomal abnormalities were observed.

Somatic hybridisation

Background The recent spectacular advances in transformation technology have considerably widened both the scope and efficiency of generating novel plant material and resulted in the high profile release of transgenic crop plants. However, the technology allows only additional single gene traits to be conferred. Rather less well publicised has been an alternative, non-genetic engineering strategy for the transfer of polygenic traits into crop plants which is both contributing a financial return by new cultivars, and providing novel material for basic genetic studies.

Though somatic hybridisation (a process where single cells or 'protoplasts' of non-related species are fused and grown back into a plant) was first reported in 1972, it is only recently that plants derived from somatic hybrid material have reached a field scale. In the most successful case, modified alkaloid and disease-resistant traits of commercial tobacco cultivars were created from the progeny of protoplast fusion products. These varieties are now grown on over 40% of the tobacco area in Ontario, Canada, representing a value in excess of $100 million[1].

Somatic hybridisation in potato This has been widely reported. Indeed, the first application of electrofusion involving plant protoplasts of any food crop, was the somatic hybridisation of *S. tuberosum* with *S. phureja*. The tetraploid nature of the potato, and the wide availability of useful related germplasm, offer a number of strategies for somatic hybridisation and improvement of existing cultivars[2].

However, widespread application of potato somatic fusion technology has been precluded by a number of factors. These include the failure to surmount the problems of genotype-dependency, thus restricting application to a narrower range of germplasm than would be desirable.

Progress within the Crop Genetics Department Within the core research programme, protocols for the isolation, culture and regeneration of protoplasts from dihaploids, diploid and tetraploid wild species and a wide range of cultivars and breeding lines have been developed since 1990. This includes SCRI material and previously recalcitrant crisping varieties. Techniques for cell fusion (electrofusion and chemically-mediated) have also been investigated and optimised.

Novel aspects of the work have included:

• accessing the Commonwealth Potato Collection for donor species. The CPC is a unique repository of germplasm, held at SCRI;

• uptake and development of molecular techniques, with the specific objective of overcoming one of the key bottlenecks in the somatic hybridisation programme, i.e. identification and characterisation of hybrid material at as early a stage as possible.

In addition to the core programme, a number of externally-funded and collaborative projects have been undertaken since 1990 (Fig. 2).

Summary: Plant cell and tissue culture techniques are not only valuable research tools but are important for the uptake and integration of novel plant material into agricultural practice. Locally, there are up to ten companies involved in applying micropropagation to the seed potato industry with important implications in both the local economy and export potential of high grade tubers.

Despite the advances made in transgenic technology - which are also heavily dependent on plant cell and tissue culture - other techniques of *in vitro* manipulation, such as somatic hybridisation will remain valuable for the creation of novel germplasm. Of particular significance is the reduction in time taken for the transfer of important resistance genes, particularly

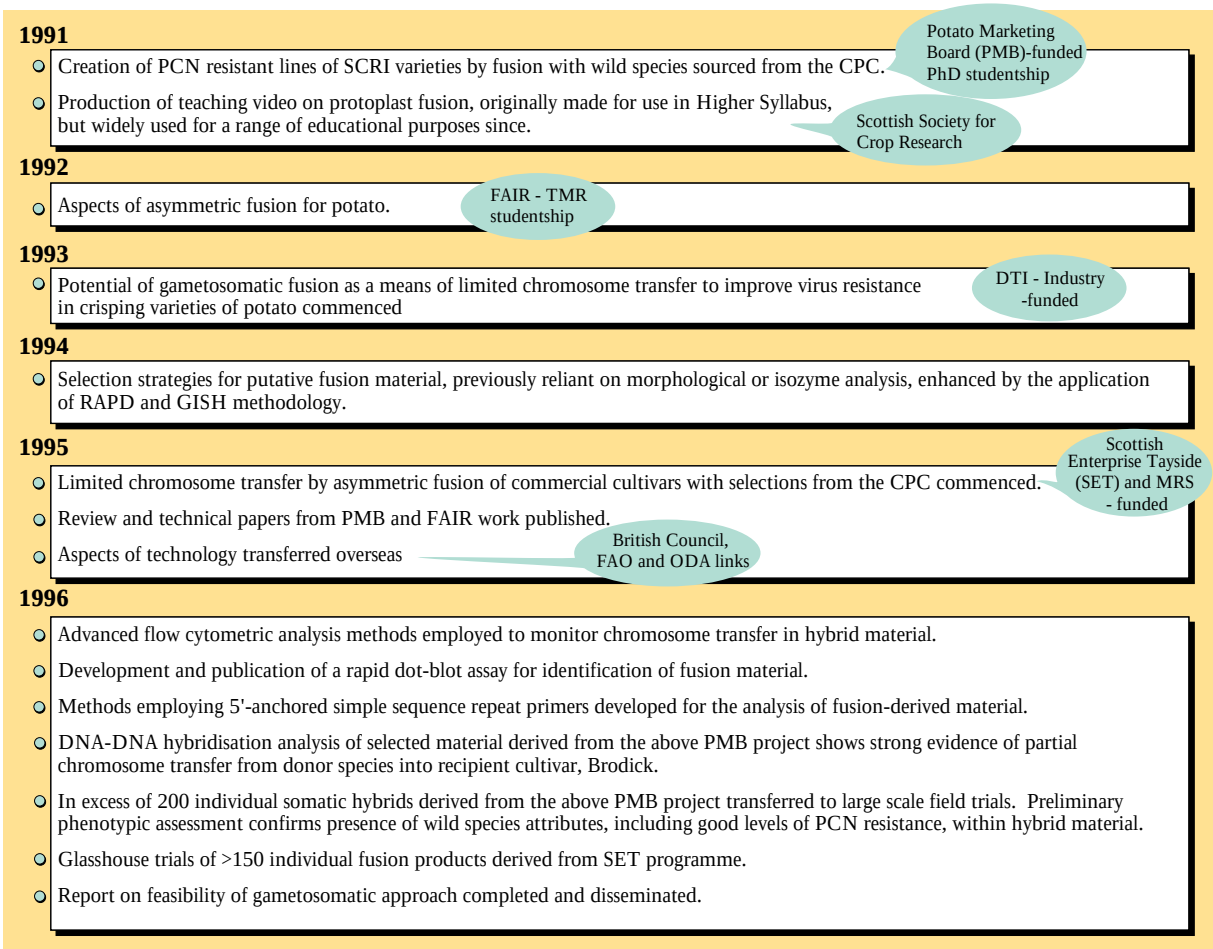

Figure 2 Progress in somatic hybridisation since 1991

quantitative characters, compared to lengthy conventional backcrossing programmes.

Progress in this area has facilitated the introgression of such material into breeding programmes, in addition to their use in projects on gene stability and expression. The circumvention of the lengthy and expensive regulatory processes involved with the release of genetically modified organisms into the environment is another factor in promoting somatic hybridisation research into potato and related germplasm.

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New brassica cultivars

New fodder kale cultivar

Caledonian (SK9): Caledonian was placed on the UK National List and granted Plant Breeders' Rights on 20 May 1996. It is a medium height, high yielding, open-pollinated, marrow-stem kale with resistance to clubroot. It came from a marrow-stem kale population constructed in 1980 from 120 plants selected for clubroot resistance from 16 cultivars of diverse geographical origin. After four generations of selection, solely for non-race specific resistance to clubroot, 36 full-sib families were mass multiplied to give cultivar Caledonian. It is being marketed by Sharpes International Seeds Ltd.

Caledonian

New turnip cultivar

Massif (ST4): Massif successfully completed official trials in 1995 and was placed on the UK National List and granted Plant Breeders' Rights on 19 January 1996. It is a high yielding, open-pollinated, green-top yellow turnip which came from a population improvement programme started in 1978. Three white-fleshed cultivars, Nepe Foll, Hvit Mainepe and Imperial Green Globe, and four yellow-fleshed cultivars, Invincible, Wallace, Green Top Scotch and Green Top Yellow were included in the foundation population. The main selection criterion was simply high dry matter yield. The sixth generation consisted entirely of green skinned, yellow-fleshed turnips and was considered uniform enough for entry into National List Trials in January 1994. Massif has a dry matter content between that of Imperial Green Globe and Green Top Scotch, but a higher dry matter yield. It is being marketed by Sharpes International Seeds Ltd.

Massif

New forage rape cultivar

Interval (SR16): Interval was placed on the UK National list and granted Plant Breeders' Rights on 19 February 1996. The parentage of Interval is a tetraploid (artificial *Brassica napus*) synthesised from *B. oleracea* (marrow stem kale) and *B. campestris* ssp. *nipposinica* and the forage rape cv. Emerald. The artificial *B. napus*, designated SV10, had been selected from a number of progeny, for good mildew *(Erysiphe cruciferarum)* resistance, good tillering and non-flowering. Interval gives high yields (both fresh weight and dry matter), has improved mildew resistance and has consistently out-performed the mean and the individual performance of its industry standards cultivars, Emerald and Hobson, in the UK. Interval has also shown great promise in other countries, including Ireland and New Zealand. It is being marketed by Sharpes International Seeds Ltd who have the worldwide rights.

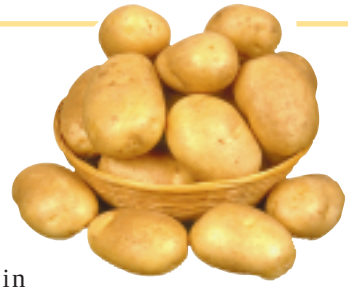
Interval

New potato cultivars

Othello

Othello is a sister clone to Buchan and Brodie (Croft x Cara), and was placed on the National List in 1996. It is a very high yielding maincrop cultivar, maturing 10-14 days earlier than Cara in Mediterranean trials, and is aimed particularly for export markets. It produces large, oval tubers with red eyes and cream flesh, and is on the Recommended List for Cyprus, where its high yield and early maturity, together with its attractive tubers, make it an ideal Cara replacement. The variety is not being developed in the UK due to scab and nematode susceptibility and large size.

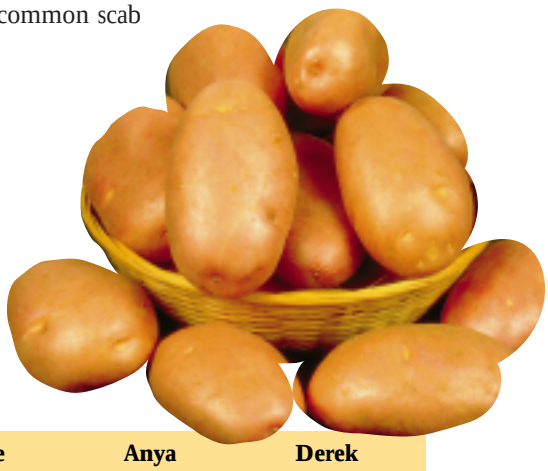
Derek



Derek is a sister clone to Buchan and Brodie (Croft x Cara), and was also placed on the National List in 1996. It is an attractive 'American Style' russet-skinned potato suitable for baking, chipping and pre-packing, with excellent resistance to common scab and both foliar and tuber late blight.

Claret

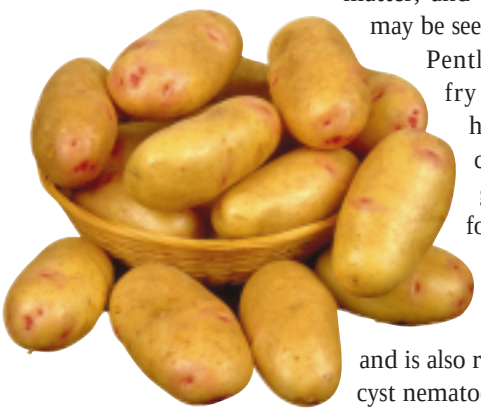
Claret is an early maincrop cultivar, placed on the National List in 1996, and produces high yields of oval, red skinned tubers, suitable for the prepack market, with low to medium dry matter and good cooking qualities. It has high resistance to common scab and virus Y,



	Spey	**Claret**	**Othello**	**Kirrie**	**Anya**	**Derek**
Origin :	12327A1 x Cara	G67551 x Cara	Croft x Cara	Spunta x Cara	Desirée x P.F.A.	Croft x Cara
Year of cross :	1981	1982	1981	1986	1985	1981
Maturity	Maincrop	Early maincrop	Maincrop	Second early	Second early	Early maincrop
Wart	Field immune	Field immune	Field immune	6	-	-
Late blight (f)	5	5	3	5	3	8
Late blight (t)	4	6	7	3	4	7
Gangrene	5	6	4	4	-	5
Dry rot	-	-	7	-	-	-
Skin spot	4	3	3	-	-	-
Common scab	6	7	4	4	8	8
Virus:						
PVY	4	8	7	7	2	2
PLRV	5	5	5	8	6	5
Spraing	1	1	-	-	Susc.	-
PCN:						
G. rost.	9	Susc.	Susc.	9	Susc.	Susc.
G. pall.	Partial R	Susc.	Susc.	Susc.	Susc.	Susc.
Blackleg	7	7	6	7	6	4

Spey

Spey is a high yielding maincrop cultivar, which was placed on the National List in 1996. It produces parti-coloured, long, oval tubers with medium to high dry matter, and good fry colour, and may be seen as a replacement for Pentland Dell for French fry production. It also has excellent table cooking qualities for general ware (score 9 for resistance to blackening and disintegration). It has high resistance to blackleg, and is also resistant to Ro1 potato cyst nematode (*G. rostochiensis*).



Anya

Anya is a second early cultivar, placed on the National List in 1996. It produces long oval tubers with a pale pink skin and white flesh, with medium dry matter and a waxy texture making it ideal for special use as a salad potato. The clone is a Desirée x Pink Fir Apple Cross, with excellent flavour and texture, and high yield. The tubers are still long, but are free of the knobbles and mature much earlier than the original Pink Fir Apple.

Kirrie

Kirrie is a first/second early cultivar, placed on the National List in 1996. It produces a high yield of parti-coloured oval to long oval tubers, with low to medium dry matter and excellent table cooking qualities. It has good resistance to leafroll and virus Y, and is also resistant to Ro1 potato cyst nematode (*G. rostochiensis*).

Soft fruit & perennial crops

Ronnie McNicol

Over the past 2 years, reorganisation within SCRI has brought most, but not all, of the fruit research at SCRI into the Soft Fruit and Perennial Crops Department. This commodity approach to research, especially when coupled with a strong intradisciplinary management matrix, has brought many advantages, not least the co-ordination and focusing of effort on the major targets within the various fruit crops of raspberry, blackcurrant and strawberry. It permits yet further improved communication between researchers in different disciplines and means that the virologists, entomologists, pathologists, biotechnologists, fruit technologists, geneticists/plant breeders and technical advisers are working alongside each other with the capability of tackling a problem from the molecular level, through the field, to the retail shelves. This co-ordinated approach, and past successes of the programme, have readily brought new partners and collaborators on both a national and international basis to our site.

The SCRI fruit transformation programme achieved another notable first, to add to its collection of being the first organisation to transform raspberry, blackberry, blackcurrant and blueberry. Following approval from the regulatory authorities, we planted the first field release of any transgenic fruit crop, not just in the UK, but for the whole of Europe. The trial involves transgenic strawberries of the SCRI-bred cultivars, Symphony and Melody. Plants of these cultivars have had the Cowpea Trypsin Inhibitor (CpTi) gene inserted into their genome. As reported elsewhere, this gene has conferred varying degrees of resistance to the pest, vine weevil, in glasshouse trials. This pest has become increasingly important throughout the whole of the horticultural industry. Existing chemical and biological controls are largely ineffective at field soil temperatures experienced throughout large areas of the UK. The introduction of a source of genetic resistance into these plants therefore offers many potential benefits to growers, consumers and the environment.

The trial, quite apart from potentially providing conclusive evidence for the utility of this gene in strawberry, has, and will also continue to be, an excellent and unique facility for the provision of risk-assessment data. That is, it can be used to assist in the determi-

nation of the best system(s) and criteria that should be considered in future risk-assessment evaluations.

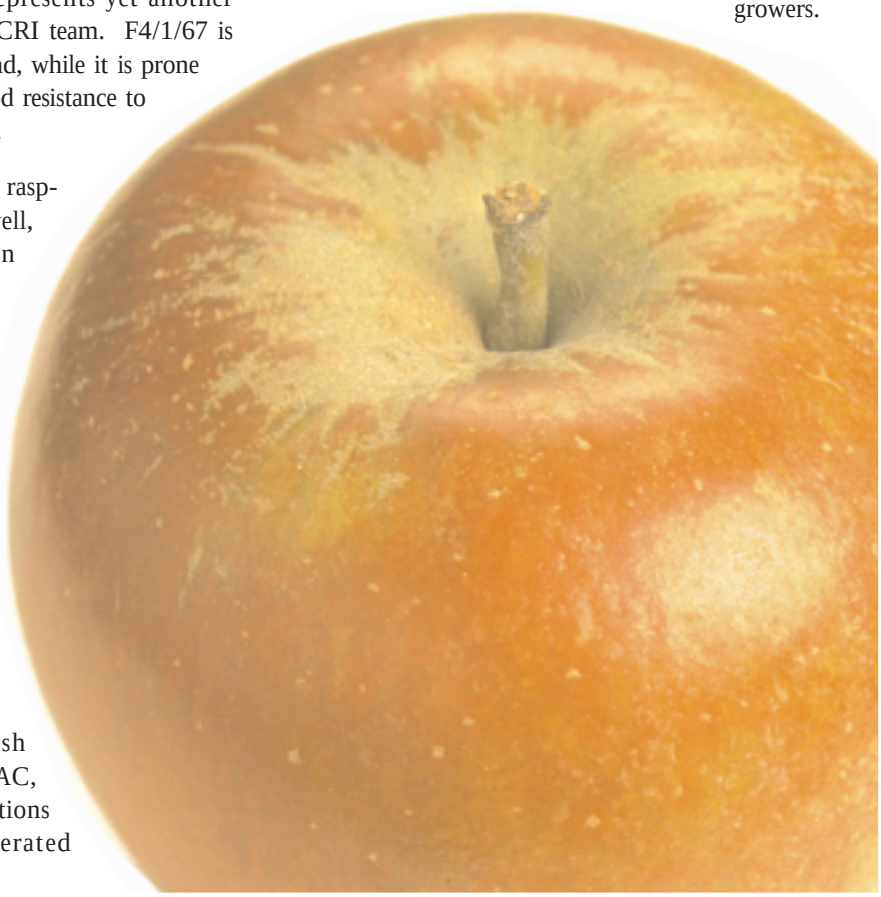
More conventional releases from all the fruit breeding programmes, with good commercial potential, have also taken place. Two advanced strawberry selections, R1P21 and RN59, were propagated and released for field and tunnel trialling. The former is of desert quality, with very high fruit sugar levels (brix >14%) and a tough bright skin. RN59 is perceived as a potential 'long-stem' or 'big-dipper' type strawberry, where long pedicels and large fruit size are the main requirements. This is seen as a new novelty market, whereby the retailers will sell fruit in packs ready to dip in, for example, chocolate.

Two new blackcurrant seedlings (C1/9/10 and F4/1/67) have also been released to industry. These are undergoing intensive fast-track propagation, some of which has been through tissue culture, on a commercial scale, with the objective of enabling the SmithKline Beecham contract growers to evaluate them on an extensive basis. C1/9/10 is similar in season to Ben Lomond and has good resistance to foliar diseases and blackcurrant gall mite. This will be the first commercial cultivar, suitable for juicing, that has effective resistance to this pest, and this represents yet another significant world first for the SCRI team. F4/1/67 is about 8 days earlier cropping and, while it is prone to gall mite infection, it has good resistance to both foliar diseases and reversion.

The 'Glen MARS' cultivars of raspberries continue to perform well, with Glen Ample and Glen Magna finding particular favour. Glen Ample has produced very high yields of relatively large fruit with excellent flavour and appears to be on course to become the new industry mid-season standard cultivar, not just in the UK, but also in other European countries. It also out-performed all other cultivars and selections in Canadian raspberry trials.

In collaboration with Scottish Soft Fruit Growers Ltd and SAC, eight advanced raspberry selections have been undergoing accelerated propagation to ensure that both machine and hand harvesting trials are established on commercial fruit farms with the minimum of delay. The number of selections being trialled is unusually high, but is a reflection of the impact that 'near market' funding cuts have had on curtailing the testing of improved genotypes. Our co-ordinated industry-wide approach (which at SCRI encompassed growers, advisory services, processors, marketers, retailers and scientists), that has been introduced over the last few years, is now becoming fully effective and we are all beginning to see the tangible benefits of our partnerships.

Our most recent strawberry cultivar release, Symphony, has continued to perform well in industry. Despite only being released in late 1994, it already occupies third position in the UK market and is confidently predicted to become number two. It is finding favour not only in the UK, but throughout Europe where it has come top in trials in Holland and Germany. Symphony has a resilient bright skin, good shelf life, juicy texture, excellent vigour and disease resistant, is later ripening than Elsanta, and in addition, does not suffer from Elsanta's proneness to fruit malformation and skin splitting after rain. All of these offer significant benefits to the growers.



Several new breeding programmes have recently been commissioned, which now must easily place SCRI as having the largest combined soft-fruit breeding genetics programme in the world. In 1996, we had *c.* 20,000 raspberry, 25,000 blackcurrant and 18,000 strawberry seedlings in the field. Whilst the raspberry and blackcurrant programmes are likely to be maintained at these levels, it is anticipated that the strawberries will be increased to about 38,000 seedlings with the three different breeding programmes (Kentish Garden, Pernod Ricard and MRS). This increased activity, particularly with strawberry, has enabled us to undertake the training of three young fruit breeders (one each from Scotland, France and America), which is vital to future genetic advancement in these crops. It has also enabled us to actively increase our international participation in co-operative research and exchange programmes. We have recently formally signed a Memorandum of Scientific Co-operation with Norway, joined the European Strawberry Cultivar Testing Network and provided reference material under the EC Plant Marketing Directive.

We have also started, on a very small scale, an apple selection and evaluation programme with the major objective of identifying a high-quality eating apple that is adapted to Scottish conditions. The first crosses were made in 1996 between locally adapted genotypes and quality desert types. The acquisition of additional new bud wood and root stocks was also initiated, together with training courses for staff and industry. Whilst a long-term project, there is every confidence that this will bring a new dawn to the rebirth of a speciality apple industry in Scotland

Genetically modified food

J. Graham

Meeting the demands of a consumer society Bigger (and smaller), sweeter, bright red fruit, fewer chemical sprays, more vitamins, longer shelf-life, cheaper, higher yielding and with better resistance to pests and diseases. The demands of consumers, growers and retailers are many, and the timescale in which these have to be delivered gets shorter. Even if genetic material to satisfy all of these criteria was available, a breeding programme may take up to 20 years to achieve some of these goals. By this time consumer demand and preference may have changed, pest and disease problems may have altered, and the industry could be under threat.

The only way to satisfy the various, sometimes conflicting demands on SCRI, is to continue the highly successful breeding practices, and to integrate into these, new technologies which can overcome some of the limitations associated with plant breeding. One technology still in its infancy but with great potential is gene transfer (sometimes referred to as genetic manipulation or genetic engineering). Like most modern molecular genetic techniques, though, it appears to be causing some controversy.

This article provides some background on gene transfer,

what it involves and what it can achieve. While soft fruit is used here to illustrate the potential of this technology, it could apply to any crop species.

From shots in the dark to controlled crossing For millennia, whether knowingly or not, humans have manipulated genetic material to their own ends. To increase food production, the hit or miss nature of early breeding attempts with no knowledge of genetics, led to the development of crops such as wheat from grass seed. For instance, many early blackberries were dioecious but through selection, have become monoecious. In the latter, the male and female flowers are on the same plant, resulting in greatly improved levels of fertility and hence fruit quality.

Unravelling the mysteries of heredity began in the 1850s with the work of the Austrian monk Gregor Mendel. He performed experiments on peas which led to a better understanding of how information is transferred from generation to generation. As early as1868, Menscher suggested what Mendel's physical units of inheritance were. However it took another 80 years or so before Avery, McCarty and McCloud carried out a classic experiment which proved that genetic information is carried by DNA. Once DNA was confirmed as the genetic material or 'units of inheritance' which transfer from one generation to the next, the structure of DNA was examined. In 1953, Watson and Crick described the now familiar, double helix, molecule which unzips and reproduces itself to pass information down the generations.

Since that discovery, scientists have been able to carry out breeding experiments with a greater knowledge of how new combinations of characteristics are achieved. This has been very successful and has led to the production of a wide variety of crops on a commercial scale.

The need for change Although plant breeding has been very successful, plant breeders are faced with a number of obstacles to the production of new, improved, cultivars. These obstacles include a lack of desirable traits in the breeding material, so that unless a source of a gene is available (for example, for pest or disease resistance), the trait cannot be transferred into the offspring. There is also a lack of control over which characteristics are transferred to future generations, leading to the passage of both desirable and non-desirable traits to the offspring. Any breeding process results in a reshuffling of genes, and it is therefore virtually impossible to make single, specific changes to a valuable cultivar. In soft fruit, there are additional complications. The length of time between generations slows down the breeding process and a number of different forms of each gene exist, requiring the evaluation of large seedling populations to identify the desirable ones. Plant breeding cannot offer a rapid solution to new problems, new preferences or new ideals. In fact, there are many examples where it cannot offer a solution at all.

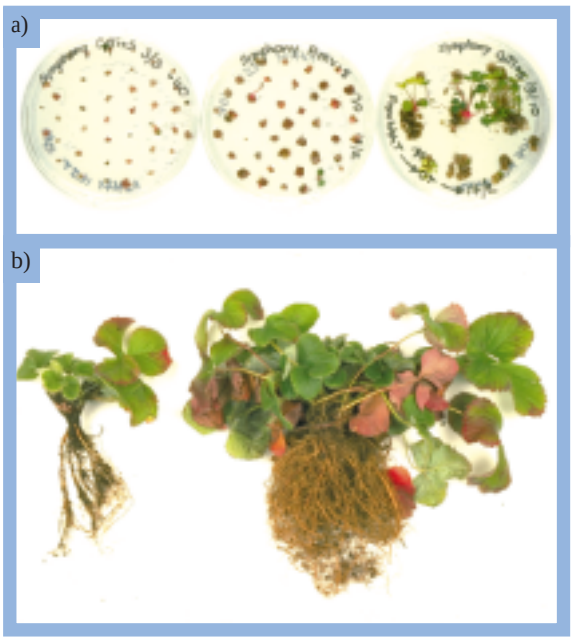


Figure 1 a) The transformation process. b) Non-transgenic (left) and transgenic strawberry plants after attack by vine weevil larvae.

A soil borne bacterium provides a new tool to transfer DNA Gene transfer refers to techniques which enable DNA to be moved from one organism to another by means other than conventional plant breeding. The information represented in DNA is recognised by all living things from Man down to the simplest virus and every organism in between. Each gene contains the information to control a particular function, or groups of genes work together to control more complex processes. Any form of genetic improvement therefore requires the transfer of information from an organism with the desired characteristic, to one without that characteristic. For a characteristic to be transferred to a plant, the gene controlling it must be inserted into the plant's chromosome in a recognisable form on which the plant can act.

In fact, nature has come up with the solution. A common soil bacterium, known as *Agrobacterium*, has the

ability to transfer its own DNA into plants, manipulating them to produce a food source for itself. Strange as it may seem, this process has probably been going on for many, many thousands of years - so gene transfer isn't really terribly new. We can therefore use this natural 'genetic engineer' to transfer the traits we require into plants by first substituting a desired DNA sequence for part of the bacterium's own genetic makeup.

Getting the gene into the whole plant is the most difficult part. *Agrobacterium* easily transfers the genes into any cell it infects. At the whole plant level though, it would be almost impossible to get *Agrobacterium* to infect every cell of a plant, and so a process of whole plant regeneration is used. *Agrobacterium* is used to transfer the gene of interest into just a few plant cells; these are then encouraged to divide forming a new plant in which every cell contains a copy of the originally inserted DNA. This DNA should behave as a normal plant gene.

As the genetic code is universal, the fact that a desired characteristic does not already exist in the breeding material does not mean that it cannot be introduced. The gene for a specific characteristic can be identified and copied from another source and inserted into the chosen plant using the *Agrobacterium* technique described above.

Gene transfer provides unlimited possibilities
Resistance to pests and diseases, reduction in chemical usage, better flavour and colour, improved nutritional value, lower processing costs, edible vaccines and novel fuel sources, are just some of the realities of gene transfer.

Resistance to insect pests and the fungal and viral diseases is an important area where gene transfer can make an impact in the short term. These cause major problems in soft fruit crops such as strawberry, raspberry and blackcurrant. The problems have been tackled by a range of approaches, including plant breeding, chemical control and biological control, with varying degrees of success. The following three examples will outline specific problems and how gene transfer can offer a solution to them.

An insect pest The vine weevil is now the major insect pest of strawberry, where the larvae damage root systems and result in severe loss of yield or plant death. Since the withdrawal of persistent organochlorine insecticides, control of adults and larvae has become difficult. Few of the currently available products are particularly effective under field conditions. Biological control has proved ineffective in many areas due to low summer soil temperatures. This, in addition to the lack of resistance in wild and breeding material, has led to the investigation of alternative control strategies, including gene transfer. By providing the plant with its own genetic-based, self-defence mechanism, the use of pesticides may be greatly reduced. One possibility for resistance to vine weevil is a gene from the tropical legume, *Vigna unguiculata*, the cowpea, which encodes an enzyme which inhibits the ability of the insect to digest food. Thus, prolonged feeding on plants with this enzyme will result in a reduction in fecundity of the insect, and eventual starvation. This gene has been shown to confer significant resistance against vine weevil in glasshouse studies on genetically modified strawberry. The gene occurs naturally in an edible plant and has been shown to be non-toxic to humans and mammals. Our ultimate aim is to have this enzyme expressed only in those plant parts on which the insect feeds and not others, thus eliminating any perceived risk to non-target organisms. We are at present studying the effect of this gene under field conditions, and examining any potential risk this gene may pose under the environmental conditions in which strawberry is grown.

Figure 2 Typical damage caused by vine weevil to a field plantation of strawberry.

A fungal pathogen The fungal disease of greatest concern in raspberry and strawberry is grey mould, caused by *Botrytis cinerea*. The fungus accounts for 50% of all fungicides applied to field-grown soft fruits. The pathogen is difficult to control because there are multiple infection sites and no sources of resistance available to fruit breeders. At present, the crop is sprayed at 7 to 10-day intervals from first flower until shortly before fruit ripening. However, many flowers are missed by sprays and therefore left unprotected. More

Figure 3 Grey mould.

frequent spraying is unacceptable because it is imperative that maximum residue levels are not exceeded and to prevent the development of strains of *Botrytis* which are resistant to the few approved fungicides. The UK soft fruit growers have an excellent, substantiated, record of following the guidelines. However, alternative control strategies are required to reduce the dependency on agrochemicals. Gene transfer can offer a number of possibilities. A number of anti-fungal genes have been identified in plants with the potential to give resistance. One route which we have taken is in switching a natural fruit gene back on (SCRI Ann. Rep. 1995, 115-116). This particular gene is naturally switched off as the fruit ripens, just at a time when *Botrytis* infects. By turning the gene back on so that the product is present as the fruit ripens, we hope to reduce infection and fungal development. A series of such genes could potentially remove the need for fungicides.

A virus disease The virus currently causing the greatest concern is Raspberry Bushy Dwarf Virus (RBDV), for which at least one resistance-breaking strain has emerged. The resistance-breaking isolate can overcome the gene which until recently gave protection against this virus. If the resistance-breaking strain of the virus enters an area of intensive cultivation, it could prove devastating and possibly wipe out an entire industry. Because this virus is pollen-borne, conventional control is virtually impossible. At the moment, the only strategy available to tackle this is the use of gene transfer technology to confer resistance. Control of viruses has involved the use of resistant varieties, where available, or the elimination of the vector, again using chemicals. The phenomenon of virus cross-protection has also been used, whereby previous infection of the plant by a mild strain of virus reduces severity of a later infection with a severe strain. The presence of parts of the mild virus is responsible for cross-protection and, through gene transfer, we can transfer just the parts required to achieve protection.

These examples show how gene transfer can be used to protect against insects, fungal pathogens and viral diseases. In the future we can change the gene insert as required to avoid resistance-breaking strains.

Perception is everything! Research by the Consumer's Association shows that few people appear to know what genetic modification actually is, even though the first products of this technology are already available on the supermarket shelves. Because it sounds so scientific and technological, it can conjure up all sorts of images e.g. 'playing with nature'. Ideas derived from Science Fiction and vociferous minority groups, make people uncomfortable with the whole gene transfer technology. Obviously, we do not all have the time or inclination to become familiar with molecular biology and shouldn't need to. The information on a genetically modified food should be freely available. Identification of such food should be seen as a very positive move, and should be accompanied with relevant information explained in simple, but accurate, terms. The issue of information transfer in an accessible form to the public domain is a real one, which can only be to the benefit of science, technology and the public in general. By and large however, the consumer simply wants the benefits of a process and if these can be explained, identified and justified, then acceptance should follow naturally.

It appears that genetically modified plants may be under threat in the short term, not because they are unsafe, but because of their nature. There is nothing inherently dangerous about the process of genetic modification. However small the risks of gene transfer appear to scientists, public perception may be very different for a whole array of reasons. These differences between the public and scientific community will polarise around notions of safety and risk, labelling and openness. A large factor in this is again the fact that information on scientific matters in general, and biotechnology in particular, is not easily accessed by most consumers. As a result, the potential for misunderstanding is enormous. Scientists must make every effort to accept people's fears of new technologies in general, and proceed in a cautious and responsible way, to ensure that all risks are examined and acted upon. As with any new technique, it needs to be carefully monitored. There is no evidence that genetically

engineered food is any less safe than conventional food. Obviously, care and consideration has to be given to what gene(s) is being inserted, for what purpose, and what effects, intentional or otherwise, it could have. It has to be evaluated very much on a case by case basis.

Risks *Crossing the species barrier* A major issue associated with the use of this technology is that it allows genetic information to cross the species barrier. However, it is not safe to assume DNA does not already cross species barriers. In nature, DNA does transfer within and between species, as is the case with *Agrobacterium* described above. DNA also transfers from viruses to bacteria, or viruses to humans. It is only now that these issues have been opened that we are starting to look and find more and more natural examples of it. A number of techniques that enable greater genetic exchange than is possible through plant breeding, have been in common practice for a number of years, and have not caused concern. However, although gene transfer can do the same job but in a much more controlled fashion, it is viewed differently. It is, however, very difficult to draw a line between a genetically modified plant and one produced using other less sophisticated or 'natural' breeding techniques. 'Natural' however is very difficult to define, as this varies between countries depending on the development of their technologies and cultural practices. Immunisation is now a natural technique for protecting people from disease in affluent countries. Edible vaccines could become the norm in developing ones (SCRI Ann. Rep. 1995, 135-137).

Gene escape Genes do not 'escape', but may transfer by normal, predictable means in pollen. Some people fear that widespread use of plants with altered genetic characteristics may threaten the environment by disturbing the existing balance between organisms. It is important, however, to realise that any genetic balance is a dynamic one, with gene mutations and rearrangements occurring as normal events in all living organisms. Transgenic technology does expand this scope, and careful examination of the transgenic plant is required before it goes into large-scale release and forms part of the food chain. Each plant and gene construct must be considered in its own right. For example, in the UK, a genetically modified strawberry cannot transfer its genes into the wild, as they are not sexually compatible. This is not the case in raspberry, so here the rate and extent of gene transfer into the wild needs to be taken into much more careful consideration.

Toxicity and allergenicity Food safety obviously has to be of great concern to both scientists and the public. In recent years, this subject has had a high profile, following a number of food scares from BSE to virulent strains of *Escherichia coli*. Extreme care must be taken during early establishment of this new technology, as irresponsible releases could set developments back by a decade. Extensive testing is essential if only to reassure the lay person of the care being taken by the scientific community.

Antibiotic resistance Most gene transfer methods rely on a second gene, known as a marker gene, to enable selection of transgenic plants. In the majority of cases to date, this marker gene has been the neomycin phosphotransferase (NPTII) gene. NPTII inactivates and provides resistance to the antibiotics kanamycin and neomycin. Some concern has been raised about the safety of the gene product. Given that humans consume an estimated 1.2×10^6 kanamycin-resistant microorganisms daily, it is unlikely that the NPTII gene product is toxic. In addition, NPTII has been shown to be non-toxic to mammalian cell lines and when produced intracellularly in *vivo*. Concern has also been raised as to whether eating NPTII may compromise oral kanamycin and neomycin therapy. However, NPTII is rapidly inactivated and degraded in the digestive system, with proteins only rarely absorbed. In addition, NPTII requires ATP in order to catalyse the inactivation of kanamycin or neomycin, and this is present only in extremely low concentrations being unstable at low pH. It has been estimated that less than 0.5% of these antibiotics administered are for oral or gastrointestinal tract use. Transfer of the gene from plant to pathogenic bacteria and the possible consequences, have also been raised as objections to its use. Considering however that the majority of pathogenic bacteria live in the gut and are already exposed to the NPTII gene, transfer from other bacteria is much more likely than transfer from plants.

Despite the fact that there is no evidence against the use of antibiotic resistance genes, public perception may weigh heavily against them. Methods have been devised to eliminate marker genes from the crop plant if deemed necessary. It might also be worth considering that as more and more desirable traits become available for transformation, this may become almost essential. The use of a marker gene in one transformation precludes its use for subsequent modification. Thus unless markers are removed, new markers will continually be required for each new trait to be inserted.

Legislation for safety The United Kingdom was one of the first countries to introduce controls on modern biotechnology. These controls were introduced in 1978, not in response to any identified health or environmental problems, but rather because of the lack of familiarity with the behaviour of GMOs and the need to ensure safety. The current legislation governing the release and marketing of GMOs aims to prevent or minimise damage to the environment. Part IV of the Environment Protection Act 1990 and the regulations made under it, are in line with recommendations made by the Royal Commission on Environmental Pollution and implement the EC Directive on Deliberate Releases into the Environment of GMOs. No GMO can be released or marketed without prior consent of the Secretary of State, acting jointly with the Minister of Agriculture, Fisheries and Food. In every case, the Secretary of State seeks expert advice from the independent Advisory Committee on Releases to the Environment, a committee composed of public and private sector experts, including representatives from environmental groups. UK regulations require a full assessment of the environmental impact and risk of any intended release, and every consent holder has to monitor the environmental effects of a release. Before GMOs can be marketed, they must also be approved at European Community level with all member states able to raise objections. In January 1997, the European parliament approved the text of the European Novel Food Regulation and this became law at the end of April. This definition of Novel Food includes food or food ingredients containing or consisting of GMOs or that have been produced from but not containing GMOs. The regulations are aimed at ensuring that food covered by this Regulation does not present a danger to the consumer, does not mislead the consumer, and does not nutritionally disadvantage the consumer.

In summary Genetically modified plants have great long-term potential and we are only in the early stages of utilising this. For public acceptance in the short term, there is a need to clearly explain the benefits of gene transfer technology. These benefits must not only be in terms of the profitability of firms and farmers; consumers must be aware of the benefits to them in terms of cost, food quality and the environment. It is clear that agriculture has to be efficient, particularly in terms of limited resources and has to be as free from negative effects on the environment as is possible. It must also meet the consumer demands who require fresh produce regardless of season. With this understood, the benefits of genetically modified foodstuff should be clear.

In conclusion therefore, genetic manipulation is not something we should fear but is a process which should be harnessed in a positive manner for not just our good, but for the good of our environment.

Rubus breeding and genetic research

R.E. Harrison, R.J. McNicol & S. Jennings

Historically, raspberry production in Scotland has been for processing through preservation as pulp for jam manufacture, canning or freezing. Although fresh fruit production in Scotland is slowly increasing, it remains a small part of the industry (c. 6 % in 1995). Current Scottish raspberry production remains focused on processing, although the market has changed dramatically. Whereas most fruit was processed as pulp in decades past, only 38% of the crop went to pulp in 1995 and 20% in 1996. The shift away from pulp is due to economics. The best prices for processed fruit now come from Individually Quick Frozen (IQF) fruit and other novel processed products.

Other changes in the economics of raspberry production in Scotland have altered the industry. As production costs increase, it has become difficult for all growers to remain competitive and this has lead to a steady decline in the Scottish raspberry area. Some fifteen years ago, raspberries were grown on around 2800 ha which produced about 13,000 to 14,000 tonnes of fruit. By 1990, this had dropped to 2300 ha producing 10,000 tonnes and currently about 1,300 ha can produce anywhere from 4,000 - 8,000 tonnes of fruit. The direct causes for this drop in production are numerous. A ban on the chemical cane-suppressant, Dinoseb, in 1987 caused dramatic reductions in the yield of the most commonly grown cultivar, Glen Clova, due to the increased incidence of cane midge and its associated midge blight[1].

The appearance of raspberry root rot in the mid-1980's has also affected much of the crop (estimated by SOAEFD at c. 30%). The fungicide, Recoil (mancozeb + oxadixyl), has been effective at keeping the fungus under control. However, many hectares were lost from the initial outbreak and many of these have not returned to raspberry production despite the successful chemical control. Healthy planting stocks and resistance breeding, in conjunction with improved management practices, will be the only long-term method of avoiding damage from raspberry root rot.

Fierce competition from eastern European pulp, selling at highly competitive prices, has kept prices low for Scottish growers. This competition is likely to increase before it diminishes, as France and Spain become established in the European raspberry market and Chilean products dominate the market during the winter. High quality fruit is likely to become the separating factor between the inexpensive eastern European fruit and UK fruit, and will allow the UK to continue production in this highly competitive market.

Possibly the most dramatic impact on the Scottish industry has been the gradual loss of a dependable work force. The Scottish crop was traditionally picked by hand, using a pool of around 40,000 people from neighbouring cities during the summer months. Various changes in welfare and tax regulations, and simply a breaking down of the summer tradition of picking raspberries, have each added to the loss of this labour force[2]. Growers have responded to this problem by adapting current cultivars and management practices to machine harvesting (Fig. 1). There are currently some 50 machine harvesters operating in the

Figure 1 Machine harvesting at a grower's site.

UK. As part of this change, the selection of cultivars specifically intended for machine harvesting is an important goal of the breeding programme. Recent cultivars from SCRI, including Glen Ample and Glen Rosa, and several older cultivars, Glen Moy and Glen Prosen, are useful cultivars for machine harvesting. However, there is still an obvious need to improve cultivars for adaptation to machine harvesting. In fact, each of these challenges for the raspberry industry identifies new opportunities for the breeding programme to develop cultivars with improved fruit texture, colour and flavours specifically selected for these new industry requirements.

To overcome some of the difficulties facing the raspberry industry, growers formed a marketing co-operative called the Scottish Soft Fruit Growers Ltd, which gained funding in part through joint UK / EU grants. The grants are to assist in many aspects of the restructuring of the raspberry industry, including capital assistance for planting and purchasing machine harvesting equipment, development of a technical advisory service as well as the funding of the raspberry breeding programme at SCRI.

Raspberries have been bred in Invergowrie, Scotland since the 1950s (Fig. 2). First by the Scottish Horticultural Research Institute (SHRI) and then by SCRI after the amalgamation of SHRI and the Scottish Plant Breeding Station. These 'finished-cultivar'programmes were originally run with government funding, but since 1991 the funding of the programme has been provided by Scottish Enterprise Tayside, the Scottish Soft Fruit Growers Ltd and the Horticultural Development Council - the latter two organisations through a UK / EU grant for the redevelopment of the Scottish raspberry industry. SCRI researchers maintain close contact with the industry

Figure 2 Early days of raspberry breeding in Scotland c.1954.

through the Scottish Soft Fruit Growers Ltd and the breeders strive to develop cultivars that meet the rapidly changing requirements of the UK raspberry industry.

Rubus breeding at SCRI is currently focused on raspberries and blackberries. Some work also continues on blackberry-raspberry hybrids, as well as novel fruits like the purple and yellow / amber raspberries. The objectives of the programme are two-fold: first to enhance the germplasm that is available to commercially oriented breeding programmes; and second to breed commercially acceptable raspberry cultivars through improvements in fruit quality, yield, plant habit, machine harvest ability and resistance to pests and diseases. The programme is essentially a recurrent

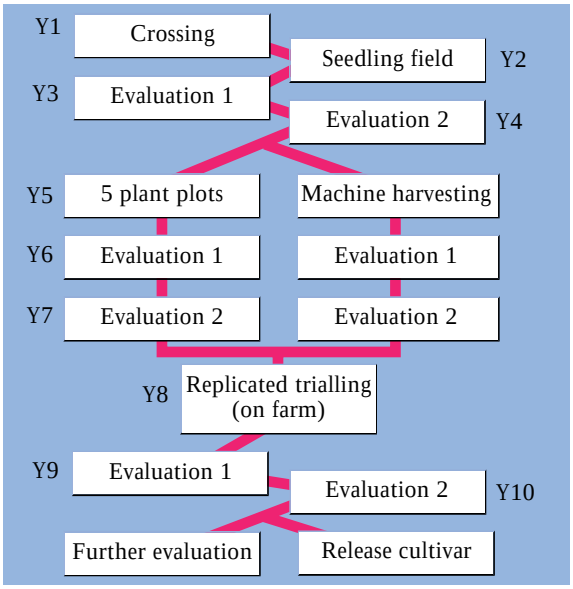


Figure 3 A diagramatic representation of the raspberry breeding programme with each level of the chart representing 1 year in the programme cycle (Y1 - Y10).

selection programme (Fig. 3). Each year selections are made and these selections form the basis of the next generation of crossing. It is not, however, a closed system. As new variability for certain traits is needed, elite cultivars and selections from outside the selection programme are included as parents.

Figure 4 Parents are lifted from the field in the autumn and forced into flower in a glasshouse the following winter. Specific parents with complementary characterisitics are cross pollinated by hand in the glasshouse.

Each year, approximately 30 selections and cultivars are chosen as parents and approximately 100 crosses are made. Dormant parent plants are brought into a glasshouse in mid-December and forced into flower by mid-January. Nearly all of the crossing is now done in glasshouses during the winter (Fig. 4) with field crosses only necessary on rare occassions. Seeds are extracted from fully-ripe fruit in April, acid-treated and then stratified at $4^{O}C$ for 4 to 6 weeks. We aim to produce between 100 and 200 seedlings from each cross. These are initially grown in a glasshouse for preliminary screening for the absence of spines and for aphid resistance. Spined genotypes are removed from segregating families, as nearly all SCRI breeding stocks now carry the recessive gene (*ss*) for spinelessness. Over 90% of the families are also segregating for the A_{10} gene for resistance to all known biotypes of the large raspberry aphid. This aphid is a vector of several important viral diseases, and resistance has played an important part in increasing the longevity of modern raspberry plantations.

The programme begins with about 12,000 seedlings. Elimination of spined and aphid-susceptible genotypes leaves approximately 5000 seedlings for field planting in late October. The plantation requires one

Figure 5 Promising raspberry selections are evaluated for machine harvesting using a Korvan raspberry harvester.

Figure 6 Machine harvested plots are assessed for yield and the quality of fruit harvested. The sample in the upper-left and upper-central positions are promising machine harvesting selections.

year of growth for plants to establish and to produce suitable primocanes on which fruit is borne in the second year. Seedlings are evaluated for numerous fruit characters during the first two fruiting seasons. Initial evaluations are on fruit size, shape, colour, firmness, flavour, shelf-life, and ease of fruit removal as an indication of suitability for machine harvesting. Some secondary assessments for fruiting-lateral characteristics are made, but more detailed evaluations of vigour, cane characteristics, and yield are not made until these selections are propagated into small plots. Each selected seedling is also initiated in tissue culture to establish a stock-plant free from root diseases. Approximately 1 - 2 % of the seedlings are selected at this stage.

Once a potential parent or new cultivar has been identified in the seedling field, it is propagated from root cuttings for further evaluation in duplicate five-plant plots. These plots are fruited for 2 - 4 years and detailed evaluations are made, including yield. Promising machine harvesting selections, based on ease of picking (removal of the plug), are also propagated into 20 m plots for machine harvest evaluation. Machine harvesting trials consist of single plots of each selection and several control plots. These plots are harvested using a Korvan machine harvester (Fig. 5). Total yield is measured and visual assessments made on these fruit (Fig. 6). Additionally, a small sample is collected from each harvest and evaluated further to quantify the proportions of fruit in various quality categories (IQF, under-ripe, over-ripe, ripe with plug, diseased and broken laterals).

Within 3 years, the best machine harvesting genotypes are selected from the trial. The tissue-culture-propagated stock-plant of each selection is then virus tested and multiplied in tissue culture to provide plants for on-farm assessment at many sites. At this time, a propagation bed is also initiated. This gives SSFG members fast access to reasonable quantities of planting stock once the best selection(s) is identified.

Although importance is placed on developing cultivars quickly, there is a balance between rapid evaluation

Species	Origin	Useful characters
R. occidentalis (Black raspberry)	*North America*	Fruit firmness; resistance to aphid and cane diseases
R. pileatus	*Asia*	Firm cohesive fruit; resistance to cane diseases and root rot
R. coreanus	*Asia*	Firm cohesive fruit; resistance to cane diseases, root rot, raspberry beetle and powdery mildew
R. spectabilis (Salmonberry)	*North America*	Earliness in summer- and autumn- fruiting raspberries; resistance to root rot.
R. cockburnianus	*Asia*	High fruit number per fruiting lateral (high yield potential); resistance to cane disease
R. crataegifolius	*Asia*	Resistance to raspberry beetle, cane midge and cane botrytis
R. phoenicolasius (Japanese wineberry)	*Asia*	Resistance to raspberry beetle

Table 1 A list of wild species, their origins, and useful characters for raspberry improvement in the *Rubus* breeding programme.

and stability of performance. Yield can fluctuate from year to year for a variety of reasons, such as establishment, climate, and damage from machine harvesting. Each of these factors can affect vigour of primocane production and reduce yield in the following season. Therefore, we believe that it is important to observe selections for a minimum of two fruiting seasons, particularly in the machine harvesting trials, before a proper assessment of the consistency of performance can be obtained. Historically, it has taken from 12 to 15 years to produce a finished cultivar following an initial hybridisation. Early identification of valuable selections, early virus testing and tissue culture each help to reduce this development time by 2 to 3 years.

Most of the characteristics required for raspberry improvement are available in raspberry cultivars introduced by SCRI and from breeding programmes in other parts of the world. Exotic species that are closely related to the raspberry are also needed as a source of novel variability (Table 1).

Germplasm enhancement, the adaptation and transfer of exotic germplasm from wild species into elite breeding lines, is an important aspect of any progressive breeding programme. Although the major emphasis within the SCRI breeding programme remains the development of commercially acceptable cultivars, research into novel genetic resources continues. In cooperation with Dr Chad Finn of the United States Department of Agriculture (USDA) - Oregon and the National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon, we are beginning to evaluate seedlings from numerous wild species of *Rubus* for root rot resistance, aphid resistance, RBDV resistance, novel fruit colour, and raspberry beetle resistance, as well as, general horticultural characters. Through the NCGR, we have access to hundreds of wild *Rubus* accessions. Dr Finn has initiated a screening programme for adaptation to a temperate climate and has reduced the number of potentially useful accessions that include *R. sachalienensis*, *R. niveus*, and *R. sumatranus* and a red-fruited form of *R. coreanus*. Preliminary research will assess small samples from each of these species for useful traits as well as cross-fertility with *R. idaeus*. Seedlings from fertile crosses will be examined further to study the inheritance of these useful traits in the interspecific hybrids.

Adaptation of exotic germplasm is vital to the rapid transfer of novel genes into cultivated species. However, locally-adapted wild *R. idaeus* material from Britain can also be a source of important traits. In cooperation with Geoff Squire (CEP), Julie Graham (SFPC), and Bruce Marshall (CEP), local *R. idaeus* populations have been evaluated for molecular and morphological variation. These plants are also being assessed for horticultural characteristics to identify useful traits for the breeding programme.

Root rot has been studied at SCRI since the mid-1980s. In the early 1990s, 12 genotypes were identified as having root rot resistance from glasshouse screening and then from an additional field test in an infected site. These resistant parents were backcrossed to elite SCRI material and seedlings from these crosses were planted into an infected site for field evaluation. It will take several years for the fungus population to build up to effective levels. However, surplus plants from some families were evaluated for resistance in the glasshouse. All families showed some levels of resistance (Fig. 7). However, our initial findings suggest that both the resistant and susceptible parent contribute to the resistance level in the progeny. Therefore care must be taken in the selection of resistant parents and test crossing with a range of elite selections and cultivars should improve our efficiency of producing resistant families.

Figure 7 A segregating family for raspberry root rot resistance. Untreated control plants are in the right-hand row and treated plants are to the left.

Root rot is a problem on a global scale and as such has provided opportunities for collaboration. Dr Patrick Moore of Washington State University (WSU) in the

United States has provided SCRI with resistance x susceptible seed lots as well as BC_3 generation seed from initial crosses to a putative accession of *R. innominatus* from Russia that has shown consistent resistance under field conditions in Washington. Currently, we are assessing this seedling material for root rot resistance and will generate BC_4 seed and share this with WSU. In addition, we have supplied WSU with samples of seed from resistant x susceptible crosses made at SCRI.

There may be no single trait as important in raspberries as quality / sensory character of the fruit or processed product. A collaborative flexible-fund project between SCRI (Rex Brennan, Rick Harrison and Ronnie McNicol), the Hannah Research Intitute (Donald Muir), and BioSS (Tony Hunter) has developed sensory evaluation protocols for fresh and processed fruit. The sensory evaluation is performed by a 12 member panel at the Hannah Research Institute under the guidence of Donald Muir. Tony Hunter of BioSS has developed user-friendly computer software to produce rapid experimental designs and multivariate analsysis of the complex sensory data.

The sensory project has now developed robust sensory vocabularies to evaluate fresh and frozen/thawed raspberry fruit and juice. Recent research using these methods has identified a particularly interesting and important relationship between the fresh and frozen / thawed character of raspberry fruit. Fresh fruit characteristics do not necessarily predict the frozen / thawed chracter. This finding has now lead to changes in the fruit quality evaluation methods that should produce better Individually Quick Frozen (IQF) products in the future. Typically, the major emphasis in the evalution of seedlings has been on fresh flavour, but beginning this season, more selections will be made at the seedling stage and fruit samples will be frozen and IQF flavour assessed as an additional selection criteria. In addition, fresh and frozen samples of all advanced SCRI selections and several control cultivars will be assessed for sensory characters to develop a better understanding of this relationship between fresh and frozen fruit. Future projects will use these methods to study heritablilty of these sensory attributes to assess if these methods could be incorporated to an even greater extent in the programme.

Successful plant breeding requires collaboration between nearly all sectors of the plant sciences, including plant pathology, entomology, virology, physiology, molecular biology and statistics. SCRI provides expertise in all these areas, which allows the *Rubus* breeding programme to flourish and remain a world leader in *Rubus* cultivar development. But possibly the most important collaboration is with the customer. The close relationship of SCRI with the growers and processors through the SSFG Ltd keeps the direction of the programme focused on the goals and objectives that will make the greatest impact for the industry.

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Interactions between plant resistance genes, pest aphid populations and beneficial aphid predators

A.N.E. Birch, I.E. Geoghegan, M.E.N. Majerus[1], C. Hackett[2] & J. Allen[3]

Insects cause estimated losses of at least 13% of world food production, despite an expenditure of approximately US $7.5 billion annually on agrochemicals. Aphids are important pests of all major temperate crops, causing economic losses by direct feeding damage, by contamination with honeydew and associated fungal pathogens and by transmitting plant viruses that decrease yield and quality. Estimates for the U.K. alone indicate that annual crop losses due to aphid attack are in the region of £100 million. Crops

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with inherent resistance to these pests, developed through conventional breeding or genetic engineering, can form the basis of a successful alternative control strategy to multiple applications of pesticides. Breeding pest-resistant varieties has yielded a 600-fold return on research investment, compared with a five-fold return on pesticide research[1].

At SCRI, we have developed and are assessing both natural and transgenic host plant resistance to aphids, as a central part of integrated pest management systems. Ideally, the pest resistance mechanisms used should be reasonably durable against resistance-breaking insect populations (biotypes) and should also complement the role of beneficial natural enemies (predators, parasites) in agricultural ecosystems for regulating pest populations. Current aphid resistance research at SCRI is focused on both of these important issues.

Natural aphid resistance genes in raspberries and the threat of a new resistance-breaking biotype

Raspberry breeders have been using genes for resistance to the virus vector aphid *Amphorophora idaei* successfully for more than 40 years. More than 12 major genes for resistance to *A. idaei* are reported in *Rubus*, differing in their effectiveness against the five known biotypes of the aphid. Currently, about 90% of the U.K. raspberry area is planted with raspberries containing *A. idaei*-resistance genes. Of these resistant raspberry cultivars, 35-40% contain gene A_{10} (effective against all documented biotypes) and 30% contain gene A_1 (effective against biotype 1 but not against biotypes 2 or X). The remainder contain minor gene resistance.

The extended use of the resistance gene A_1, for more than 30 years in U.K. raspberry cultivars, has exerted a strong selection pressure on *A. idaei* biotypes. This is reflected in a comparison of surveys of U.K. *A. idaei* populations from the 1960's with a recent survey, carried out by SCRI scientists in the early 1990's. Over this 30-year period of exposure to the A_1 resistance gene, there has been a swing from about 3% to over 75% of *A. idaei* samples having A_1-resistance-breaking ability. This effectively means that raspberry cultivars containing the gene A_1, which were resistant to *A. idaei* (e.g. Glen Prosen, Glen Moy, Malling Delight) are now susceptible to *A. idaei* attack and to the four viruses transmitted during aphid feeding.

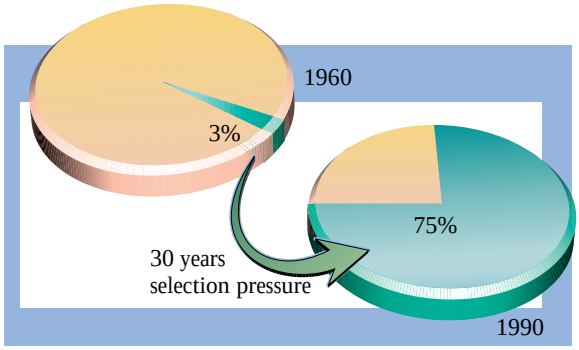

Figure 1 Cultivation of aphid-resistant raspberry varieties for more than 30 years has resulted in a selection pressure for resistance-breaking biotypes of *Amphorophora idaei*, increasing from <3% of the U.K. population in the 1960's, to 75% in the 1990's.

Over the past 5 years in England there has been an increase in the cultivation of later-fruiting raspberry cultivars which also contain the stronger A_{10} resistance gene (e.g. Malling Leo, Autumn Bliss). These two cultivars now represent about 42% of English plantations but are not widely grown in Scotland. Because of the prevalence of A_1-breaking *A. idaei* biotypes in England and Scotland, plant breeders and growers are becoming increasingly reliant on A_{10}-based resistance for non-insecticidal aphid control in existing and new raspberry cultivars. No further *A. idaei* resistance genes, effective against all biotypes have been discovered. Together with ADAS advisors, we have been monitoring A_{10} - containing raspberries over the past 3 years. Recent analysis of U.K. *A. idaei* populations using ribosomal DNA probes revealed considerable genetic variability within and between biotypes, suggesting that increased exposure to the A_{10} gene could lead to a rapid selection of a new resistance-breaking biotype[2].

The first positive identification of an *A. idaei* population colonising A_{10}-containing cv. Autumn Bliss was in November 1994. This observation was viewed with caution because previous experiments and field observations had shown that *A. idaei* can settle and feed on old senescing leaves of Autumn Bliss.

Figure 2 A new biotype of *Amphorophora idaei*, capable of breaking the strongest form of aphid resistance (conferred by resistance gene A_{10}) has been recently discovered feeding on the formerly *A. idaei*-resistant raspberry cv Autumn Bliss.

However, further observations in 1995 and 1996 confirmed the development of a new biotype, when *A. idaei* colonies were found covering the upper and lower leaves of Malling Leo and Autumn Bliss in a small number of English raspberry plantations from July to November each year. Additional confirmation of the new *A. idaei* biotype was obtained from chromosome karyotype analysis (R. Blackman, pers comm.) and from culturing field samples taken from Autumn Bliss on other A_{10} cultivars for more than 30 generations in a controlled environment room at SCRI.

Currently in the U.K., 85-90% of raspberry plantations, valued at more than £ 28 million contain *A. idaei* genes which are no longer effective against the *A. idaei* biotypes now prevalent (biotypes 2 and X). This problem is likely to increase due to emergence of the new biotype, able to overcome A_{10} resistance in the increasing English plantings of Autumn Bliss and Malling Leo. Although the new biotype is not currently widespread in England, based on the historical pattern of spread of biotypes 2 and X in response to cultivation of cultivars containing resistance gene A_1, the new A_{10}-breaking biotype is likely to become much more widespread in the next 5-10 years. This means that direct damage and virus diseases caused by aphid feeding are likely to increase in raspberry from the current level of £0.3-£1.0 million each year, decreasing fruit quality and viable raspberry plantation lifespans by up to 50%. In the short term, more insecticides will need to be applied to raspberries, and growers will need to monitor their plantations more closely for aphid and associated virus problems. For the longer term, plant breeders are searching for alternative resistance genes, to keep pace with the development of new resistance-breaking biotypes. Future raspberry aphid control based on host plant resistance may involve the combined use of aphid resistance genes from *Rubus* with genes from other plants, through genetic engineering. Screening of suitable new transgenes for aphid resistance and the assessment of their environmental impact is now underway at SCRI (see below).

Transgenic resistance to aphids in tri-trophic interactions involving crop plant, aphid pest and ladybird predator Recent advances in biotechnology have greatly expanded the opportunities for transferring pest resistance genes from one plant species to another. A number of insecticidal proteins, including protease inhibitors and lectins, have been identified as candidates for genetically engineering new insect resistance traits into crops. Although several plant-derived lectins (proteins that exhibit non-catalytic binding to a specific carbohydrate) have been identified as having efficacy against aphids and other homopteran insect pests, little is known about the mode of action and specificity. It is vitally important that any new mechanism of transgenic insect resistance not only is effective in controlling the target pest (e.g. aphids), but also does not harm other non-target organisms in the environment. For integrated pest management to be successful, each of its components (e.g. resistant crop plants, pest predators and parasites, targeted pesticides, insect traps) should be carefully chosen to avoid disruptive interactions, and ideally achieve additive or synergistic effects for suppressing pest populations.

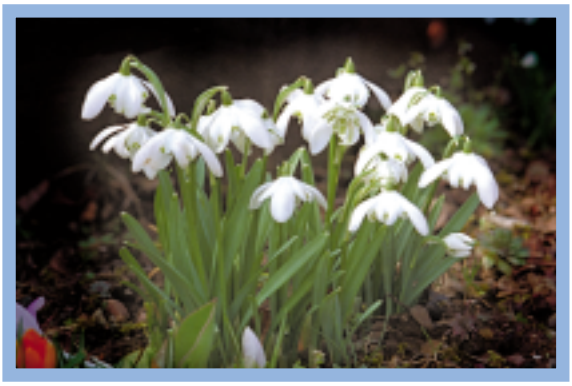

Figure 3 The snowdrop, *Galanthus nivalis*, produces a natural plant lectin (sugar-binding protein) which confers resistance to a range of sucking insects including aphids. The gene for this lectin has been genetically engineered into potato, to reduce the adverse effects of aphid attack on the crop. The effects of the lectin on beneficial aphid predators was unknown.

As a model system for studying tri-trophic interactions involving an insect resistance gene, we have chosen the anti-insect gene encoding for the snowdrop lectin (*Galanthus nivalis* agglutinin - GNA), genetically engineered into potato. This lectin has been shown to have useful activity in suppressing aphid feeding, growth and reproduction but does not appear to have lethal effects on aphid pests. This work forms part of a larger collaborative risk assessment project involving SCRI, the Rowett Research Institute and the University of Durham. For the tri-trophic interactions experiments, we designed special experiments with the University of Cambridge, to assess any potential risks of using this type of transgenic aphid resistance to beneficial aphid predators. The 2-spot ladybird, *Adalia bipunctata* (Fig. 4), was chosen as the model aphid predator, feeding on the peach-potato aphid (*Myzus persicae*). Potato aphids were initially reared on either control or transgenic potatoes, genetically engineered to express the GNA lectin. Aphids from control and GNA potato were fed to sexed sibling groups of ladybirds for 14 days (phase 1) under controlled environmental conditions. After this exposure period, individual pairs of ladybirds from different sibling groups were mated in reciprocal crosses and their ecological fitness and survival were monitored. For the remaining experimental period (phase 2) all ladybirds were fed on an optimal diet, consisting of pea aphids (*Acyrthosiphon pisum*) reared on non-transgenic bean plants. This simulated the likely change of aphid diet experienced by actively flying ladybirds in an agricultural environment, where a transgenic crop would be one of several plants searched for aphids during an adult ladybird's lifespan.

Figure 4 The 2-spot ladybird, *Adalia bipunctata,* was used as a test aphid predator to assess the possible effects of consuming aphids colonising transgenic potato expressing the snowdrop lectin. Ladybirds fed on aphids from either control or transgenic potatoes were mated and monitored for several weeks, to check for adverse effects on ladybirds' life-cycle.

A number of ecological 'fitness indicators' were used to monitor the ladybirds fed on aphids from control or GNA potatoes:

- Daily aphid consumption over the first 14 days (aphids reared on control or GNA potato)
- Duration of ladybird mating behaviour
- Ladybird fecundity (daily egg production) over 30 days
- Ladybird egg viability (fertility and hatch) over 30 days
- Ladybird adult longevity during phases 1 and 2.

Table 1 The ecological 'fitness indicators' used to asssess the impact on 2-spot ladybird of eating aphids from transgenic potato producing the snowdrop lectin.

The pilot experiments have produced a number of biologically-interesting findings concerning the tri-trophic interactions of this particular transgenic potato line with aphids and ladybirds[3]. Firstly, expres-

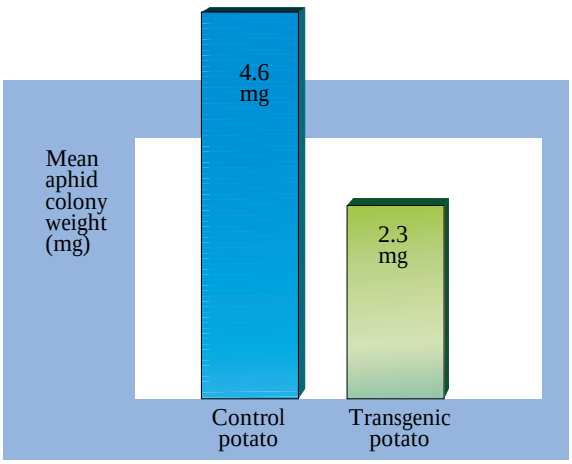

Figure 5 In glasshouse tests, transgenic potatoes expressing the snowdrop lectin have 50% less aphids than control potatoes (cv. Désirée). The lectin interferes with aphids' growth and reproduction but does not kill the pests, so ladybirds and other aphid predators are needed to complement the partial resistance of the transgenic potato plants.

sion of the GNA lectin in potato, even at low levels, does reduce aphid populations by up to 50% in glasshouse bioassays (in the absence of ladybird predators). This level of crop protection is not sufficient on its own, so the role of aphid predators to complement partial resistance would be potentially important for IPM using transgenic aphid resistance genes for GNA lectin expression. (Fig. 5). Although the lectin appears to act as an anti-feedant for aphids and other pest insects, over the 14 day feeding test (phase 1) ladybirds consumed similar quantities of aphids, regardless of whether the aphids were reared on control or GNA potatoes. This indicates that *M. persicae* reared on the transgenic potatoes are not less palatable to ladybirds than aphids reared on normal (non-transgenic) potatoes.

However, once the ladybirds were mated, some effects of feeding on aphids from GNA potato on fecundity and egg fertility were observed. By the third week of egg production in phase 2 of the experiment, the fecundity of female ladybirds previously fed on aphids from GNA potatoes was reduced by 38%, compared with the control potatoes aphid-fed females. Further checks on the viability of the ladybird eggs laid during phase 2 also showed effects of the source of aphid diet. For example, the number of fertilised eggs failing to hatch in the first week of egg production was almost three times higher for female ladybirds fed on aphids from GNA potatoes than those females fed on aphids from the control potatoes (Fig. 6). Similarly, male ladybirds fed on aphids from GNA potatoes then used in matings with females fed on control aphids resulted in four times the number of unfertilised eggs compared with matings using males fed control aphids. In addition, female ladybirds fed on *M. persicae* from control potatoes lived twice as long as females fed for 14 days on *M. persicae* from GNA potatoes. The longevity of male ladybirds was not significantly reduced after feeding on aphids from GNA potato, indicating different effects depending on the ladybird's sex.

In general, the viability of ladybirds' eggs appeared to be most affected for the first 7-14 days after switching from feeding on GNA potato-reared *M. persicae* to the optimal diet (*A. pisum* reared on non-transgenic beans). This suggests that careful management of transgenic and non-transgenic crops in adjacent fields could reduce any possible adverse effects on ladybirds observed in these short-term experiments. Strategies for the safe release of transgenic crops must be devised and validated under field or closely simulated conditions. Longer term experiments, looking at the effects of lectins and other proposed insect resistance gene products on the fitness of ladybirds over several generations are now needed. Our current experiments highlight the importance of assessing all transgenic crops genetically engineered for pest resistance in this way, to be sure that any new type of pest-resistant crop plant does not jeopardise the delicate balance between pests and beneficial insects in agricultural ecosystems.

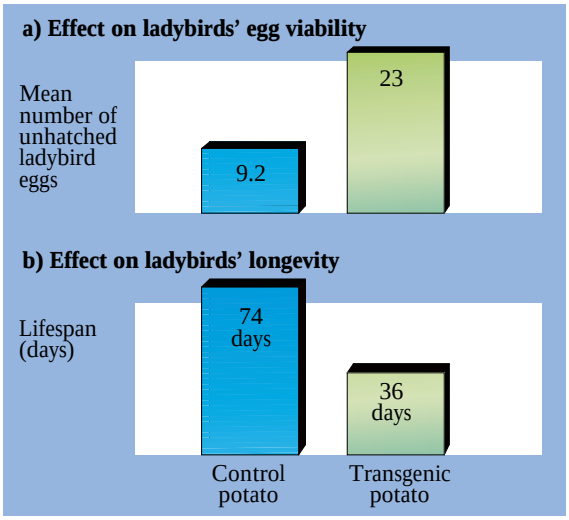

Figure 6 After female ladybirds fed for 14 days on aphids from transgenic potatoes expressing snowdrop lectin, a) their egg viability and b) adult lifespan were reduced, compared with female ladybirds fed on aphids from control potatoes.

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Transgenic resistance to raspberry bushy dwarf virus in *Nicotiana* species

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Raspberry bushy dwarf virus (RBDV) occurs in *Rubus* species world-wide and in some red raspberry cultivars (*R. idaeus*, *R. strigosus*) it induces yellows disease and/or crumbly fruit. In mixed infections with some aphid-borne viruses it can cause a serious degeneration in vigour and yield. Several of the recently released UK red raspberry cultivars are susceptible to the virus and in some, RBDV can induce severe crumbly fruit that greatly affects fruit quality (Fig. 1).

Because RBDV is transmitted between plants in nature through pollen, growing cultivars resistant or immune to the virus is the only means of controlling its spread and effects. Some red raspberry cultivars contain such resistance which is conferred by a single dominant gene, *Bu*. However, in recent years the presence of RBDV isolates able to overcome such resistance (RB, resistance-breaking isolates) has been identified in raspberry fields in England, including commercial raspberry and blackberry crops. The occurrence of such RB isolates, able to infect all existing raspberry and blackberry cultivars grown in commerce in the UK, is a serious threat to the cultivation of *Rubus* here and elsewhere. As there are no suitable sources of resistance to such RB isolates for use in *Rubus* breeding programmes, transgenic resistance using viral gene sequences seems the best, possibly the only, means of protecting future crops from infection.

Figure 1 Crumbly fruit in RBDV-infected Autumn Bliss raspberry.

The efficacy of such an approach to control RBDV was assessed firstly in *Nicotiana* species, as the technology for the transformation of *Nicotiana* species is much further advanced than that of *Rubus*. However, a significant impediment to this approach was that RBDV does not readily infect tobacco and some other *Nicotiana* species systemically, and does not usually induce symptoms in such infected plants. However, the discovery at SCRI of a variant of RBDV (Can-S) able to infect several *Nicotiana* species systemically and which induced noticeable symptoms in most, was a significant finding that overcame this initial difficulty.

RBDV has a bi-partite RNA genome packaged in quasi-isometric particles. RNA-1 encodes a protein with methyltransferase (mtr), NTP-binding (NTP) and RNA-dependent RNA polymerase (pol) motifs. RNA-2 encodes the coat protein (CP), which is expressed by translation of a sub-genomic messenger RNA, and a putative movement protein (MP) (Fig. 2). Of these different genes and gene sequences for possible use as transgenes, we have tested the efficacy of the CP gene in the sense and antisense orientation and in a non-translatable construction, and the pol gene sequence in the sense orientation.

Constructs were made by reverse transcription-PCR of viral RNA to produce cDNA corresponding to the required virus gene sequence. These were then cloned in pGEM-T and subsequently sub-cloned into

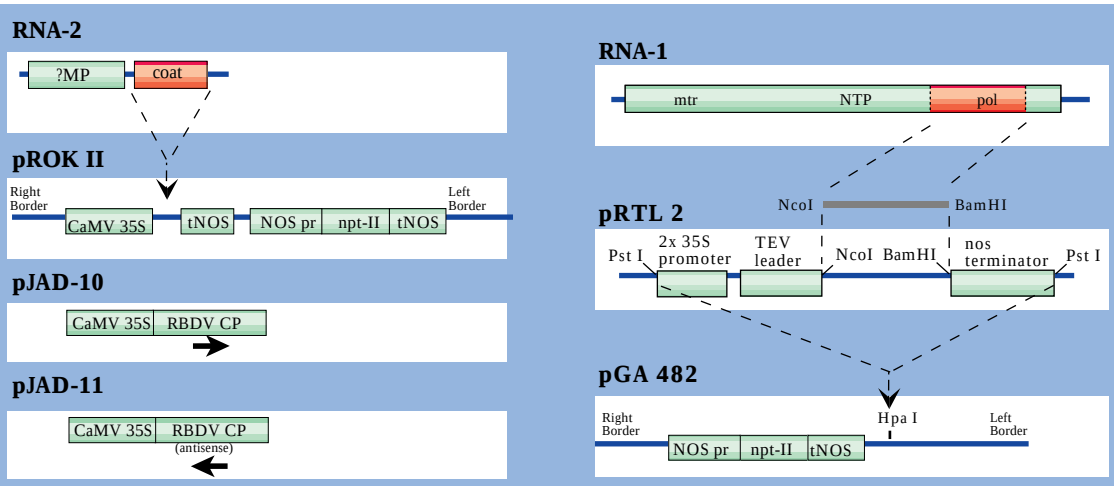


Figure 2 Diagram of the genome map of RBDV and of the cloning of RBDV genes for plant transformation. cDNA was obtained by PCR of the genome RNA-1 or RNA-2 (red) and finally inserted into pROKII (coat protein (CP) gene) or pRTL2 (polymerase gene sequences). The CP gene was in positive orientation (messenger sense) (pJAD-10) or in the inverse orientation (antisense) (pJAD-11). The polymerase gene sequences together with promoter, leader and terminator sequences were excised from pRLT-2 and cloned in pGA 482.

pROKII- or pGA-482-based vectors (Fig. 2). Vectors were introduced into disarmed *Agrobacterium tumefaciens* by triparental mating and the resulting cultures used to transform leaf pieces of plants of *Nicotiana tabacum* cv. Samsun and *N. benthamiana*. Plants regenerated from kanamycin-resistant callus were allowed to set seed, and seedlings of the F1 generation were tested for resistance to RBDV by manually inoculating expanded leaves with the Can-S isolate of the virus in sap of infected *N. clevelandii*. Inoculated leaves were assayed for RBDV in some experiments in tobacco by counting the numbers of local lesions and determining the ELISA values 7 days after inoculation. In all other experiments, inoculated leaves were assayed by ELISA 10 days after inoculation. These results were compared with those of inoculated plants that were not transformed, to assess them for any resistance to RBDV infection and invasion. More than 20 lines were obtained from transformations with each type of vector and construct, and the majority of lines showed some resistance to RBDV.

Several lines of one CP construct in tobacco, in which no viral gene transcript was detectable, showed only

Tobacco line	No. local lesions/leaf	A_{405} values
A1	8.8	1.44
A10	34	1.53
A17	8	1.31
A26	4.7	1.01
Control	85.6	1.71

Table 1 Mean numbers of local lesions per leaf and A_{405} values in ELISA for RBDV in four lines of tobacco transformed with the CP gene of RBDV.

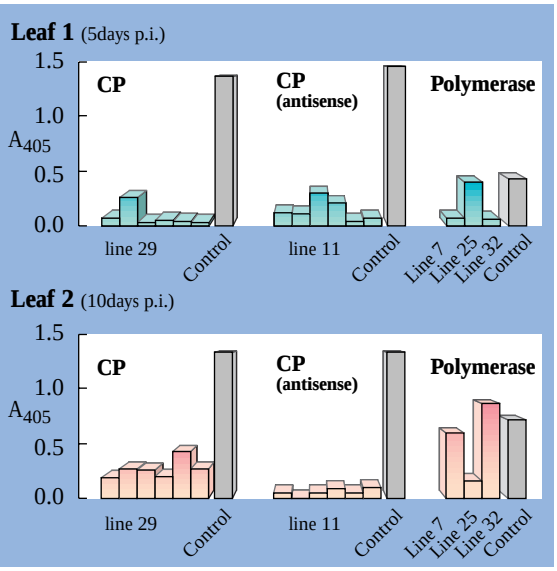


Figure 3 The effect of transformation with RBDV sequences on virus titre as evidenced by A_{405} values in ELISA for RBDV of inoculated tobacco leaves assayed 5 and 10 days post-inoculation with RBDV. The histograms show six R1 plants of CP gene-transformed (29) and CP-antisense-transformed (11) and three individual R0 plants transformed with the pol gene sequence (7, 25, 32).

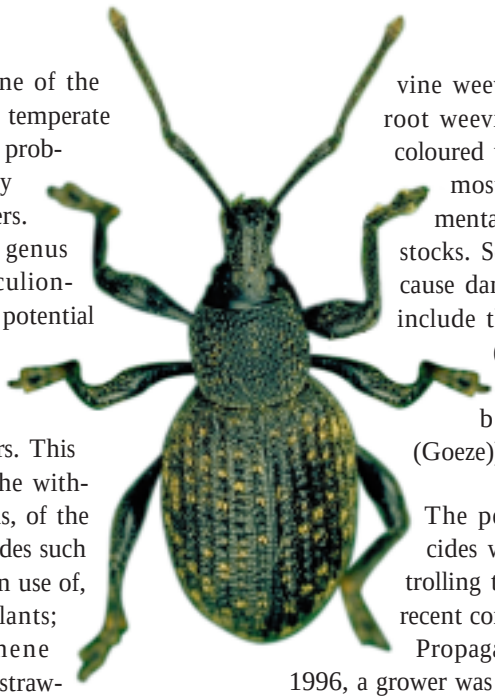
Figure 4 Symptom-bearing and symptomless *Nicotiana benthamiana* plants inoculated with Can-S.

10-35% of the number of local lesions present on comparable control plants but ELISA values were 65-94% of controls (Table 1). This suggests that these transformants may have some resistance to inoculation with RBDV but little or no resistance to its multiplication once plants are infected.

This work has demonstrated the effectiveness of transformation with the RBDV-CP gene or -pol gene sequences to induce resistance to RBDV infection in *Nicotiana* species. Furthermore, it demonstrates that this resistance can be conferred whether the CP gene is in a translatable or non-translatable form, and whether it occurs in the sense or anti-sense orientations. These results are encouraging and, if equally successful when applied to *Rubus* plants, offer the prospect of providing possibly the best means of protecting future *Rubus* crops against this difficult-to-control pathogen.

The increasing importance and control of wingless weevils as pests in temperateWorld horticulture

S.C. Gordon, J. Graham & D.C. Gordon



Wingless weevils are now one of the most troublesome pests of temperate horticulture, causing considerable problems to fruit, ornamental nursery and forest nursery stock producers. Wingless weevils belong to the genus *Otiorhynchus* (Coleoptera: Curculionidae) and have been recognised as potential pests of a wide range of crops for many years, but they have become particularly troublesome in horticulture in the last 15-20 years. This follows, amongst other factors, the withdrawal, on environmental grounds, of the persistent organochlorine insecticides such as aldrin and DDT; the increase in use of, and trade in container grown plants; and the reliance on polythene mulches, especially in soft fruit (strawberry and blackcurrant) production. Three species, the vine weevil (*O. sulcatus* (F.)), strawberry root weevil (*O. ovatus* (L.)) and the clay-coloured weevil (*O. singularis* (L.)), are the most damaging to fruit, hardy ornamentals and in commercial tree nursery stocks. Several other *Otiorhynchus* species cause damage to soft fruit crops and they include the red-legged weevil (*O. clavipes* (Bonsdorff)), and *O. rugifrons* (Gyllenhal)) and the rough strawberry weevil (*O. rugosostriatus* (Goeze)).

The persistent organochlorine insecticides were particularly effective in controlling this group of insects. Indeed, at a recent conference of the International Plant Propagators Society in Cork in August 1996, a grower was quoted as saying 'Life after aldrin is difficult and expensive....Control of vine weevil is

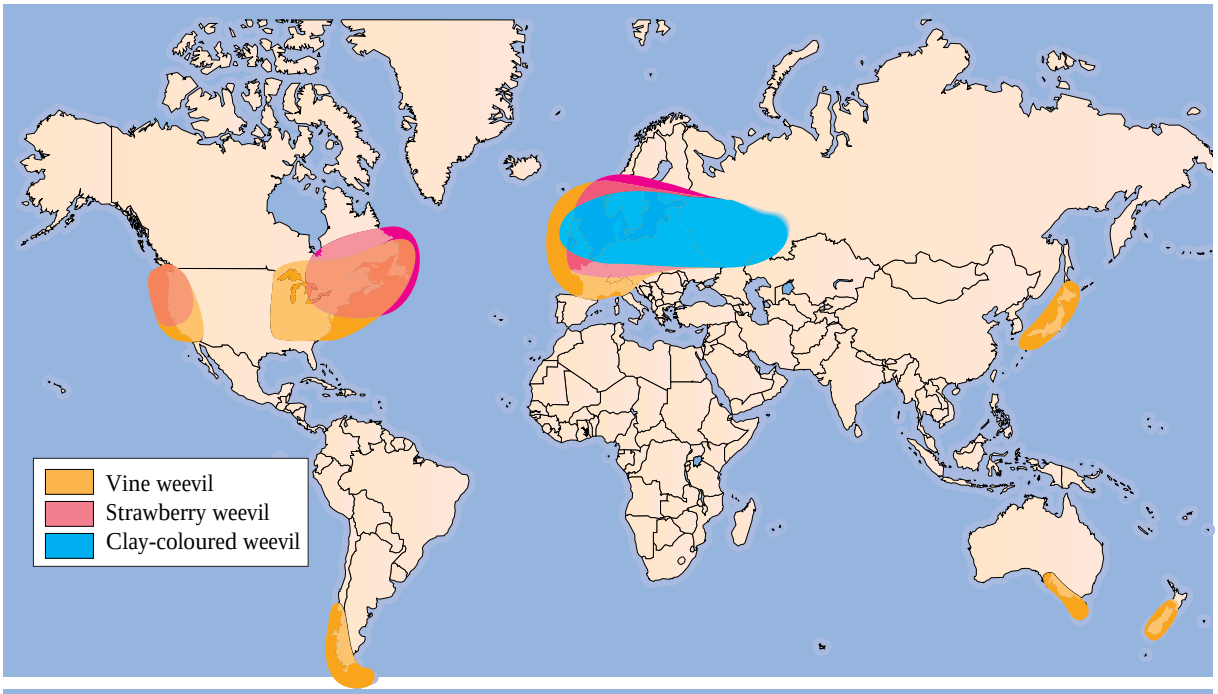


Figure 1 The approximate geographic distribution of the main temperate Otiorhynchid weevil species (after Moorhouse *et al*, (1992). *Annals of Applied Biology* **121,** 431-454)

very, very difficult....It takes number one priority on our nursery.'[1]

The genus *Otiorhynchus* is thought to be of European origin but *O. sulcatus*, in particular, is now widespread in most temperate regions of the World (Fig. 1). *O. singularis* is largely confined to north western Europe, whereas *O. ovatus* is widespread in both Europe and North America.

Biology of wingless weevils As their name implies, wingless weevils cannot fly because their elytra (wing cases) are fused but they can disperse easily by walking, as contaminants sheltering in pots or crates used to transport plants or produce, and as eggs or larvae in the compost of containerised plants. The most numerous wingless weevil, *O. sulcatus*, has one generation per year, but there may be some overlap between the generations. Adult weevils are all parthenogenic females (Fig. 2a), no males being found, and some species are relatively large (7-11 mm). Adult weevils feed at night soon after emergence, hiding in the soil or within dense foliage during daylight hours. After emergence from the pupal stage, all weevils need a period of intensive feeding before they start to lay eggs and foliar feeding becomes most noticeable at this time. The number of eggs laid varies considerably but on average, each female can lay several hundred. Although only a small proportion of the eggs may survive to complete their development, this is sufficient to allow for a rapid increase in the population. The larvae (Fig. 2b) of all the species feed on the roots of their host plants, but the response to feeding varies

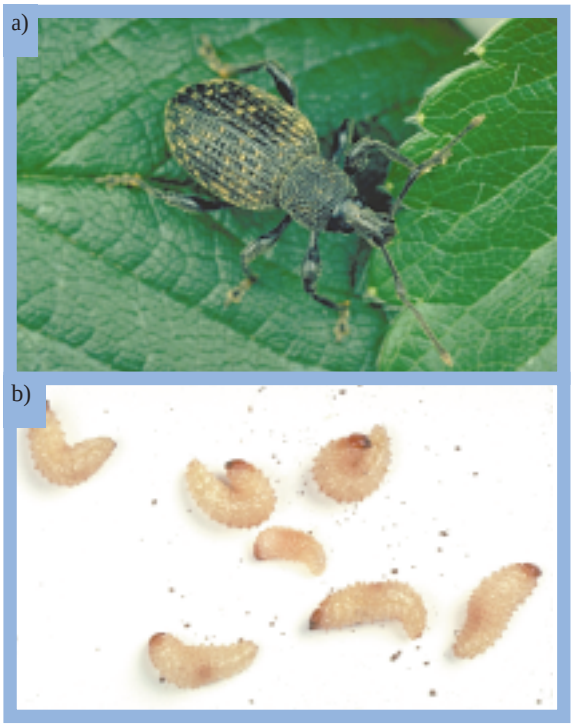


Figure 2 a) Adult vine weevil b) Clay-coloured weevil larva.

Figure 3 Clay-coloured weevil feeding damage to raspberry plants.

considerably. Single larvae can kill very susceptible plants, whereas large populations may be tolerated on others e.g. clay-coloured weevil larvae appear to cause little root damage to raspberry. The different species may have different larval feeding preferences, e.g. vine weevil larvae tend to feed on roots close to the surface, whilst clay-coloured weevils have been observed feeding on raspberry roots at depths below those reached by conventional pesticide application techniques.

Laboratory observations indicated that adult clay-coloured weevils were able to survive for up to 3 years. *O. singularis* adults emerge from the soil in May and June and feed after dark on bursting raspberry buds and developing fruiting laterals (Fig. 3), but rarely feed on fully developed raspberry foliage. Laboratory studies indicate that they avoid feeding on damaged raspberry foliage, probably because of the release, or induction, of anti-feeding compounds[2].

Host ranges of Otiorhynchid weevils The vine weevil is extremely polyphagous, with larvae and adults found feeding on many perennial and semi-perennial plant species grown in both glasshouses and in the open. The hosts range from maidenhair ferns, through perennial fruit crops (e.g. strawberry, grape vine and blackcurrant), to perennial ornamentals such as *Taxus* and *Rhododendron*. The host ranges of the other Otiorhyncids are thought to be narrower, e.g. clay-coloured weevils cause extensive feeding damage to raspberry and girdle stems of newly planted trees or feed on buds and grafts of apple and pear.

Control strategies present and future *Present control* The current range of commercially available insecticides is not particularly effective against adult weevils and few are effective against larvae. Some success in controlling larvae in nursery stock has been achieved by using a slow release granular formulation of chlorpyrifos and a microencapsulated formulation of fonofos. However, to be fully effective, all the compost in a pot needs to be treated with a precise dose of insecticide. When properly incorporated into compost, these products have been shown to be effective for up to two years. These products are unlikely to be used to control field infestations of vine or other weevil because of difficulties of incorporation into the soil around the growing plants and the relatively high cost involved.

In recent years, much effort has been given to develop a biological control strategy for wingless weevils. Some success has been achieved using the entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.) against larvae. These nematodes work well against vine weevil larvae in protected cultivation, but have proved to be less reliable under field conditions, especially when soil temperatures are low. Several centres are currently striving to isolate and develop strains of nematodes which will be effective at temperatures below 10^{o} C. Similarly, the insect parasitic fungi, *Metarhizium* sp. and *Beauveria* sp. have been investigated and shown to be effective under some conditions.

Future control Control of wingless weevils will remain problematic in the future. It is unlikely that any one control strategy will prove to be effective. Populations of weevils will have to be managed in such a way as to reduce the risk of spread from infected areas into 'clean zones'. The fact that wingless weevils do not fly could be exploited by the use of physical barriers, either on their own, but more likely in combination with adhesive strips. Where possible, clean land should be selected for growing susceptible crops or a lengthy period of crop rotation practised.

Suitable monitoring systems for the presence of adult weevils need to be developed to warn the grower of

the problem at an early stage of infestation. The use of appropriate control measures at an early stage of infestation will be more effective than trying to manage large damaging populations. Monitoring of the insect, use of appropriate insecticides, and selection of growing sites all form part of an Integrated Pest Management (IPM) strategy for weevils. In addition, recent research at SCRI has show that the insertion of the cowpea trypsin inhibitor (CpTi) gene from the tropical legume (*Vigna unguiculata*) into strawberry, may protect plants from vine weevil attack in glasshouse trials. Field trials are currently underway to establish the effectiveness of genetically modified plants when challenged by weevil attack under field conditions[3]. If these prove successful, there is now the opportunity to modify other high value fruit crops to protect them from vine and other wingless weevil damage.

Natural plant resistance to wingless weevils Much effort has gone into finding sources of natural plant resistance to vine weevils. Resistance has been identified in the beach strawberry (*Fragaria chiloensis*) from the Pacific Northwest of America and in some *Rhododendron* stocks, particularly the lepidote type. Observations on the feeding behaviour of clay-coloured weevil on raspberry have shown that this insect will avoid mature or damaged raspberry leaves and will preferentially feed on stems, bud or developing canes. It is thought that they have developed this feeding strategy to avoid potential anti-feeding compounds that may be present in raspberry leaves. In a feeding study where weevils were exposed to healthy and wounded raspberry leaves in complete darkness (video images obtained using an infra-red video technique), adult weevils rapidly moved away from the wounded leaves to shelter under the undamaged healthy leaves[2].

References

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Molecular biology

Wayne Powell

Since the formation of the Cell and Molecular Genetics Department in 1987, the framework for conducting research within the UK has changed considerably. Sponsors and funding agencies place greater emphasis on scientific deliverables and 'added value'. However, this requirement for greater accountability has not obscured our main objective of conducting internationally competitive programmes of research. In the current scientific environment, it is essential that we create a research environment where innovative individual talent can flourish, assemble functional multi-disciplinary teams that transcend traditional departmental boundaries, and access funding from a diverse range of sources. This philosophy must operate in a climate where new scientific opportunities abound. For example, programmes to sequence random complementary DNA clones are revolutionising the biomedical sciences and associated industries. Similar exciting opportunities exist for our own experimental systems and crop plants. Therefore, we must remain alert to new technological advances, and have sufficient scientific confidence, vision and commitment to exploit selectively these opportunities. I have every confidence in my colleagues' abilities to meet these challenges.

The Genetic Manipulation Group, within an inter-departmental effort, is assessing the role of invertase, and other key enzymes of carbohydrate metabolism, in low temperature sweetening. This currently involves the trialling of over 500 unique transgenic lines generated from a series of novel constructs that we have engineered. Detailed analysis of the function of invertase promoters in reporter-gene assays has indicated that one invertase gene is expressed specifically in pollen. This discovery raises the possibility of engineering male-sterility into existing potato cultivars, either by interfer-

ing with the function of this particular invertase, or by using its promoter to express cytotoxic agents specifically within pollen cells. If the specificity of expression from this promoter is retained in near-relatives, male-sterile phenotypes thus generated might be exploited in other crops in which hybrid seed production is important, as well as in potato breeding programmes.

The RNA Processing Group continues to provide basic insights into splicing in plants. The demonstration that branchpoint sequences were indeed important to efficient splicing in plants is a major consideration in understanding the mechanisms by which introns are removed from precursor messenger mRNAs. In addition, the value of information being generated by the international *Arabidopsis* community has been shown by an examination of mutations affecting the pre-mRNA splicing of a number of different *Arabidopsis* genes. The splicing behaviour of these mutants not only confirms previous research but has also provided information pointing to communication across exons in plant splicing. These novel observations in plant pre-mRNA splicing are vital to our understanding of this important aspect of gene expression and are essential to future examination of how splicing is regulated. In a second area of research, the RNA Processing Group has demonstrated a novel snoRNA gene organisation which is unique to plants. Multiple, different snoRNA genes are found tightly linked in the plant genome. They are expressed polycistronically and processed by endonuclease activity. This mode of expression is distinct from that of vertebrates and raises many interesting questions in terms of the evolution of the organisation and how the snoRNAs are regulated, along with other ribosomal components. This area of basic research underpins the development of gene promoters for transgene expression and a novel, generic marker system with potential for use in all eukaryote systems.

Within the Plant Genome Analysis Group, the isolation of microsatellite markers in both potatoes and barley has progressed to a stage where more than 120 working primer pairs have been developed for both species. In barley, approximately 100 of these have been mapped to individual linkage groups on a doubled haploid population derived from the cultivar Lina and a *Hordeum spontaneum* accession. These SSRs are now beginning to permeate through the rest of the barley projects in the Department, offering a superior alternative to the RFLP technique which has been the mainstay of plant genetics research for the last 15 years. In potato, just over 60 loci have been mapped in a single population. In both species, this represents the largest available collection of mapped SSRs and these 'universal mapping reagents' are in demand with several requests for primer sequences and map locations. A future, exciting challenge will be to integrate information on map position of SSRs with studies of genepool variation.

A sample of these mapped SSRs has been used by Joanne Russell, John Fuller and Alan Booth to examine the consequences of domestication on genetic variability in a collection of *H. spontaneum* genotypes from the Fertile Crescent, European landraces and commercial cultivars. By comparing the levels of allelic variability at SSR loci in the three genepools, a major bottleneck associated with the domestication of barley (*H. vulgare*) from *H. spontaneum* has been identified. The distribution of allelic variability is shown in Figure 1 where 45% of the alleles identified are unique to *H. spontaneum*, whereas only 5% are unique to the landraces studied. The cultivars appeared to possess the majority of the alleles present in the primitive landraces, indicating that barley breeders have been particularly successful in manipulating the genetic diversity available in the *H. vulgare* genepool. The results from this study are consistent with results obtained using RFLPs but bring greater resolution to quantifying the patterns of genetic variability in crop genepools. Our results have major implications for barley breeding, indicating that any

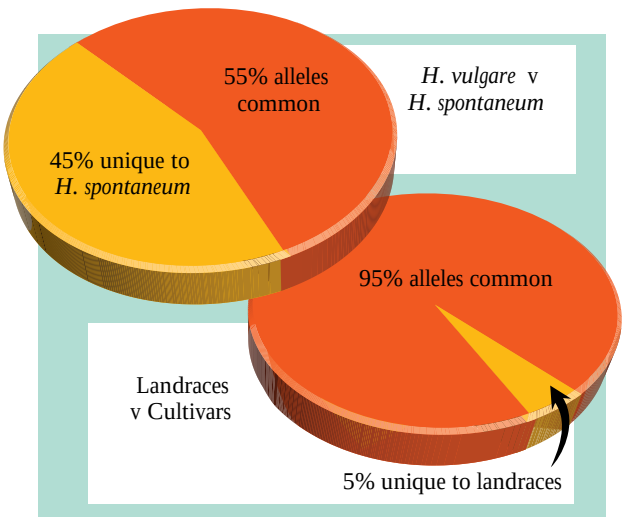


Figure 1 Distribution of allelic variability detected with SSR loci in three genepools: cultivars, landraces and *Hordeum spontaneum*. This demonstrates that approximately 45% of the alleles identified are unique to *H. spontaneum*.

'quantum leaps' in trait improvement will probably be made through exploiting the allelic richness of the *H. spontaneum* genepool. Microsatellites have also been used to genotype uniquely and therefore discriminate between barley cultivars. Three separate combinations of four microsatellites could be used to genotype uniquely 24 of the most widely grown winter and spring barley cultivars. The level of resolution is very high with overall probabilities of identity of less than one in 1000. The microsatellites were shown to be able to distinguish cultivars with the same pedigrees and also demonstrate different patterns of discrimination to those obtained from botanical descriptors. A parallel study in potato, conducted by Hilary Dewar, is based on the use of fluorescently- labelled microsatellite primers to genotype potato cultivars. This approach has the advantage of being non-radioactive and allowing multiplexing of informative SSRs. This research provides a practical example of how molecular markers may be used to evaluate distinctness, uniformity and stability (DUS) of cultivars. In future, this programme will provide underpinning, enabling technology for the protection of proprietary germplasm and retrospective genetic analysis of barley and potato cultivar development in multi-generation pedigrees.

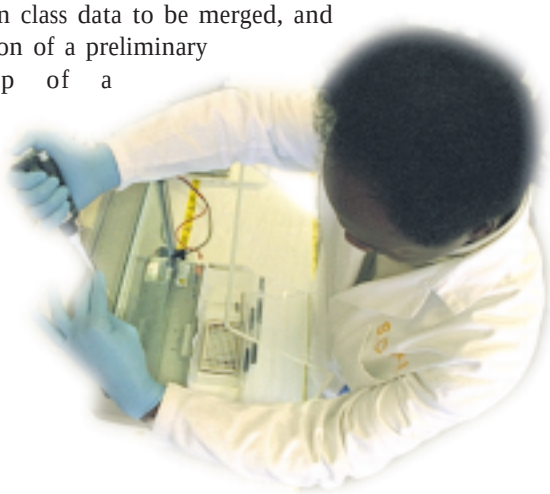
Amplified Fragment Length Polymorphism (AFLP) - another powerful molecular polymorphism assay - has been used extensively over the last year and a few properties of this assay have been identified which will aid in the design of future experiments and interpretation of experimental data. For example, it has been demonstrated conclusively that within the barley cultivated genepool, similar sized products are homologous and can be used for anchor markers in *de novo* mapping exercises. We have also shown that this correlation tends to break down as more distantly related accessions are included (i.e. the chance of obtaining false matches increases).

Further evidence of our commitment to integrating barley genomics research with breeding and diversity studies is provided by the work of Roger Ellis and colleagues. More than 40 barley cultivars were characterised with genome-wide AFLP markers, providing new insights into the development of modern barley cultivars and pinpointing the need to enrich the current advanced breeding genepool with alternative sources of genetic variation. Brian Forster (in collaboration with Linda Handley, David Robinson and Christine Hackett) is using both multi-locus (AFLP) and single locus (SSR) assays to establish associations between markers and genes determining abiotic stress tolerance. The molecular breeding lab has played a crucial role in facilitating this work by providing the expertise and stimulus to unite previously divergent research activities. A particularly rewarding feature of this laboratory's activities has been the productive collaborations established with Oregon State University (P. Hayes), University of Haifa (E. Nevo) and ICARDA (M. Baum, S. Ceccarelli and S. Grando). Interactions with these organisations have involved hosting and training visiting workers, which provides further recognition of the importance of this research internationally.

Commercial sponsorship of our breeding programme under the leadership of Bill Thomas continues to produce exciting germplasm for commerce and research. For example, Livet has been granted Plant Breeders Rights and placed on the National List (see accompanying information). Closer realignment of some of our research activities with the needs of industry has provided new opportunities for establishing partnerships with international agbiotechnology and seed companies.

A molecular assay procedure, sequence-specific amplification polymorphism (S-SAP), has been developed which when used in conjunction with specific retrotransposon sequences, provides an increase in the level of polymorphism detected in barley with AFLP. When used with other conserved sequence motifs from genes or gene families, the method provides a rapid and efficient way of mapping 'functional sequences'. The approach has been used successfully to convert diagnostic AFLP products into polymorphic single locus assays.

The genetics of tetraploid potatoes has been addressed by following the inheritance of dominant AFLP markers in a segregating population. Because there are a number of unique features of tetrasomic inheritance, new methods of analysing the linkage data have had to be developed. This has allowed the 1:1, 3:1 and 5:1 segregation class data to be merged, and the construction of a preliminary linkage map of a tetraploid population. Markers linked to genes determining polygenic resistance to PCN and



late blight have been identified, converted to co-dominant single locus assays and mapped on a reference diploid population.

The Tree Genetics Group continues to focus on the genetics of fragmented populations with the objective of integrating genomics and population genetics research. The genetic consequences of habitat fragmentation are complex and this group is particularly interested in the relationship between fragmentation and gene flow. Microsatellite markers are being deployed extensively because of their multi-allelic, co-dominant nature, allowing individuals to be genotyped uniquely. The high resolving power of microsatellites has been demonstrated by Gemma White working with *Swietenia humilis*, where 26 alleles were detected at one microsatellite locus. Microsatellites are being used in this species to undertake a hierarchical analysis of paternity at the levels of the tree and individual capsule. Information emerging from this study on the extent and pattern of gene flow and genetic differentiation, has important practical relevance for understanding the relationship between habitat fragmentation and levels of genetic diversity.

Joanne Russell has initiated a study to investigate pollen-mediated gene flow in *Eucalyptus globulus* and *E. grandis*. Small insert genomic libraries enriched for microsatellites, have been created and the most informative microsatellites will be used to describe the pattern of pollen-mediated gene flow in seed orchards. For this purpose, individual trees and progeny arrays will be genotyped with selected microsatellites.

Jim Provan, Nicole Soranzo and Neil Wilson are attempting to unravel the complex history and origin of *Pinus sylvestris* in Scotland. Native populations of Scots pine in Scotland represent the only natural woodland in the UK and are therefore of considerable ecological and recreational importance. In pine, the chloroplast is inherited via the male gamete and the mitochondrial genome is maternally transmitted. The development of organellar molecular markers in Scots pine will therefore provide pollen- (chloroplast) and seed- (mitochondrial) specific markers. To date, chloroplast- and mitochondrial-specific markers have been identified and these are being used to examine patterns of genetic variability detected in Scottish and mainland European populations of *P. sylvestris*. Already this research is producing new insights into the genetic relationships between Scottish and mainland European populations. Preliminary data indicates that Spanish populations of *Pinus sylvestris* are genetically different from the other material sampled.

Based on this overview and subsequent articles, it will be evident that the past 12 months have been an exceptionally active period of productive research. Significant findings have been published in premier scientific journals, a number of important recordals filed, and exciting new cultivars are reaching the agricultural marketplace. These achievements demonstrate vividly that multi-skilled researchers working on fundamental strategic and applied research problems can function in parallel, to enhance the scientific standing of the Department and Institute.

Development of a simple sequence repeat-based linkage map of barley

R. Waugh, M. Macaulay, K. McLean, J. Fuller, N. Bonar, L. Ramsay & W. Powell

Over the last few years, several examples of how the introduction of molecular markers into plant improvement schemes can increase the speed and precision of cultivar development have been documented. This has had two major impacts. First, sceptics of the early promises made about the application of molecular markers have been forced to change their opinion and second, the bulk of the plant breeding community has become convinced that marker-assisted, plant breeding can yield significant advantages over current methodologies. This latter decision to apply molecu-

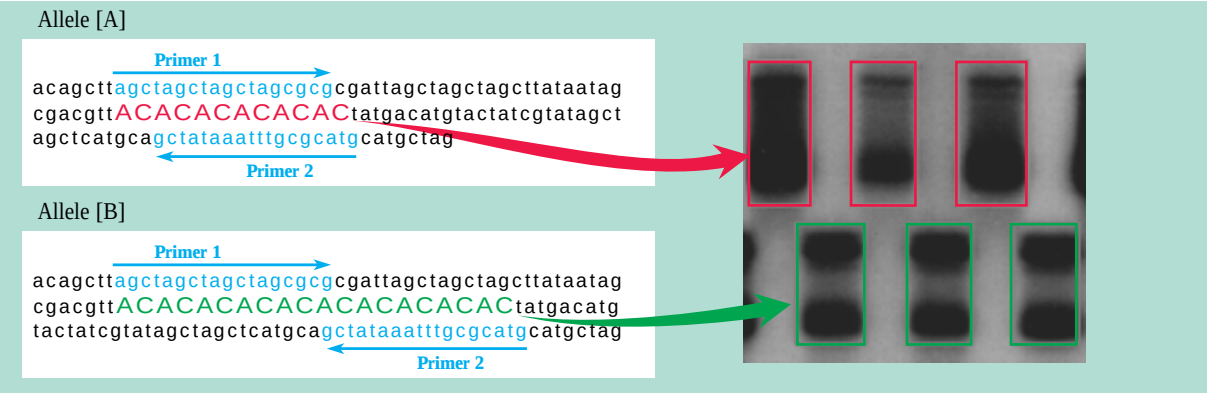


Figure 1 The basis of SSR polymorphism. (a) SSRs consist of tandem arrays of simple sequence motifs such as $(AT)_n$, $(AC)_n$, $(ACT)_n$, $(GATA)_n$ etc. Changes in the number of repeats at a given microsatellite locus result in a change in the length of a PCR product generated by primers 1 and 2 which are homologous to sequences in the unique flanking regions.
(b) The different products can be detected simply, following size separation by gel electrophoresis.

lar markers has identified a number of problems, such as the use of radioisotopes or cumbersome nature of the assays with established molecular technologies, and a need for simple, highly informative approaches has been recognised. Simple sequence repeats (SSRs; Fig. 1) are one molecular approach which satisfies many of the demands of the end-user community. SSR development is both time-consuming and costly but the product is considered to be of sufficiently high value to merit the investment required for their isolation and characterisation. We have adapted a process which involves constructing enriched SSR-containing, small insert genome DNA libraries. These identify clones which contain a microsatellite motif, determining the unique DNA sequences flanking the microsatellites and designing oligonucleotide primers to these sequences which can be used in a PCR to amplify the corresponding sequence from a complex total genomic DNA preparation. However, this is only the first step and is in many respects the easy part. Once this has been achieved, the informativeness of effective primer pairs must be determined by establishing the number and frequency of different alleles detected and then by determining the genetic location of each of the microsatellite loci. This latter step involves following the inheritance of polymorphic

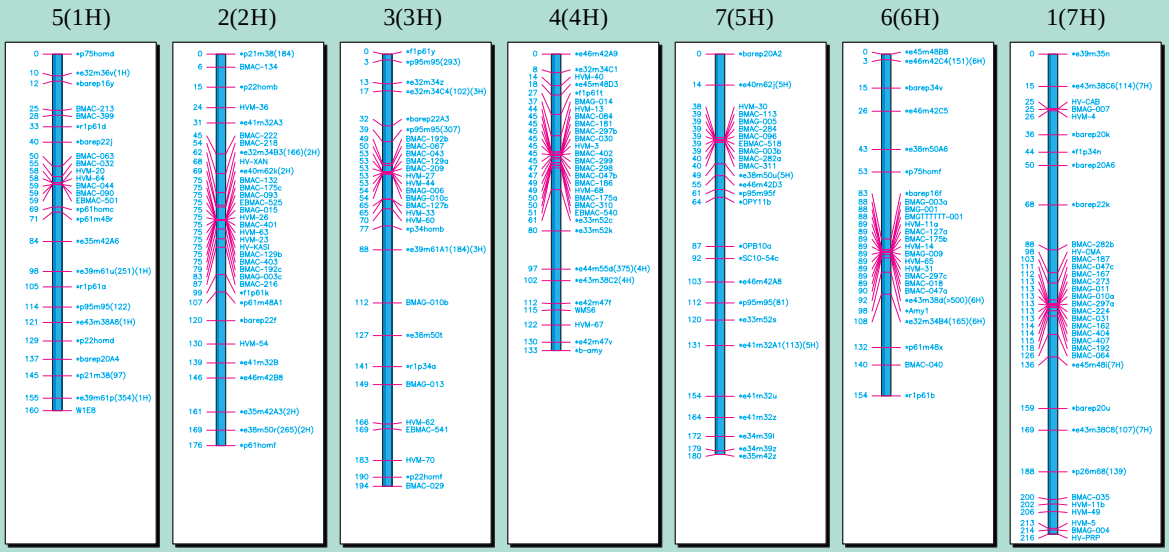


Figure 2 A SSR-based framework map of barley. This figure shows the relative genetic location of SSR markers on the genetic linkage map of barley. Loci on the right of each chromosome have been mapped in the Lina x *H. spontaneum* doubled haploid population. Loci on the left have been mapped in a number of different populations by Liu *et al.* (1996).

SSR loci in a defined plant population and statistical analyses of the data to establish linkage order and chromosomal designation.

To date, we have sequenced over 300 unique clones selected as containing a microsatellite motif. Of these, 11% were identified as false positives and in 12% the SSR motif was too close to the cloning site to design primers. Primers have been designed and synthesised to the remaining 77% of the sequences and these are currently being evaluated as outlined above. To date. around 100 loci have been mapped on to the barley genetic linkage map (Fig. 2). It is envisaged that these microsatellite markers will largely replace the predominant RFLP marker assay which has been the mainstay of contemporary molecular genetics for the last 10 years. Our objective is to obtain >500 informative SSRs for barley which will be made freely available to the academic and research communities in order to facilitate the innovation and successful application of marker-assisted crop improvement.

Reference

Liu, Z.W., Biyashev, R.M. & Saghai-Maroof, M.A. (1996). *Theoretical and Applied Genetics* 93, 869-876.

The use of AFLPs to examine genetic relatedness in barley

R.P. Ellis, J.W. McNicol, E. Baird, A. Booth, P. Lawrence, W.T.B. Thomas & W. Powell

Since 1950, progress in spring barley has been remarkable with a great improvement in farm yield (Fig. 1). In part this is a result of changes in farming practice, with better use of fertiliser and consistent control of plant diseases but of equal important has been the genetic enhancement of the crop by breeders. Techniques of selection and field trialling have been finely honed and now allow efficient identification of superior lines. The performance of a new cultivar is well understood by the time it reaches the farmer's field because it has been subject to a programme of National List Trials, which includes a demonstration that the new line can be uniquely identified.

In contrast, breeders rarely know anything substantial about the genes that they are manipulating. In particular instances of disease resistance, there will be deliberate use of single or multiple genes, but even in such cases, the position and relationships of different resistance genes in the genome are not well known. Improvements in straw strength, yield and malting quality have been made without detailed knowledge of their genetic control, though height reduction has been achieved in spring barley by the use of major dwarfing genes.

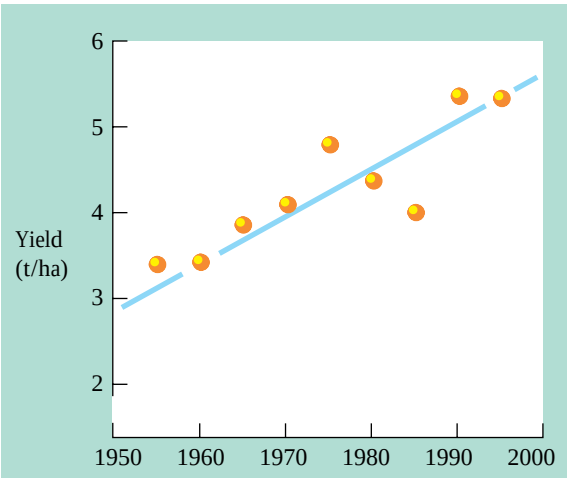


Figure 1 Yield improvement in spring barley 1950-1995.

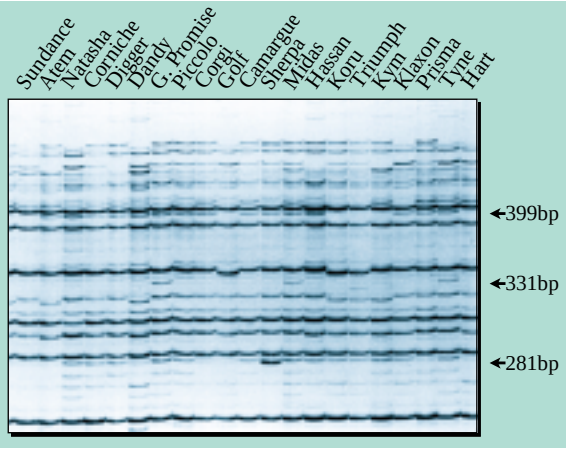


Figure 2 AFLPs showing discrimination between genotypes that allow fingerprints.

Historically, the development of genetic knowledge has fed into plant breeding practice as improvements in techniques. The discovery of genes and their linear assembly into chromosomes in the early 1900s, eventually led to techniques to maximise the reassortment of the genes into new combinations. We have now reached the point at which it is possible to routinely fingerprint barley cultivars (Fig. 2) so that we can begin to understand the detailed interactions that occur at the gene level. This opens up the prospect of manipulating gene by gene interactions to enhance barley performance, and to adapt the crop to new husbandry systems such as conversion to organic farming or to meet the challenges posed by global warming. Detailed understanding of gene regulation leads to the possibility of developing new techniques for genotype development such as transformation.

We have generated genetic fingerprints in a retrospective analysis of the breeding progress in the UK since 1950, using a multi-locus molecular marker technique - Amplified Fragment Length Polymorphism (AFLP). Profiles of 41 barley cultivars uniquely identified all the genotypes, showing the discriminatory power of AFLPs for cultivar identification. Additionally, the linkage relationships between the AFLPs and other genetic markers can be used to create detailed genetic maps of crops such as barley (*Ann. Rep. 1995, 59-62*). A number of the AFLP markers used to screen the 41 cultivars were also segregating in the Blenheim x E224/3 mapping population. Those AFLPs were scored in the Blenheim x E224/3 population and it was shown that six of the seven barley chromosomes

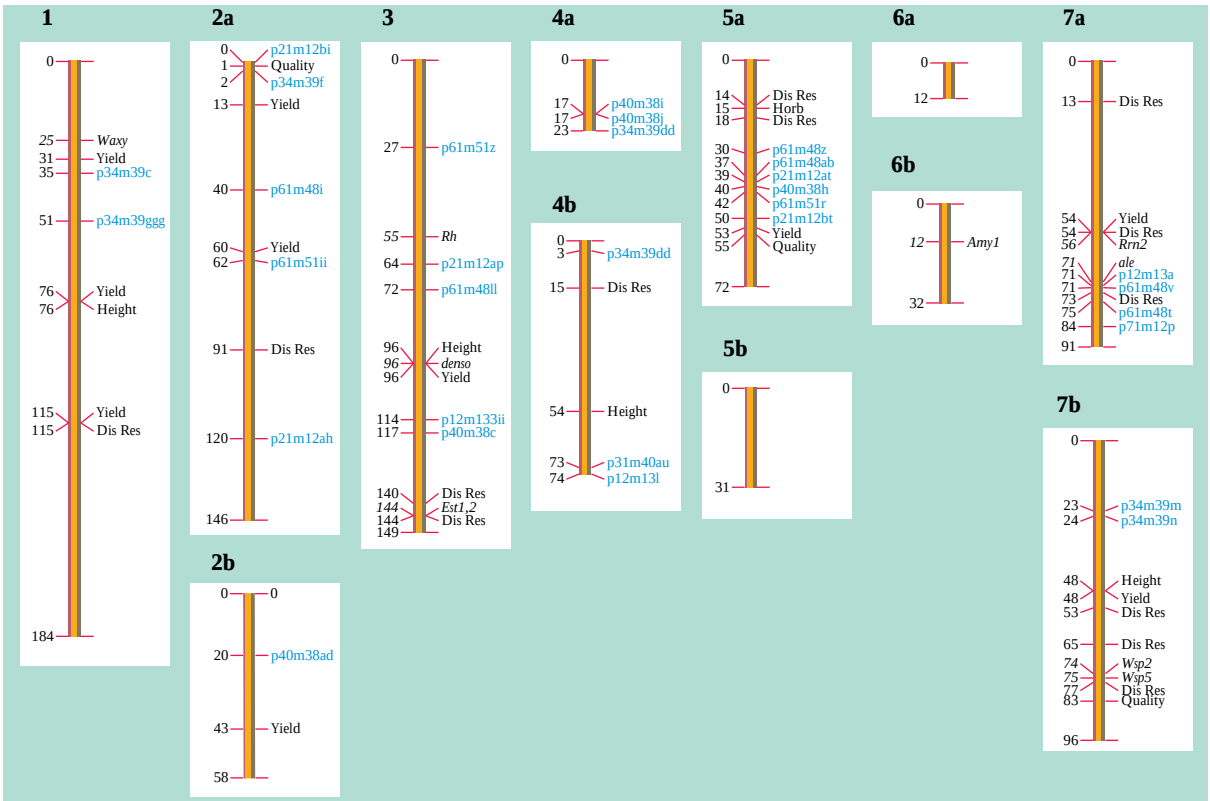


Figure 3 Chromosome map of barley showing the distribution of the AFLPs used to fingerprint selected barleys grown in the UK between 1950 and 1995.

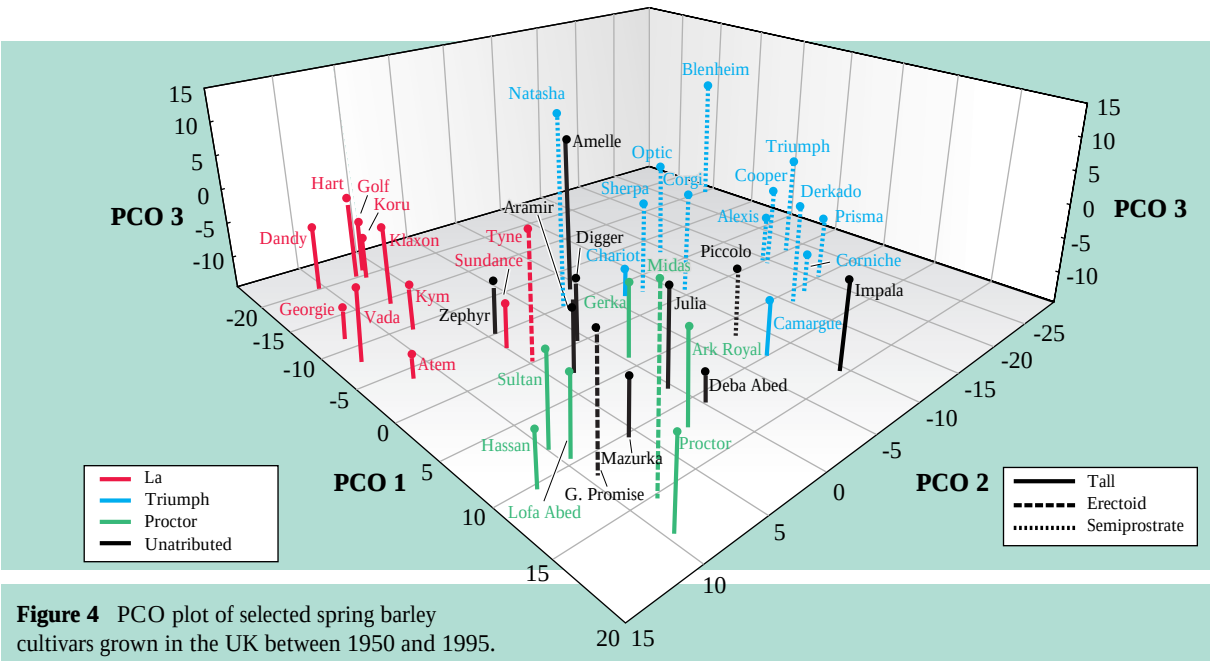


Figure 4 PCO plot of selected spring barley cultivars grown in the UK between 1950 and 1995.

had been assayed in the genome scanning project (Fig. 3). The AFLP based plot (Fig. 4) shows groups based on cultivars widely used as parents in the 1960s and 1970s. Firstly, the attempts to improve the yield of Kenia and Proctor led to poorer malting quality, as seen in Midas and Hassan. The use of the La mildew resistance resulted in the formation of a distinctive group containing high yielding feed types such as Georgie and Sundance. Finally, the introduction of Triumph from Germany and its wide use as a parent, resulted in a sustained increase in malting quality.

Our assay of genetic variability in the UK spring barley crop suggests that there is a possibility of the genetic base of the crop becoming much narrower. In Europe, malting barley breeding has exploited germplasm derived from Triumph and it would appear that a point has been reached where it is necessary to assess the available genetic variability and to determine how important it is to increase diversity. In this context, global environment change will present breeding programmes with serious challenges which require an extension of the range of crop response to biotic and abiotic stresses. We conclude that there is urgent need to continue the analysis of the barley genome with DNA assays, to seek out new genetic variation in *H. vulgare* spp. *spontaneum*, and to develop marker-assisted, selection techniques to transfer efficiently the new genes into the UK crop.

Molecular breeding: applications to barley

W. Powell, W.T.B. Thomas, A. Booth, E. Baird, T. Toojinda[1], P. Hayes[1] & H. Vivar[2]

The integration of molecular breeding technologies with conventional breeding can reduce the timescale of breeding programmes and enhance knowledge of the genetic composition and diversity of breeding genepools. One of the first examples of molecular breeding in barley is the transfer of quantitative trait loci (QTL) determining resistance to stripe rust (*Puccinia striiformis* f.sp. *hordei*).

Barley stripe rust is an important disease and emphasis is being given to adult plant, or quantitative resistance, which is likely to be more durable than race-specific resistance. Its importance in the UK, however, has declined over the past 15 years and the disease is now rare[1,2]. This may reflect good levels of non-specific resistance in current cultivars, or climatic change preventing epidemics of the disease. Genetic analysis of

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[2] ICARDA/CIMMYT, Mexico

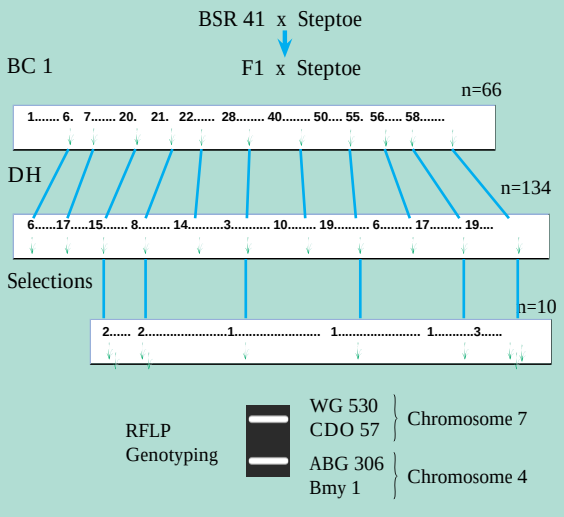


Figure 1 Modified molecular backcross conversion programme for introgression of genes controlling adult plant resistance to stripe rust.

adult plant disease reaction, phenotype data[2], located QTL for stripe rust resistance to chromosomes 4 and 7. Based on this information, a modified backcross conversion strategy was devised (Fig. 1) to introgress stripe resistance into adapted genetic backgrounds. From the first backcross (BC1), a total of 66 genotypes was produced and evaluated with RFLP markers flanking the QTL on chromosomes 4 and 7. These flanking markers can be used to identify individual BC1 genotypes, heterozygous for the targeted genomic region. A total of 11 BC1 genotypes was identified and 134 doubled haploids (DH) were produced from these genotypes, using the *Hordeum bulbosum* technique. These DH were evaluated for stripe rust infection in several environments in Mexico during 1995. In parallel with this field evaluation, each DH was genotyped with AFLPs. The objective of this phase of experimentation was to identify recombinant individuals that have genome compositions significantly better than would be predicted from theoretical expectations. A total of 111 polymorphic AFLP products was identified and an example from one *Eco*RI/*Mse*I primer combination is shown in Figure 2.

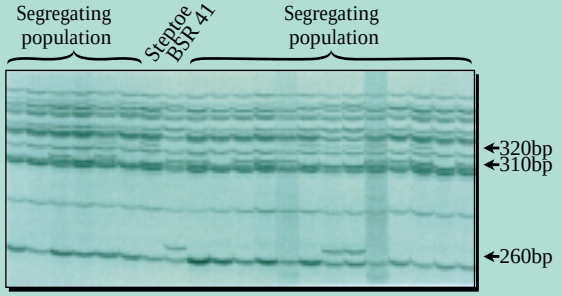


Figure 2 AFLPs showing discrimination between genotypes that allow fingerprints.

High multiplex assays such as AFLPs, are particularly useful for recurrent or donor parent analysis and this is illustrated in Figure 3 for the 134 BC1 DH genotypes. The percentage donor DNA composition varies from 7% to 60%, which illustrates the spectrum of genotypic values obtained and emphasises the benefits of applying a genetic analysis to introgression programmes. Thus, selection of DH line 39 with 7% donor DNA would be equivalent to advancing this population to a BC3 generation. Furthermore, reduction in the number of individuals processed in a backcrossing programme would reduce the cost associated with further testing and evaluation.

This data also provided an opportunity to search for associations between AFLP products and stripe rust resistance. For this purpose, the frequency of each AFLP product arising from the donor parent (BSR 41) was determined in each of the 11 BC1 DH families. This data was then regressed against mean stripe rust infection scores for each BC1 DH family to identify significant ($P<0.05$) marker:phenotype associa-

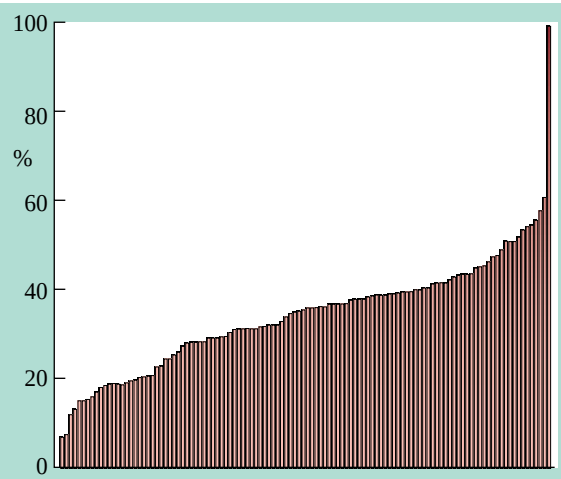


Figure 3 Percentage donor DNA determined by AFLP analysis in 113 BC1 DH genotypes.

AFLP product	Chromosome	R^2(%)	$\hat{b}$ [2d]*
e3850j	7	43	-31.0±12.0
e36m33a	3	40	-27.4±11.3
e41m33i	?	43	39.4±15.0

* A negative number indicates that BSR 41 possesses the resistant allele

Table 1 Association of AFLP products with percentage stripe rust infection.

tions. Eight AFLP products were identified that exhibited significant associations with stripe rust sensitivity and three of these are shown in Table 1. The regression co-efficient (R^2) provides a measure of the total phenotypic variation accounted for and the regression slope ($\hat{b}$) estimates the effect of allelic substitution at the locus defined by the AFLP product. Furthermore, the slope of the regression co-efficient ($\hat{b}$) indicates that the donor genotype (BSR 41) is contributing both resistant and susceptible QTL alleles.

In conclusion, we have deployed both single locus and multi-locus molecular assays in conjunction with doubled haploidy, to transfer loci determining quantitative inheritance to stripe rust, into an adapted genetic background (cv. Steptoe). During the course of this molecular backcross conversion programme, we have also identified AFLP products linked to genes controlling stripe rust resistance and enhanced our understanding of the genetical control of polygenic forms of resistance to this disease.

References

1 Meadway, M.H. & Hutton, W.C. (1996). *UK Cereal Pathogen Virulence Survey Annual Report for 1995.*

2 Chen, F., Prehn, D., Hayes, P.M., Mulrooney, D., Corey, A. & Vivar, H. (1994). *Theoretical and Applied Genetics* 88, 215-219.

Locating genotypes and genes for abiotic stress tolerance in barley: maps, markers and the wild species

B.P. Forster, H. Pakniyat, R.P. Ellis, L.L. Handley, D. Robinson, C.M. Scrimgeour, C.A. Hackett, R. Keith, D. Gordon & W. Powell

Barley (*Hordeum vulgare* L.), is one of the oldest crops, being first cultivated some 8,000 to 10,000 years BC. The wild progenitor is *Hordeum spontaneum* C. Koch, which has its centre of diversity in the Fertile Crescent of the Middle East. Wild barley is able to colonise a wide range of habitats, from high rainfall to desert, from cool to hot areas, and from sub-sea levels to altitudes in excess of 1,700 metres. Both *H. vulgare* and *H. spontaneum* are diploid species, carrying seven pairs of chromosomes, and there is no biological barrier to crossing or meiotic recombination between them. Consequently, there is great interest in exploiting the rich genetic variation of the wild species for crop improvement.

We have used 39 genotypes of wild barley from three geographically separated areas of the Fertile Crescent: Israel, Turkey and Iran (Fig. 1). Ecogeographic data were collected from each site-of-origin and subjected to principal component analysis (PCA). The PCA

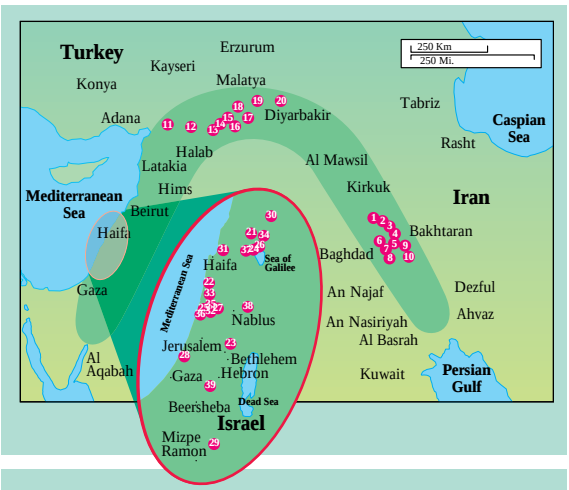


Figure 1 Location of wild barley populations used.

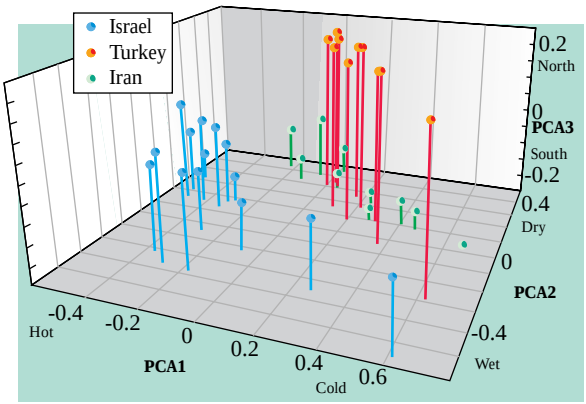


Figure 2 PCA plot of wild barleys from Israel, Turkey and Iran.

plot (Fig. 2) shows that the sites from Israel, Turkey and Iran form separate clusters. The first axis, PCA1, accounts for almost 50% of the variation, and separates high altitude and cold sites, from those of low altitude and hot sites. PCA2 accounts for about 30% of the environmental variation. The components in PCA2 are easterly longitudes, low humidity and low rainfall *versus* more westerly longitudes, high humidity and high rainfall. PCA3 accounts for approximately 10% of the variation and is essentially a North/South divide which separates the Turkish sites from those of Iran. PCA is useful, therefore, in identifying sites covering a range of abiotic stresses which can be selected for study. By traversing PCA1, genotypes can be identified which are adapted to cold growing conditions (e.g. high frost tolerance and marked vernalisation requirement) or hot conditions. Variation for drought tolerance is expected along the PCA2 axis, and daylength response along the third PCA axis (North/South divide). These three axes thus separate genotypes carrying major traits of interest to plant breeders in adapting and improving cultivated barley to specific agricultural conditions around the world.

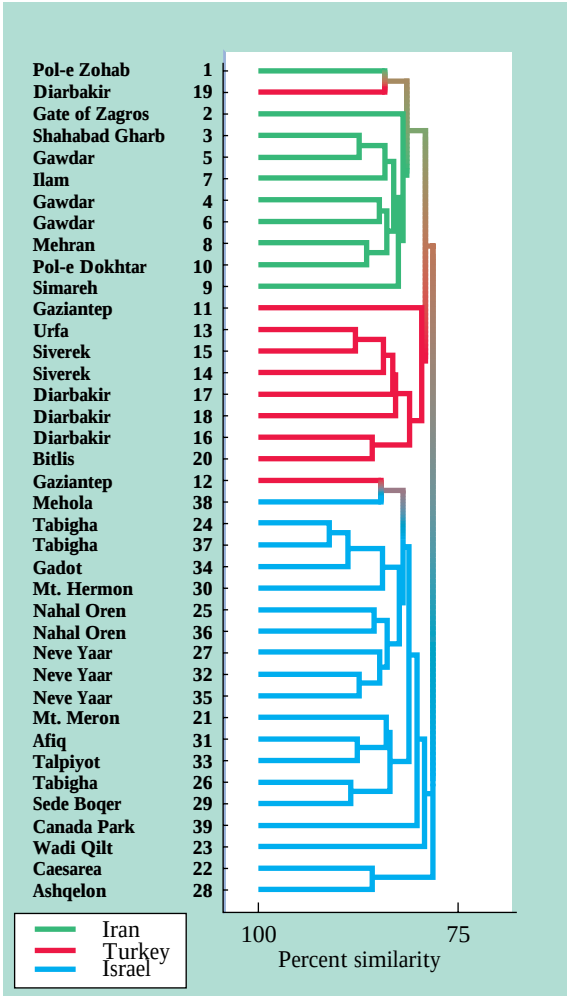


Figure 3 Dendrogram of wild barleys from Iran, Turkey and Israel based on average linkage cluster analysis.

Of the 39 lines, 30 were genotyped using amplified fragment length polymorphisms (AFLPs). Using 12 primer combinations, 204 polymorphic bands were found which uniquely fingerprinted each genotype. The genetic diversity is illustrated in a dendrogram (Fig. 3). With two exceptions (lines 12 and 19), all of the wild barleys from Israel are clustered together and are distinct from those of Turkey and Iran. As might be expected, the most similar lines are those collected from the same site-of-origin; Tabigha 24 and 37 had 91% similarity. The greatest difference was found between Ashqelon 28 (Israel) and Gwadar 5 (Iran) which share only 70% of AFLPs. Although Tabigha 24 and 37 were very similar, a third genotype from the Tabigha site (26) was more distinct. This finding is interesting because Tabigha 26 was collected from the same 100 metre transect as

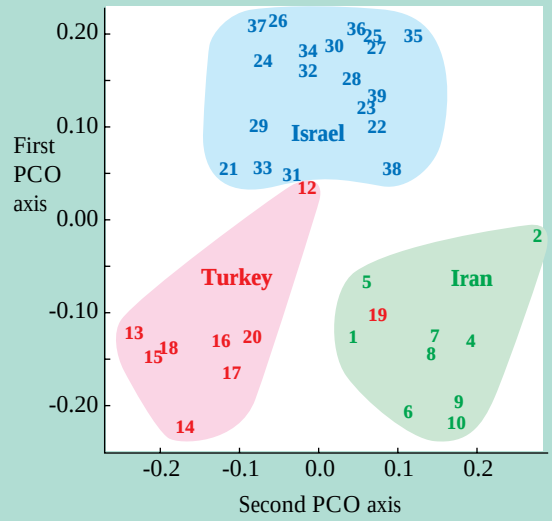


Figure 4 Principal co-ordinate plot of wild barleys from Iran, Turkey and Israel based on AFLP.

the other Tabigha genotypes. However, the transect incorporates two markedly different soil types. Tabigha 24 and 37 were collected from terra-rossa soil, whereas genotype 26 was collected from basalt soil. There is, therefore, some evidence that genetic variation within wild barley populations is associated with local ecological variations.

The AFLP similarity data were also used to generate a principal co-ordinate (PCO) plot (Fig. 4). With the exception of the two odd genotypes mentioned above, PCO1 separates the wild barley lines of Israel from those of Turkey and Iran. The latter two areas are separated by PCO2. These data show that the wild barleys from Iran are genetically more closely related to those from Turkey than to those from Israel. Significantly, the genetic fingerprinting data plotted as a PCO, mirrors that of the ecogeographic PCA plot (Fig. 2). Thus the opportunity exists to relate specific AFLP fingerprints with abiotic stressors and site-of-origin ecogeography. We have demonstrated this for salt tolerance in 30 fingerprinted lines.

The lines were grown for 4 weeks in a saline, hydroponic system containing 100 mol m^{-3} salt (NaCl). Salt tolerance was measured in terms of shoot Na^+ content and the natural abundance of the carbon isotope ^{13}C ($\delta^{13}C$) in shoots, after 4 weeks of stress. Salt tolerance in barley is known to be associated with low shoot Na^+ accumulation and more negative $\delta^{13}C$ values. As expected, these were highly correlated in the study. Of the 204 polymorphic AFLP bands, 17 were found to be significantly associated with shoot Na^+ and $\delta^{13}C$ ($p<0.05$). Ideotypes combining the most favourable and the most unfavourable combinations of AFLPs, are compared with those of the most salt-tolerant and -susceptible genotypes determined by experimentation (Fig. 5). In the most salt-tolerant line, Ilam 7, five of the 17 AFLP markers are associated with salt tolerance. The most salt-susceptible line, Tabigha 26, has the AFLP fingerprint of the salt-susceptible ideotype, but with two miss-matches. Thus, it is possible with these 17 markers to seek even more tolerant (and susceptible) genotypes in the wild barley genepool.

The AFLPs associated with salt tolerances, were themselves associated ($p<0.05$) and formed three subgroups; bands 2, 7 and 9 were significantly associated, as were bands 6, 8 and 11, and bands 3 and 4. AFLP bands 7 and 11 were found to have the greatest individual effects accounting for 32% and 27% of the variation for shoot Na^+ respectively (with similar effects on shoot $\delta^{13}C$). Together, they accounted for over 53% of the variation. Further stepwise regression showed that markers 7, 11 and 10 account for 60.7% of the variation for shoot Na^+, and 62.6% of the variation for shoot $\delta^{13}C$.

The analysis was then taken further to test whether the AFLP bands associated with salt tolerance were

AFLP marker (bp)	Salt tolerant Ideotype	Salt tolerant Ilam	Salt sensitive Ideotype	Salt sensitive Tabigha
P17M62(194)	-	-(✓)	+	+(✓)
P17M62(93)	-	-(✓)	+	+(✓)
P16M47(170)	-	+(✗)	+	+(✓)
P95M95(175)	-	-(✓)	+	+(✓)
P26M68(266)	-	-(✓)	+	+(✓)
P26M68(100)	-	-(✓)	+	+(✓)
P46M53(145)	-	-(✓)	+	+(✓)
P46M53(106)	-	-(✓)	+	+(✓)
P25M50(249)	-	+(✗)	+	-(✗)
P17M62(137)	+	+(✓)	-	-(✓)
P55M38(345)	+	+(✓)	-	-(✓)
P55M38(304)	+	+(✓)	-	-(✓)
P55M38(186)	+	+(✓)	-	-(✓)
P95M95(395)	+	+(✓)	-	+(✗)
P95M95(307)	+	+(✓)	-	-(✓)
P46M53(296)	+	+(✓)	-	-(✓)
P21M38(135)	+	+(✓)	-	-(✓)

Negatives and positives indicate relative effects of the presence of the band

Figure 5 Ideotype of the most salt-tolerant and salt-sensitive AFLP marker combinations compared with the two extreme wild barley genotypes.

also associated with site-of-origin ecogeography. Of the 12 tested, eight were associated with longitude, three with humidity and two with mean August temperature (Fig. 6). In a stepwise regression on all the ecogeographic variables, longitude was found to have the largest effect on shoot Na^+ and $\delta^{13}C$. The inference is that salt tolerant, wild barleys tend to originate from the south-eastern portion of the Fertile Crescent. This is expected because the climate becomes warmer and drier, and the soils more saline as one travels south and east from the Mediterranean. The south-eastern portion of the Fertile Crescent is, therefore, a target site for collecting wild barleys for crop improvement for salt tolerance, and markers are now available to aid selection.

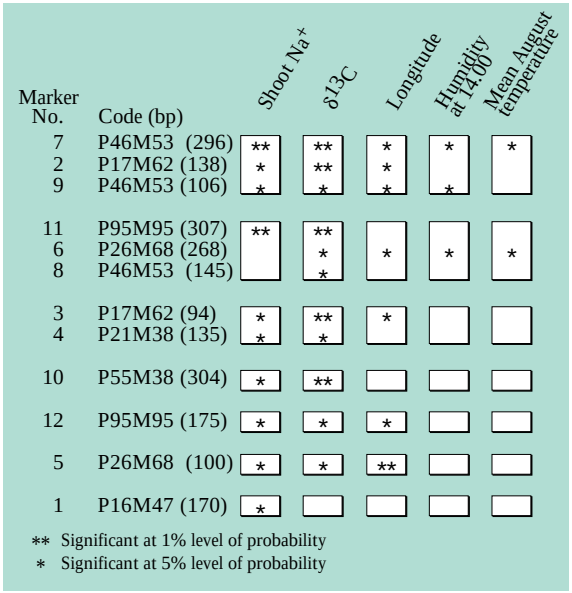


Figure 6 AFLPs associated with shoot Na^+ and $\delta^{13}C$ and their associations with site-of-origin ecogeography.

The results between the populations of the Fertile Crescent were also reflected within some populations. For instance, variation for salt tolerance was found among the three individual genotypes from the Tabigha site. The two genotypes from the terra-rossa soil were significantly more salt tolerant that the genotype from basalt soil (which was the most salt-susceptible line tested) (Fig. 5). Terra-rossa soil is much drier than basalt soil, and the rapid summer drying of terra-rossa soil makes it more saline than basalt soil. The phenology of barley plants growing on terra-rossa soil is also markedly different from neighbouring basalt plants. They mature 2-3 weeks earlier than the former. AFLPs therefore have potential in defining ecotypes within barley populations.

We are currently mapping AFLPs and locating genes controlling tolerance to salt. We have, therefore, tested a barley genetic mapping population for tolerance to salt. This population, a set of doubled haploids, was derived from a cross between a salt-susceptible, barley cultivar and a salt-tolerant wild barley. AFLP markers associated with shoot Na^+ and $\delta^{13}C$ have been located. As part of this work, we are very interested to see if the AFLP markers associated with salt tolerance are genetically linked; i.e. do markers, identified by population associations, reflect linkage disequilibrium of AFLP markers, determining tolerance to stress? By combining genetics with physiology, we are beginning to identify genetic markers which are associated with specific physiological traits. By mapping these markers, it is possible to identify genomic regions and identify genes of specific physiological/biochemical function involved in the trait, in this case salt tolerance. Similar work is being conducted on tolerance to drought, low nitrogen supply, frost, aluminium and hypoxia.

BarleyDB - a new genome database

L. Cardle & R. Waugh

A new project to construct a genome database for barley has begun under the BBSRC Plant and Animal Genome Analysis special initiative. The project is part of the UK Crop Plant Bioinformatics Network (UK CropNet)

which aims to construct several specialist databases for a number of plant species, and provide high level training in bioinformatics. Emphasis has been placed on the development of tools to facilitate comparative genome analysis and chromosome structure.

At the SCRI, the main objective is to take information currently in lab books, ledgers, and on various computers and integrate it into a useful tool to assist crop breeders and molecular biologists.

The new database, BarleyDB (Fig. 1), will be accessible to researchers within the SCRI and will be made available to the wider scientific community through the World-Wide Web. Information will also be deposited in the UK CropNet Node at the Nottingham Arabidopsis Stock Centre, and from there exchanged with the Graingenes database at Cornell. The database is being constructed using ACEDB software, and will be compatible with Graingenes.

The main forms of data in the database include:

- Molecular marker linkage information, currently available for a number of populations with *c.* 100 to over 1000 markers per population.
- Phenotypic trait scores collected over several seasons and at several locations for these populations.
- Genotypic information on a wide range of representatives from the primary gene pool.
- A molecular marker-derived genotypic database of barley cultivars.
- Genotypic information on wild barley populations and associated ecogeographical data.
- Molecular and traditional pedigree information.
- Images of autoradiograms and phenotypes.

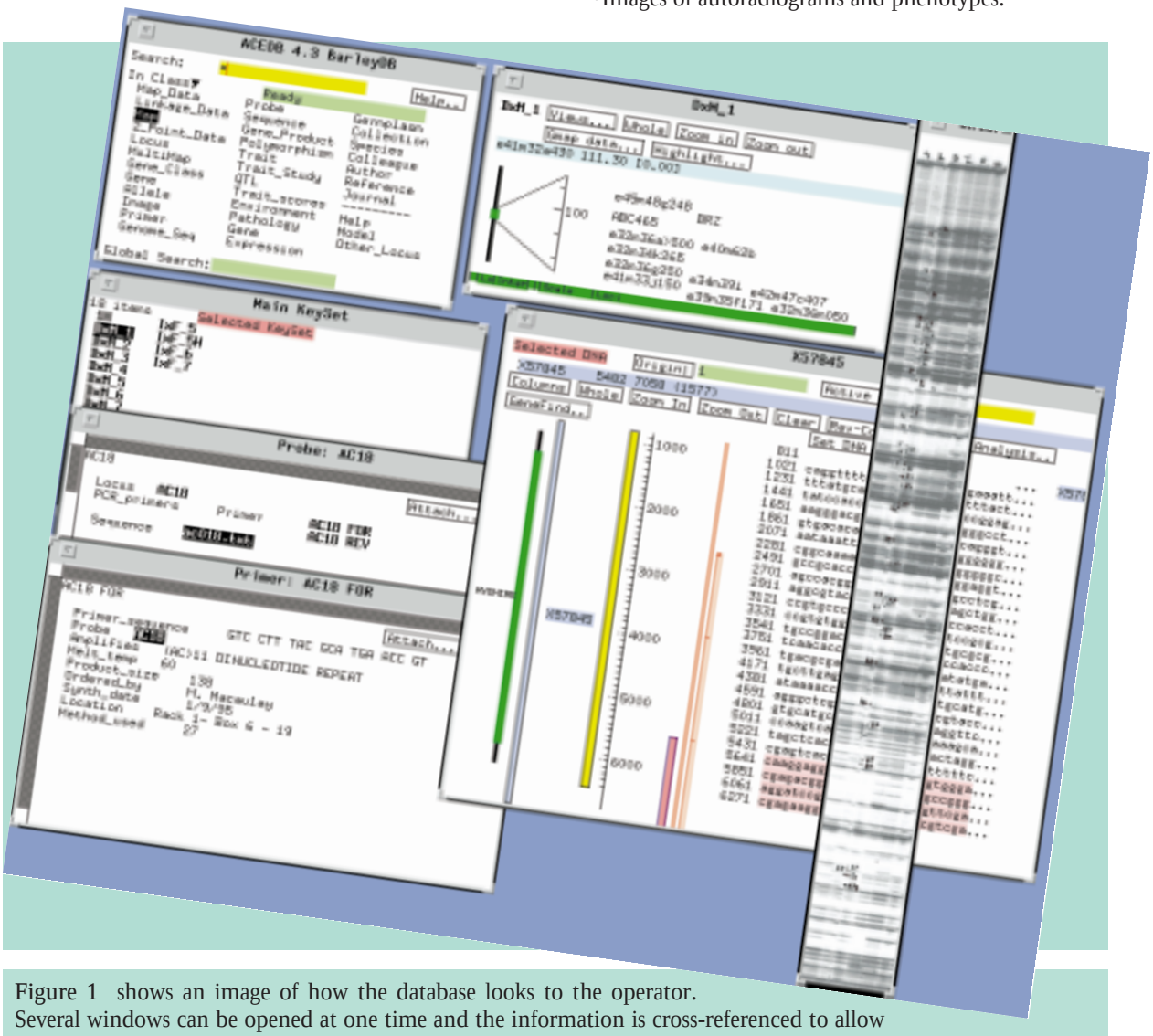


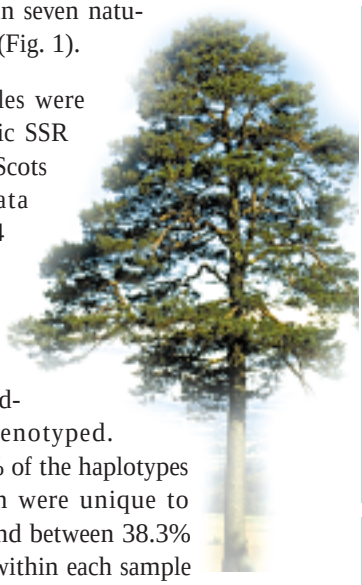
Figure 1 shows an image of how the database looks to the operator. Several windows can be opened at one time and the information is cross-referenced to allow the data to be browsed through easily. The information is manipulated through 'models', which define how the pieces of data should be linked together within the database. These models are being written and modified to allow the structure of the database to be altered and accept new types of data. The overall structure of BarleyDB has been created and is now operational.

Chloroplast simple sequence repeats: applications to the population genetics of Scots pine

J. Provan, N.J. Wilson, N. Soranzo, J.W. McNicol & W. Powell

The native populations of Scots pine (*Pinus sylvestris*) in Scotland represent the only remaining natural woodland in the UK. As a result of excessive exploitation and mismanagement, only a few dozen small woodlands covering less than 11,000 ha remain. Recently, the historic, scientific and recreational importance of these woodlands has increased and the conservation of native pinewoods has aroused both public and scientific interest. The most important issue in the conservation of present-day populations of native Scots pine is to maintain high levels of population diversity whilst protecting the genetic integrity of the species. Knowledge of the genetic structure of existing populations should form the basis for a rational and sustainable conservation programme.

Current legislation on the management of Scots pine is based on 15-year old monoterpene data. Since these guidelines were established, more advanced molecular techniques for the quantification of genetic variability have become available. DNA-based polymorphic assays provide a more detailed means of genetic analysis of both individuals and populations, and foremost among these are techniques based on the polymerase chain reaction (PCR). We have used PCR amplification of chloroplast simple sequence repeats (cpSSRs; *Ann. Rep. 1995, 57-59*) to study the levels of genetic variation in seven natural *P. sylvestris* populations (Fig. 1).



Between two and six alleles were detected at 17 polymorphic SSR loci in the chloroplast of Scots pine. The combined data from these loci gave 174 haplotypes in the 330 trees studied, of which 124 (72%) were unique. This means that 38% (124/330) of the trees studied could be uniquely genotyped. Between 47.1% and 80.0% of the haplotypes found in each population were unique to that particular woodland and between 38.3% and 63.8% of individuals within each sample

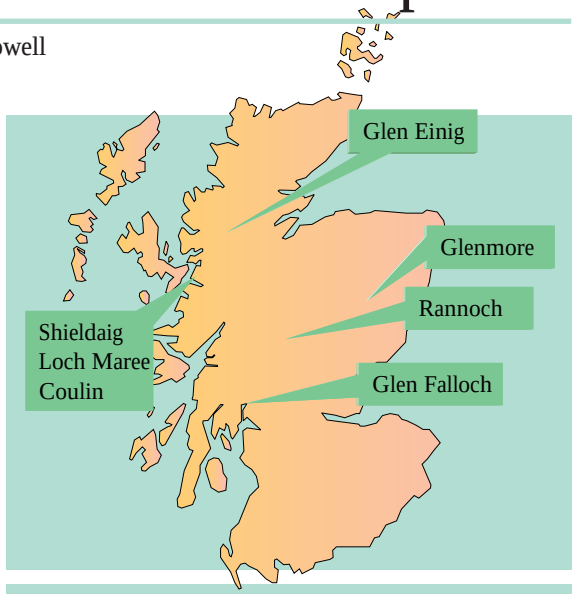


Figure 1 Native Scots pine *(P. sylvestris)* populations studied using cpSSRs.

contained population-specific haplotypes. The distribution of cpSSR haplotypes suggests that, at the haplotype level, cpSSRs offer potential for the identification of population-specific markers. Diversity values based on haplotype (0.930-0.968), are much higher than earlier monoterpene (0.272-0.378) and isozyme (0.291-0.311) analyses carried out on the same populations, highlighting the high resolving power of cpSSRs in genotyping studies. Haplotype data are summarised in Table 1.

Population	Number of haplotypes	Unique haplotypes	Population specific haplotypes	% individuals with population specific haplotypes	$\hat{H}$
Glen Falloch	30	24	24	43.8	0.930
Rannoch	34	26	16	40.4	0.967
Coulin	35	27	19	44.7	0.967
Shieldaig	33	22	19	59.6	0.963
Glen Einig	35	30	23	55.3	0.968
Loch Maree	36	28	17	38.3	0.965
Glenmore	37	30	26	63.8	0.966
Total	174	124	144	42.8	0.982

Table 1 Diversity values ($\hat{H}$) in *P. sylvestris* populations based on haplotype frequencies.

Variance component		Observed partition Variance	%	Φ-statistics
Among groups	$\sigma^2(a)$	0.0029	0.58	Φ_{CT} = 0.006
Among populations within groups	$\sigma^2(b)$	0.0014	0.28	Φ_{SC} = 0.003
Within populations	$\sigma^2(c)$	0.4890	99.14	Φ_{ST} = 0.009

Table 2 Hierarchal analysis of variance showing partitioning of total diversity within and between populations of *P. sylvestris.*

Hierarchal analysis of variance using Φ-statistics, showed that >99% of the total observed variation exists within, rather than between, populations (Table 2). This suggests extensive gene flow between the populations, but since they would appear to be isolated by distance, it is more likely that these figures reflect the historical existence of a single continuous population covering the present limits of distribution in which levels of gene flow were extensive. The fact that population differentiation through allele fixation has not occurred to any great degree, coupled with the long generation time of *P. sylvestris*, suggests a relatively recent fragmentation of the present day populations.

Since SSR loci undergo bi-directional mutation, allelic distribution at these loci generally exhibit an approximately symmetrical, uni-modal distribution. At one locus, however, we detected an unusual rare allele (Fig. 2). The bi-modal, allele distribution at this locus (108/109 bp *vs.* 116 bp) and the absence of intermediate allele sizes, probably suggests that the 116 bp allele has not evolved naturally. Of the 13 trees containing the 116 bp allele, 10 were found in the Wester-Ross populations (Shieldaig, Loch Maree and Coulin). These trees could be descended from non-native material, possibly introduced into the region in small numbers. Alternatively, this allele may be indicative of a separate refugial origin of the Wester-Ross group of populations. We are presently comparing the occurrence of this allele in native populations with samples from non-native provenances to establish if this is diagnostic of one or more European populations of *P. sylvestris*.

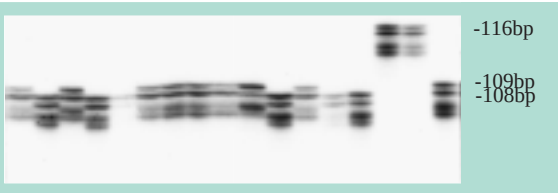


Figure 2 An atypical cpSSR polymorphism detected at locus PCP36567.

Principal co-ordinate analysis was used to analyse levels of intra-population, genetic structuring within each woodland. In general, the accessions within a woodland formed a single major cluster. The exception to this was the Rannoch population, which was clearly divided into two sub-groups (Fig. 3). The reasons for this are not immediately apparent since there is no spatial or age correlation between the trees in each sub-group. It is possible that these two sub-groups represent two original progenitors or seed lots, since it has been documented that plantations were often located in close proximity to native stands and that gene flow could occur between the two. Alternatively, it has been suggested that the Rannoch woodland was replanted in the last few hundred years using material from a different population, or that biased seed collection from a restricted number of trees was used as a source of planting material. Any of these scenarios could give rise to this apparent genetic sub-structuring.

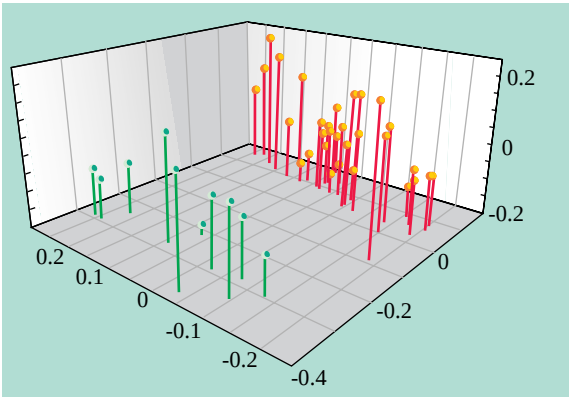


Figure 3 Apparent genetic substitutioning in the Rannoch population.

We have shown that cpSSR polymorphism can be used to analyse diversity in the chloroplast genome of *P. sylvestris*. In addition, multiple cpSSR loci can be analysed to give haplotypes which may have value as population-specific markers. These insights into the history and genetic architecture of our native pinewoods represent an invaluable asset when considering future legislative decisions concerning the management of existing populations of Scots pine.

Conservation genetics of a tropical tree: mahogany (*Swietenia humilis* Zucc.)

G. White, A. Booth & W. Powell

Swietenia humilis Zucc. (Meliaceae) is one of the three important tropical hardwood species, commonly known as mahogany, belonging to the genus *Swietenia*. *S. humilis* (Fig. 1) is indigenous to the Pacific coast of Mexico and Central America. Due to high deforestation rates in its native range, it is considered an endangered species and was listed in Appendix II of the Convention on International Trade in Endangered Species (CITES) in 1973, becoming the first hardwood tree of commercial significance to have been listed. To develop a strategy for the sustainable management and conservation of mahogany, information on the level of gene flow, genetic variation and extent of genetic differentiation within and between natural populations is vital. The development of microsatellite markers, also known as simple sequence repeats (SSRs), for *S. humilis* provides a tool to fulfil such criteria.

We have developed a method of constructing an enriched microsatellite library for *S. humilis*. This method is based on a pre-cloning enrichment of SSRs, using synthetic oligonucleotide probes bound to magnetic beads and hybridising to complementary microsatellite core sequences in digested genomic DNA. This enrichment protocol increases the efficiency of microsatellite isolation compared to previous methods of isolation from tropical tree species by 80-125%, thus representing a very efficient and successful procedure. Ten of the developed SSR loci have been used to study genetic diversity within a natural population of *S. humilis*; an area of 55 ha located in the dry forests at Punta Ratón, Choluteca, Honduras. This population is bisected into two habitat terrains; forest and pasture, and the level of sub-population differentiation is determined by measuring levels of heterozygosity and allelic diversity.

Figure 1 *Swietenia humilis* from a population at Choluteca, Honduras.

A total of 97 alleles were identified among the 88 individuals in the population, with an average of 10

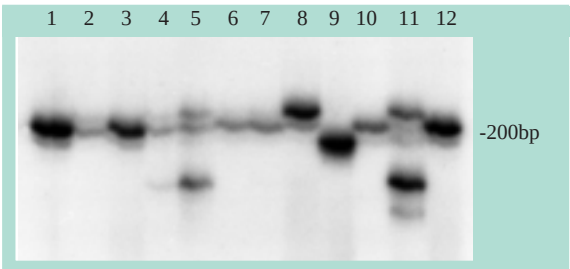


Figure 2 Allelic variation revealed at a single SSR locus, MAC44; an $(AC)_{13}$ repeat in a subset of 12 trees from a population at Choluteca, Honduras.

alleles over all loci. Very high levels of allelic polymorphism were detected at individual loci, with 23 alleles observed at the most variable, indicating the informativeness of SSRs as genetic markers for population studies. An example of allele polymorphism is shown in Figure 2. The mean observed heterozygosity was 0.346 (range of 0.038-0.815), exceeding levels of diversity detected in related species which used isozymes as the marker system. Sub-population differentiation at a micro-geographical scale was low (F_{st}=0.052) and probably reflects extensive gene flow occurring between individual trees.

In addition, the ability of these microsatellites to amplify products in 13 related Meliaceae species was tested. Cross-species amplification was observed, indicating that a high level of sequence conservation exists within the primer regions of the tested species, thus demonstrating the potential of using these heterologous microsatellite primers in investigations into other ecologically and economically interesting timber species in the Meliaceae family. We are currently using the SSRs to assess the levels of genetic variation and extent of gene flow in relation to forest fragmentation in natural populations of *S. humilis* to endeavour to understand the effects of forest disturbance on the spatial pattern and structure of genetic variation; vital information required for the sustainable management of existing, disturbed mahogany stands.

Simple sequence repeat marker location on a genetic linkage map of potato

R.C. Meyer, D. Milbourne, A.J. Collins, L.D. Ramsay, C. Gebhardt[1] & R. Waugh

Simple sequence repeats (SSRs) or microsatellites, are tandemly repeated DNA motifs such as $(AC)_n$ or $(ATT)_n$ that are highly polymorphic and abundant in vertebrate and plant genomes. They appear to be randomly distributed throughout the genome and occur intra- and inter-genically. They can be analysed by PCR with primers specific to the sequences flanking the microsatellite and generally behave as co-dominant markers. Because of their ease of use and distribution, SSRs are rapidly becoming a reference marker system in many species. We are isolating and characterising SSRs from potato for application in a wide range of genetical studies. To date, SSRs have been developed from a number of sources. First, the

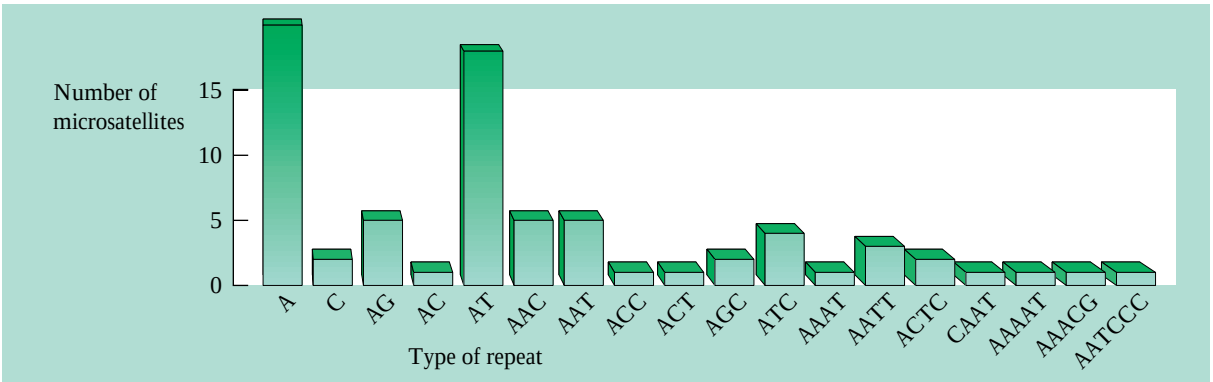


Figure 1 Microsatellites in potato database sequences. Seventy one microsatellites were detected in potato genes in the EMBL database. A/T based microsatellites account for 60% of the repeats found in the database.

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	No. of SSRs	Primers designed	Primers tested	Product agarose	Polymorphic polyacrylamide	Monomorphic polyacrylamide
Potato sequences	138	122	105	93	78	14
Tomato sequences	17	11	7	7	2	2
Total	155	133	112	100	80	16

Table 1 Microsatellites identified in database and libraries.

EMBL database was searched for sequences that contain microsatellites from Solanaceous species. The 'findpatterns' program from the GCG package was used with a data file of all possible mono-, di-, tri-, tetra-, penta-, and hexa-nucleotide repeats: $(N)_{15}$, $(NN)_7$, $(NNN)_5$, $(NNNN)_4$, $(NNNNN)_4$, $(NNNNNN)_4$. The database searches identified SSRs in the translated and untranslated regions of potato genes. A total of 71 SSRs was detected with A/T based repeats accounting for 60% (Fig. 1). Based on this information, we screened a potato cDNA library for SSRs, and constructed genomic, small-insert, libraries enriched for GT or AG repeats by ssDNA hybridisation and by triplex affinity capture. Overall, 155 unique SSRs have been identified (Table 1). Primer pairs have been designed to 133 sequences, and so far 112 have been tested in three potato populations, of which 89% amplified the correct product. Of these, 80 SSRs were poly-

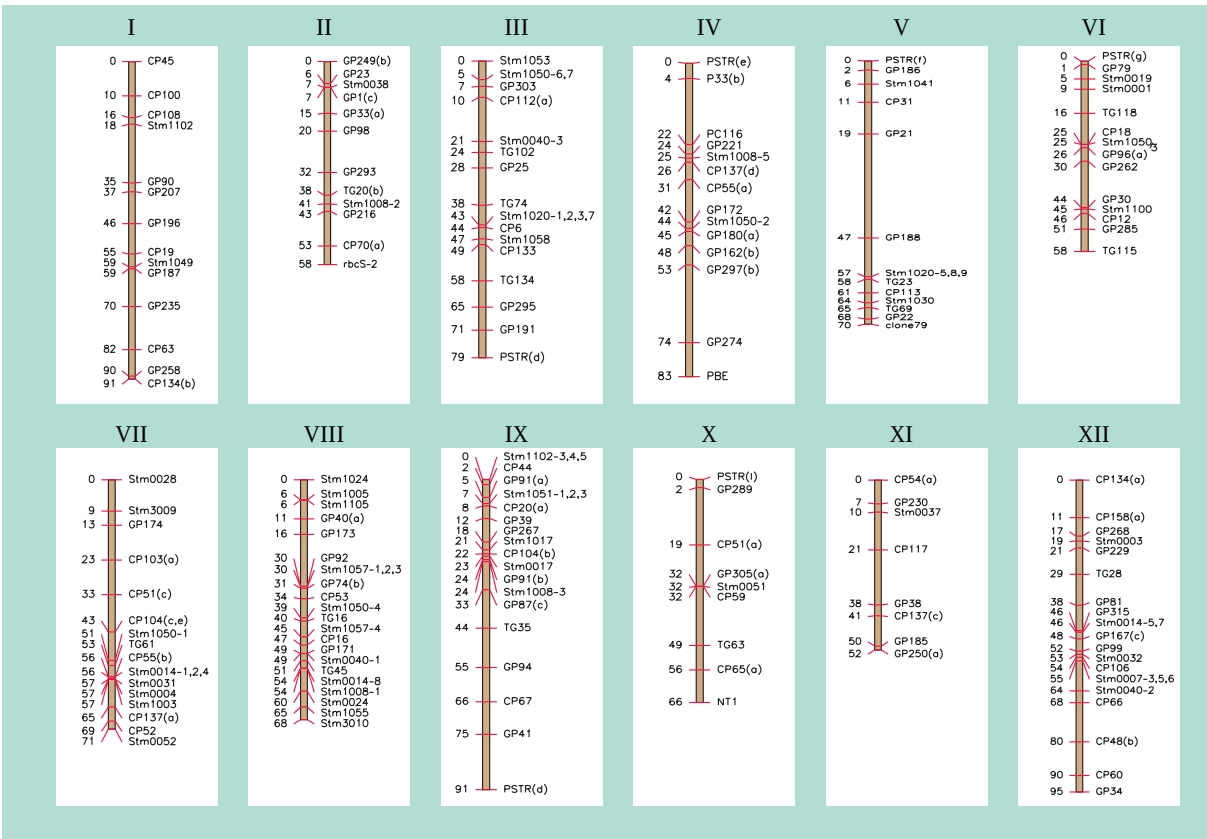


Figure 2 SSR anchors (Stm = *Solanum tuberosum* microsatellite) on a diploid RFLP reference map.

Primers derived from	Primer number tested	DNA: Potato	Tomato	Tobacco	Petunia	Aubergine	Pepper
Potato	18	18	11	12	12	9	5
Tomato	5	4	5	4	5	5	4
Tobacco	1	1	1	1	1	1	1
Petunia	1	1	1	0	1	0	0

Table 2 Interspecies amplification of microsatellites. The table indicates the number of primer pairs that amplify correct size products in various Solanaceous species as revealed by agarose gel electrophoresis.

morphic and 16 monomorphic in six surveyed accessions. As the first aim was to construct an SSR base anchor map in a reference population, a diploid population previously used to construct a detailed RFLP map of potato[1] was chosen for this purpose. This population is a backcross between heterozygous parents [(L9 x L16) x L16] and contains 70 lines. Forty-two polymorphic primer pairs which amplify a total of 66 loci, have been examined to date. Of these, 49 loci have been integrated with the existing RFLP map data using Joinmap 2.0 (Fig. 2). The number of SSR loci per linkage group varies from 1-10, and each linkage group contains at least one SSR locus. The level of polymorphism of the mapped SSRs was then examined in two further populations. The high number of shared polymorphic markers confirms that the SSRs mapped in the diploid reference population can be effectively deployed in other populations at high frequency. Finally, database comparisons showed that sequences within related species are often highly conserved. To evaluate the potential of interspecies amplification of SSRs, we tested 25 potato, tomato, tobacco and petunia-derived SSR primer pairs on a panel of Solanaceous species (Table 2). The results indicate that a number of the SSRs can also be used in related species.

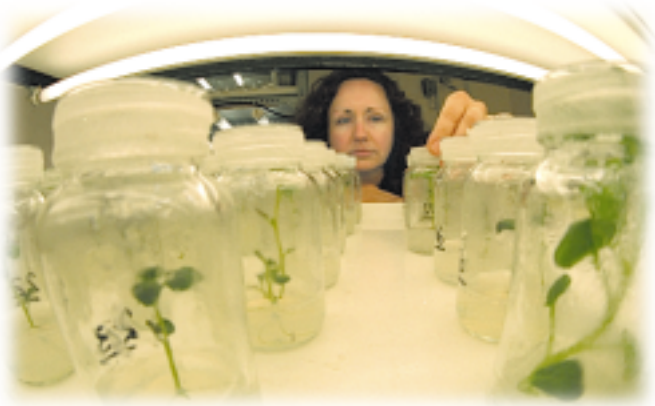
Reference

1 Gebhardt, C., Ritter, E., Debener, T., Schachtschabel, U., Walkemeier, B., Uhrig, H. & Salamini, F. (1989). *Theoretical and Applied Genetics* 78, 65-75.

Identification of genetic markers linked to quantitative resistance to late blight and white potato cyst nematode in tetraploid potato

D. Milbourne, R.C. Meyer, C. Hackett[1], J.E. Bradshaw, H.E. Stewart, M.S. Phillips & R. Waugh

A major objective in potato breeding is the combination of durable resistance to late blight (LB) and to the white potato cyst nematode (PCN). This type of resistance is controlled by numerous interacting genetic loci (QTLs) and influenced by the environment, resulting in quantitative trait variation. Quantitative traits are not inherited in a simple Mendelian fashion (although component parts are) and are therefore difficult to select for in breeding programmes. Nevertheless, the application of molecular marker analysis has facilitated the genetic dissection of QTLs and established the possibility of 'indirect selection' based on genotype as opposed to phenotype. While currently being applied in diploid inbred species such as barley and tomato, QTL dissec-



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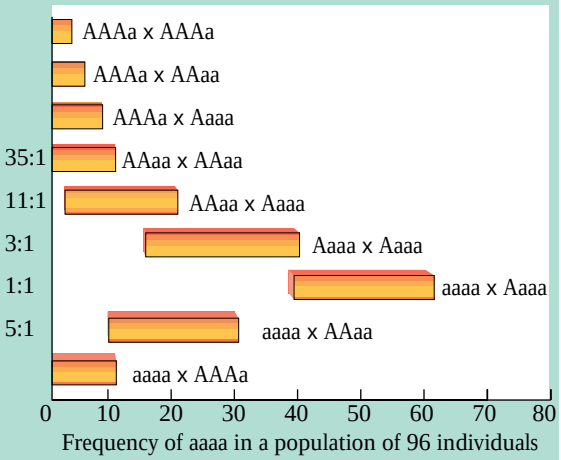


Figure 1 95% confidence intervals for the observed segregation ratios in a population of 96 individuals (allowing for the phenomenon of double reduction). Only non-overlapping classes are distinguishable. In a population size of 96 we can reliably distinguish 1:1 and 5:1 segregation classes (given parental genotypes).

tion and indirect selection have not yet been established for tetraploid potatoes. One prerequisite is the ability to follow a sufficient number of markers in a single, defined segregating population. This is now achievable with molecular markers such as AFLPs[1].

A tetraploid F_1 population of *c.* 300 lines, segregating for quantitative resistance to LB and PCN, has been developed within the SCRI potato breeding programme from a cross between the advanced breeding clone 12601ab1 (PCN resistant) and the cultivar Stirling (LB resistant). Markers which are present in only one parent, are expected to segregate in a 1:1

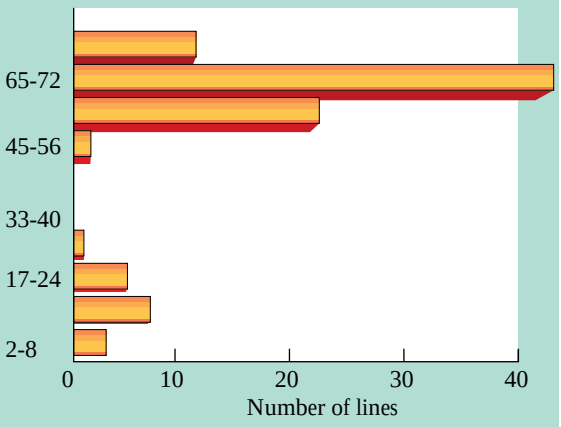


Figure 2 Low number of markers from Stirling in 16 lines. The histogram shows the number of F1 genotypes against the number of male parental bands they contain.

	Aaaa x aaaa	AAaa x aaaa	Aaaa x Aaaa	AAaa x Aaaa	? x ?	Total
Ratio	1:1	5:1	3:1	11:1	Other	All
Number	216	106	33	62	154	571
%	37.7	18.6	5.8	10.9	27.0	100

Table 1 Segregation ratios in 12601ab1 x Stirling F_1 population of 78 lines. Number of AFLP markers falling into each of the expected segregation classes for a tetraploid cross according to Chi^2 tests at the 5% level.

ratio if they are derived from just one of the four chromosomes (simplex), and in a 5:1 ratio if they are from two of the four chromosomes (duplex). Markers present in both parents should segregate in a 3:1 ratio if they are present on a single chromosome in both parents. On the basis of the predicted number of individuals required to distinguish between the 1:1 and 5:1 segregation classes (Fig. 1), a subset of 96 lines was chosen at random from the F_1 population. Approximately 3,000 loci in this population have been surveyed with AFLP, and 571 segregating mark-

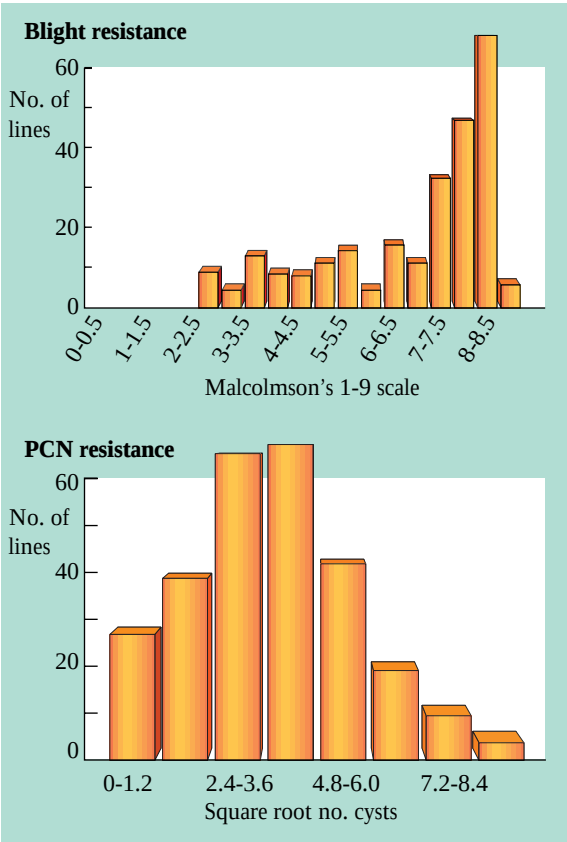


Figure 3 Distribution of disease resistance scores. The blight resistance data are presented untransformed. The PCN data have been square root transformed.

ers were scored. Analysis of 1:1 and 5:1 markers originating from the male parent (Stirling), highlighted a group of 16 lines that had an unusually low number of male parental products (Fig. 2). The 16 anomalous genotypes were omitted from the mapping experiment and subsequent linkage and QTL analyses were carried out on the remaining 78 lines (Table 1). A preliminary linkage map was constructed using the 1:1 and 3:1 marker data. Twenty-six (Stirling) and 28 (12601ab1) parental linkage groups of at least two markers were obtained, with 18% of the markers remaining unlinked. In a parallel set of experiments, the entire F_1 population was screened for resistance to late foliage blight (LB) and PCN resistance. The distribution of the resistance scores over the population is shown in Figure 3. Associations between markers and resistance were then tested. For LB resistance, one 5:1 marker from Stirling accounted for 31.6% of the total variation, and for PCN resistance, two 5:1 markers from 12601ab1 accounted for 27.8% and 23.8% of the total variation. In the absence of known chromosomal location markers, the AFLP products strongly associated with quantitative resistance from both Stirling (LB) and 12601ab1 (PCN) were cloned, sequenced, and primers designed in order to produce single locus markers transferable to a diploid mapping population. Using these primers, PCR with DNA from Stirling, 12601ab1, and individuals from a diploid potato population previously used to construct a detailed RFLP map[2], generated a single product of the expected size. The DNA polymorphism responsible for the presence/absence of the AFLP bands in the tetraploid parents is therefore not detected by these primers. Further analysis is currently being undertaken in order to map these three resistance QTLs.

References

[1] Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. & Zabeau, M. (1995). *Nucleic Acids Research* 23, 4407-4414.

[2] Gebhardt, C., Ritter, E., Debener, T., Schachtschabel, U., Walkemeier, B., Uhrig, H. & Salamini, F. (1989). *Theoretical and Applied Genetics* 78, 65-75.

A potato pollen-specific promoter

A. Maddison, R. Meyer, P. Hedley & G.C. Machray

In plants, the cleavage of sucrose to glucose and fructose is a key reaction in carbohydrate metabolism. The primary product of photosynthesis is sucrose, an important storage carbohydrate and precursor for the synthesis of other storage carbohydrates such as starch and fructans. In addition, sucrose is the predominant translocated carbohydrate, playing a major role in sink/source relationships and photoassimilate partitioning. Glucose and fructose, produced by its cleavage, are utilised to meet the fundamental energy requirements of the cell through the glycolytic cycle. Two families of enzymes, the invertases and sucrose synthases, catalyse this cleavage. In potato, as in other plant species, a family of genes encodes a variety of related invertase enzymes. We have characterised four genes encoding acidic invertases found in the plant cell wall, two genes encoding vacuolar acidic invertases have been described elsewhere, and it remains possible that further invertase genes exist. This complexity suggests a functional significance which is being assessed by a more detailed examination of the organisation and expression of the invertase gene family in potato.

We have isolated a clone which contains sequences for two linked invertase genes from the potato genome (Fig. 1). The intervening 1.8 kb of sequence constitutes the promoter of the downstream gene.

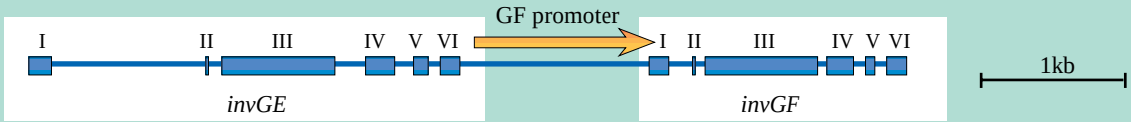


Figure 1 Tandem organisation of invertase genes (invGE and invGF) in the potato genome. Corresponding exons are boxed and numbered, the invertase promoter is arrowed.

Expression of the upstream gene was detected in source and sink leaf, flower bud and flower by RT-PCR, using sequence-specific primers. Using this gene-specific method, expression of the downstream gene was not found in vegetative tissues but was subsequently detected in floral tissue. Dissection of mature flowers from potato indicated that this expression originated in stamens. To determine the precise location of expression, the entire promoter of the downstream gene was fused to a reporter gene, the *uidA* gene from *Escherichia coli* which encodes the enzyme ß-glucuronidase, and the fusion construct transformed into potato. These transformants allowed the histochemical localisation of ß-glucuronidase, expressed under the control of the invertase promoter. This analysis revealed clear and specific expression from the invertase promoter in pollen (Fig. 2), with no expression elsewhere in the stamen including the tapetum and filament. A second series of transgenic lines was generated which contain deletion derivatives of the invertase promoter fused to the same reporter. Results from these transgenic potato lines have indicated an upstream region of the promoter necessary for high-level expression in pollen. A more targeted deletion analysis of the promoter is required to confirm this and to identify any sequences which may determine pollen-specificity.

These results suggest a role for one of the potato invertase enzymes exclusively in pollen cells. Further insight into this enzyme may be gained by a more detailed histochemical analysis throughout pollen development and interference in this process by the expression of invertase antisense RNA from the promoter. Specific down-regulation of the expression of this invertase or expression of an heterologous gene encoding a cytotoxic agent, might be expected to result in a male sterile phenotype. If the specificity of expression from this promoter is retained across species, this phenotype might be exploited in near relatives in which hybrid seed production is important, as well as in potato breeding programmes.

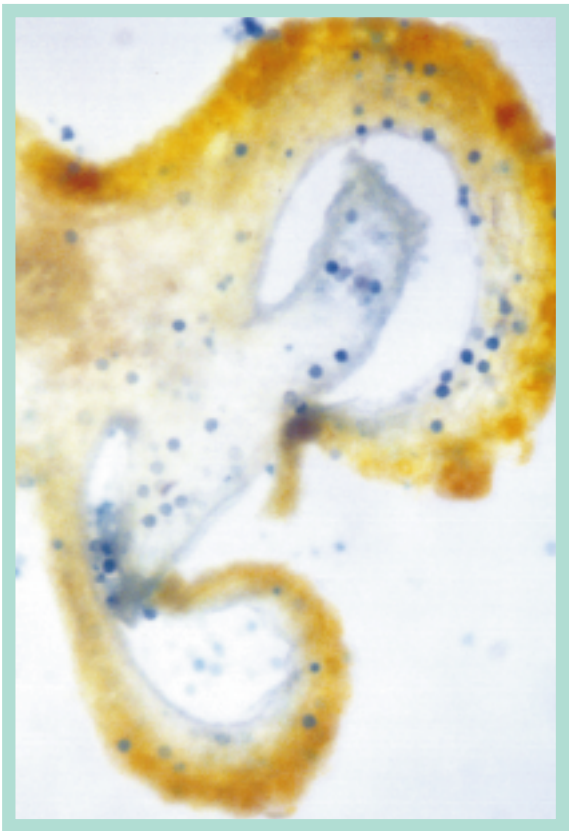


Figure 2 Cross-section of part of a potato anther with expression from the reporter gene revealed as blue staining of pollen cells.

Exon definition and co-operativity in plant pre-mRNA splicing

C.G. Simpson, J. Lyon, C. McQuade & J.W.S. Brown

Precursor messenger RNA (pre-mRNA) splicing is the process by which intron sequences are removed from pre-mRNA transcripts to generate mRNAs which are translated into proteins. The majority of plant protein coding genes contain multiple introns, and one of the key questions in plant pre-mRNA splicing is how introns and exons (the protein coding regions) are defined and recognised by the splicing machinery. Over the last six years, research into plant pre-mRNA splicing has suggested that the intron is the basis of definition in plant pre-mRNA splicing. The RNA Processing Lab at SCRI has shown that plant introns contain preferred branch-point sequences required for efficient splicing (*Ann. Rep. 1995, 48*) and that scanning from the branch-point to select the 3' splice site occurs in plant intron splicing[1]. These findings realign models of plant intron splicing with those of vertebrates and yeast, and point to the differences in plant intron splicing lying at early stages of intron recognition.

One of the predictions of the model where the intron is the unit of definition of splicing, is that introns will be spliced independently of one another. In vertebrate intron splicing, the concept of exon scanning is well established and involves early definition of exon sequences by interactions between splicing factors at the splice sites (Fig. 1a). Such interactions stabilise factors at each splice site across the exon (exon definition), prior to interactions across the intron leading to splicing of the intron sequence. Strong evidence for such interactions has come from a number of human genetic disorders, where mutation to one or other splice site can lead to removal of both intron and exon sequences (exon skipping) (Fig. 1b).

The world-wide research effort into the model plant *Arabidopsis thaliana* has led to sequencing of many genes and gene mutations. A number of mutations affect splicing and of these, some show exon skipping. We have recently characterised the sequence mutations of three *cop1* mutants: *cop1-1*, *cop1-2* and *cop1-8*, providing six examples in *Arabidopsis* where mutation to a splice site leads to exon skipping (Fig. 2). The mutations and their splicing behaviour, directly parallel those found as the basis of a number of human genetic disorders, and suggest that exon definition is an important process in determining intron/exon borders in plant pre-mRNA splicing.

Exon definition demonstrates communication between splice sites of adjacent introns. To investigate whether communication between introns in a multi-intron transcript can enhance the splicing process, intron constructs were generated where inefficiently- and efficiently-spliced introns were placed

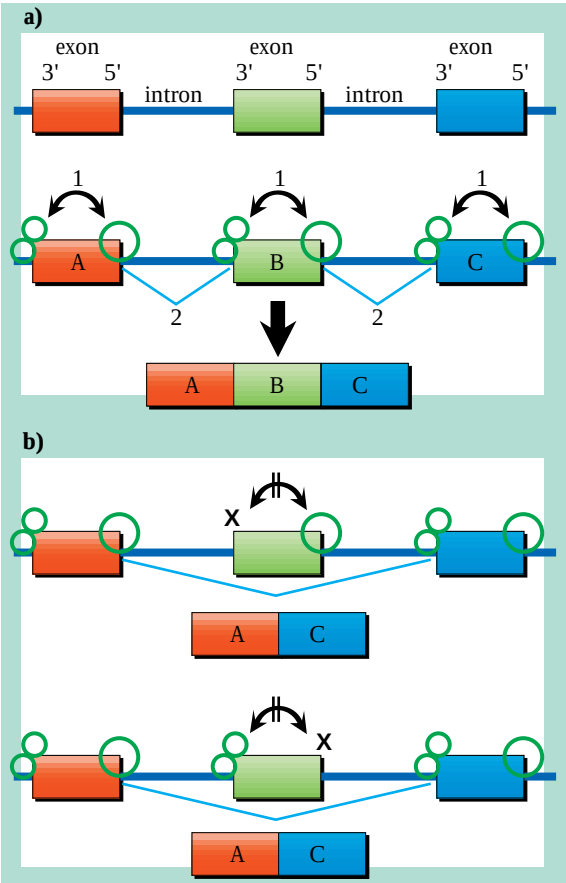


Figure 1 Exon definition in plant intron splicing. (a) A multi-intron transcript showing the exons (boxes) and 5' and 3' splice sites. Factors at each splice site can interact across the exon (double-headed arrow), defining the exon, prior to intron splicing (line). (b) Mutation (X) at one or other splice site disrupts factor association and the exon bridging interaction leading to removal of the internal exon B and introns (exon skipping).

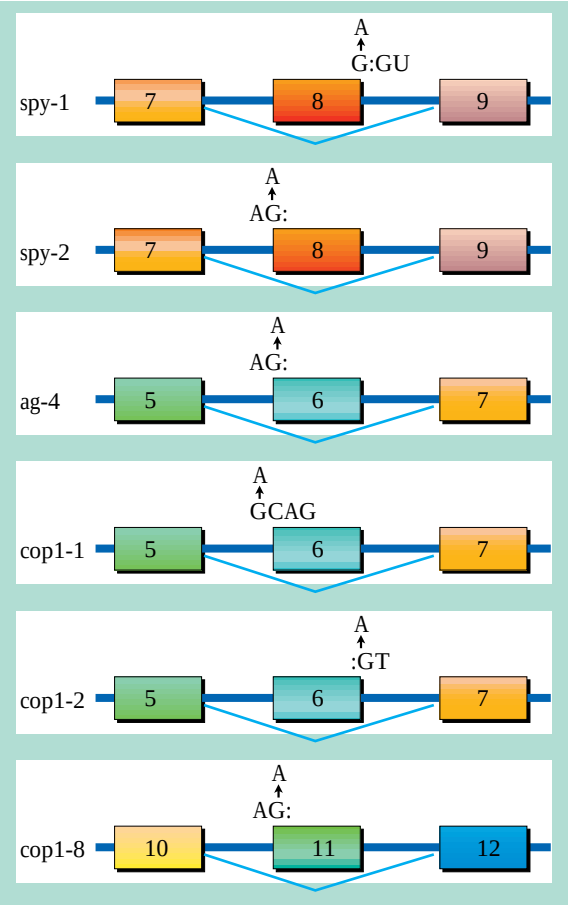


Figure 2 Exon skipping examples in *Arabidopsis* mutants. Mutations in either the 3' or 5' splice site flanking the internal exon lead to exon skipping.

adjacent to each other (Fig. 3a). If splicing of each intron is independent, the amount of final spliced product should be limited by the efficiency of splicing of the poorly spliced intron. However, when placed next to the efficiently-spliced legumin intron, approximately 50% of the transcripts were fully spliced, representing a 2.5-10-fold enhancement of splicing of the amylase intron (Fig. 3b). The elements essential to

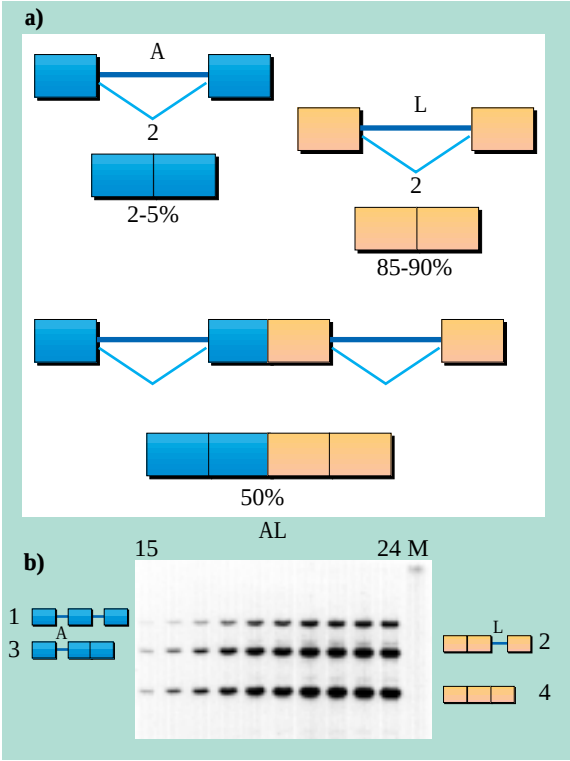


Figure 3 Co-operative enhancement of splicing. (a) The amylase intron (A) is spliced with 5-15% efficiency in tobacco protoplasts. The legumin intron is spliced with 85-90% efficiency. When joined together, final spliced product is produced at around 50% efficiency showing greatly improved splicing of the amylase intron in this context. (b) Quantitative RT-PCR analysis of double amylase and legumin intron splicing in tobacco protoplasts. Unspliced (1), partially spliced (2 and 3) and fully spliced transcript (4) is shown over a PCR cycle course of 15 to 24 cycles.

this co-operative enhancement are now being investigated in mutants of the double intron constructs.

Reference

1 Simpson, C.G., Clark, G., Davidson, D., Smith, P. & Brown, J.W.S. (1996). *Plant Journal* 9, 369-380.

Processing of plant snoRNAs is splicing independent

D.J. Leader, J. Watters, G.P. Clark & J.W.S. Brown

Small nucleolar RNAs (snoRNAs) are involved in many aspects of processing of precursor ribosomal RNA (pre-rRNA) and ribosome assembly. In vertebrates and yeast, the majority of snoRNAs are encoded within introns of protein coding genes. Production of snoRNAs is a splicing dependent process where the snoRNA-containing intron is spliced from the pre-mRNA and, after being linearised, excess intron sequences are removed by exonucleases (*Ann. Rep. 1995, 49*). When an snoRNA is expressed, in animal cells, without flanking exon sequences (non-intronic transcript), virtually no snoRNA is produced (Fig. 1a).

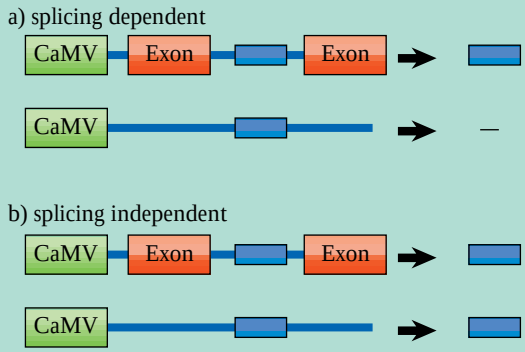


Figure 1 a) Splicing dependent processing of vertebrate snoRNAs. When an intron, the snoRNA is produced via splicing but in the non-intronic construct, virtually no snoRNAs are produced. b) splicing independent processing of plant snoRNAs. Plant snoRNAs are produced from both intronic and non-intronic constructs.

The RNA Processing Group at SCRI have shown a novel organisation of snoRNA genes in plants. Multiple, different snoRNA genes are found in clusters and are expressed as a polycistronic transcript from an upstream promoter. Individual snoRNAs are then produced by processing, presumably involving endonucleolytic activity (Fig. 2). This organisation is unique to plants and suggests that processing of snoRNAs is splicing independent. To demonstrate this, a plant U14snoRNA and flanking sequences was cloned behind the Cauliflower Mosaic Virus (CaMV)

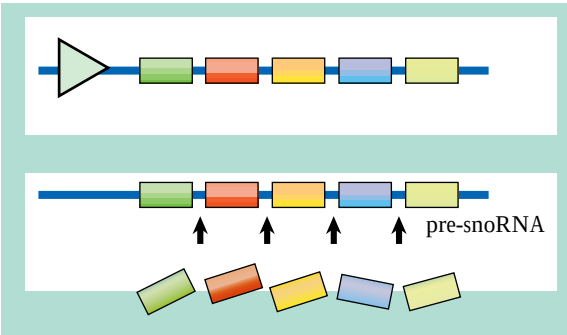


Figure 2 Novel organisation of plant snoRNA genes. Multiple plant snoRNA genes (boxes) are tightly linked and lie downstream of a promoter (triangle). A pre-snoRNA transcript is processed presumably via cleavage between snoRNAs.

35S promoter (non-intronic) and within a plant intron in a gene being transcribed from the CaMV 35S promoter (intronic) (Fig. 1b). Fully processed U14snoRNA was produced from both the intronic and non-intronic transcripts. The latter result contrasts the situation in vertebrates where splicing is required to produce snoRNAs (Fig.1a). Thus, plants have evolved a different gene organisation which reflects the ability of the plant cell to process multiple snoRNAs from single pre-snoRNA transcripts by virtue of the particular nuclease activity in the plant cell. In contrast, the gene organisation which has evolved in animals, reflects the lack of accessibility of snoRNA-containing transcripts to endonucleases.

New barley cultivar

Livet Livet is a selection from a cross between two SCRI breeding lines, 22746Co41 and TSS311/54. 22746Co41 is a cross between Dera and Digger, made at the Welsh Plant Breeding Station but handed over to SCRI as an unselected bulk on the cessation of its barley breeding work, and TSS311/54 was a cross between TS42/3/5 and Apex. The combination of the disease resistances from 22746Co41 and TSS311/54 resulted in Livet possessing the Digger, major gene, *Rhynchosporium* resistance and the Apex *mlo* mildew resistance. It also possesses good resistance to brown and yellow rust. Good all-round disease resistance gives Livet a high yield in the absence of fungicides but it also produces a high yield in fungicide treated trials. It appears to be particularly well adapted to Scotland, being the highest yielding entry in trials from 1995/96. Preliminary malting tests are promising. The combination of high hot water extract and high fermentability may make Livet particularly suitable for the distilling industry. It is a medium maturity cultivar and has very short, stiff straw. Livet was awarded Plant Breeders Rights and placed on the National List in 1996 and is in the UK Recommended List Trials in 1997.

Livet

Cellular and environmental physiology

Howard V. Davies

Despite the pressures imposed by reviews of public sector research centres this year, the department's major research programmes have been pushed forward with vigour, and substantial success stories have emerged with regard to external funding from diverse sources. Major links have been forged with industry on the back of basic research findings, bearing testimony to the entrepreneurial skills of several scientists operating in our research units. The implementation of focused and novel research plans for the immediate future will require further integration of effort within and across units. We look forward to the challenge and the rewards for success.

In the Unit of Integrative Bioscience, considerable progress has been made on several fronts. The rhizosphere microbial community structure of plants, with altered below-ground carbon flows has been determined using a broad-scale community DNA analysis. The results show no detectable change in rhizosphere microbial community structure, as a result of altered C-flow patterns induced by increased atmospheric CO_2 concentrations, in a number of experiments carried out over timescales of both weeks and years. However, the effects of elevated CO_2 are modulated by the nutrient status of the soil in longer term experiments. Investigation of the interaction between soil microorganisms mediated by volatile organic compounds, has shown that all bacterial isolates are capable of influencing the growth rate, either by stimulation or inhibition, of at least one other soil fungal species. Some bacteria are capable of influencing the growth rate of several test fungi.

It has long been known that the microbial community in soil influences soil structure. Theories and experiments developed at SCRI have shown how soil structure affects microbial dynamics. These have facilitated links with theories and experiments on soil structural genesis to show how the soil/plant/microbe system is self-organising. The microbial biomass

affects the heterogeneity of structure and the connectivity of pore space in quite different ways - enhancing the former, while decreasing the latter.

A central question in ecology is the role of spatial heterogeneity in promoting diversity. Soil is clearly a heterogeneous environment, and the diversity of the soil community is central to its natural fertility. In collaboration with the University of Abertay, Dundee, a combined theoretical and experimental programme has been established to understand how heterogeneity promotes diversity in a community of soil fungi. One of the most significant findings is that heterogeneity gives rise to 'emergent behaviour' as the size of spatial coherent communities increases. Thus the dynamical behaviour and diversity depend on patch-size, and continual fragmentation of communities will tend to suppress diversity.

Studies continue on the ecology of oilseed rape, both as a feral plant and volunteer weed. These studies combine molecular, physiological, and mathematical techniques to understand the dynamics of populations from field to regional scales. The timing of germination is identified as an important factor affecting both the competition for resources, and the impact of pests in feral populations. The molecular studies have identified markers associated with timing, and the work is being expanded to study the consequences of this finding for the stability of feral populations at a regional scale. Research into weed seedbank dynamics has demonstrated extreme spatial and temporal variability in the populations from year to year. A complex mathematical model has been produced to identify the key factors responsible for such variability. As a result of the sensitivity analysis, a greatly simplified model has been produced which demonstrates that much of the behaviour is attributable to the form of density dependence in fecundity and forcing, due to environmental heterogeneity. The result of these findings are being combined in a theoretical study of the implications of the release of herbicide-resistant, transgenic cultivars on the dynamics of feral and volunteer populations. For further information on the vegetation dynamics programme, see p. 121.

Mathematical approaches are used to dissect the complexities of flux control in higher plants. Despite the considerable incentives and increasing allocation of resources to the problem, targeted up-regulation of fluxes by genetic engineering of metabolic pathways continues to prove extremely difficult. Mathematical techniques are being developed to deal with the complexity of metabolic regulation. An important facet of these theories is the understanding of how biochemical systems behave when subjected to 'noise' such as irregularities in substrate supply, and imperfect mixing of metabolites within cells. Considerable progress has been made in modelling complex biochemical systems subject to environmental noise. Theories have been developed to facilitate an understanding of how such systems remain stable, and the effect of large perturbations on the dynamical states. New types of dynamical behaviour have been discovered, and the implications of noise for the energetic efficiency of pathways have been calculated. It is clear that noise can dominate the behaviour, and that a full understanding of the functioning can only be appreciated after the role of noise is properly accounted for.

Additional successes emerging from the application of mathematical and related skills are exemplified by significant external contracts: (a) to develop a management advisory package for potato; and (b) to develop further a predictive model of potato size distribution and procedures to optimise its operation.

In the Unit of Stable Isotopes, collaborative research work with the Cell and Molecular Genetics Department continues on the use of the natural abundances of stable isotopes of C and N ($\delta^{13}C$ and $\delta^{15}N$) and molecular markers to estimate genetic diversity in barley populations. It has been shown that $\delta^{15}N$ is as useful as $\delta^{13}C$ in trait analysis. The physiological and genetic interpretations of plant $\delta^{15}N$ have not been explored previously. Another major programme, in collaboration with the University of Western Sydney, Australia, has discovered that in a C4 grass, *Panicum coloratum*, biomass increases in response to CO_2 are not confined mainly to the root system. However, within the root system, most of the 'extra' biomass is in the form of nodal roots arising from the base of the stem. This was also suspected to be the case in wheat (*Ann. Rep 1995, 75-77*). In dry soil, growth of nodal roots in *P. coloratum* is inhibited, an effect abolished at elevated CO_2. This results from the soil remaining wetter, since plants (even C4 species) use less water under elevated CO_2. Genera such as *Panicum*, *Zea* and *Sorghum* produce only a single primary root from the embryo. The developing seedling depends on nodal roots for proper establishment. If nodal growth is inhibited by dry soil, the crop often fails. Elevated CO_2 may indirectly increase the probability of good establishment should rainfall become less frequent in a future climate.

Pilot studies exploiting $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, have been initiated in two areas - (a) to study the sources and fates of these elements in water, algae and invertebrate populations, and (b) to study the timing of S uptake in wheat, using natural gypsum sources of different $\delta^{34}S$.

The Unit for Industrial Crops continues to make extensive use of spectroscopic methods, especially FTIR and NMR, to assess the quality of plant fibres and materials produced after suitable processing. Using tobacco as a model plant along with FT-IR microscopy, it has been shown that down-regulating of the gene for cinnamyl alcohol dehydrogenase (CAD) produces plants in which the lignin is less condensed and in which there is a significant increase in cinnamaldehyde-like absorbancies. Such lignins are easier to remove in subsequent processing. This work is continuing using multiple gene inserts for greater changes in lignin content and structure, as well as in more economically important fibre sources than tobacco. The NMR work has concentrated on the lignins from a range of economically important fibre-producing species such as hemp, sisal, jute and reed Canary grass. This work (in collaboration with the Agricultural University, Wageningen and the University of Edinburgh) has demonstrated some of the key structural difference found between these materials. Research on methods to improve the storage of cereal and other straws (in collaboration with Silsoe Research Institute and Cranfield University) continues and progress has been made on the conditions necessary to optimise use of biocides to reduce the extent of biodegradation.

Protocols for the efficient extraction of peroxidases and oxidases from lignifying tissues have been improved and have yielded both types of enzymes with high affinities for the oxidation of monolignols which are precursors of lignin in both tobacco and Sitka spruce. A purification step using Concanavalin A is of particular benefit and sufficient quantities for sequencing and antibody production can now be obtained.

The theme of starch biosynthesis and starch quality is central to programmes in the Unit of Cell Physiology, where considerable progress has been made in identifying novel glucan synthases and branching enzymes. Transgenic potato plants have been generated which carry genes encoding glucan branching enzymes from heterologous species, including higher plants, bacteria and fungi. Other potentially novel genes are undergoing scrutiny at present. Having cloned a potato α-glucosidase gene, attempts are also being made to modify polymer structure by directing the gene product to the amyloplast. Starches extracted from the transgenic tubers will now undergo detailed chemical and rheological testing to assess significant changes in starch behaviour. In terms of the quantity rather than quality of starch, a significant body of work has been completed in collaboration with the Monsanto Company, USA, on potato tubers expressing a non-regulated *E. coli* ADPglucose pyrophosphorylase. Glasshouse and field trials at SCRI have confirmed that the gene can elevate the starch and dry matter content of tubers by between 10% and 25%, and that the effect is dependent upon growing conditions. Radiolabelling experiments have demonstrated quite convincingly a higher capacity of the transgenics to convert sugar into starch, both in developing tubers and in stored tubers. The latter is believed to contribute to lower sugar levels in stored transgenic tubers together with a longer dormant period. A detailed description of the work will appear in a future Annual Report.

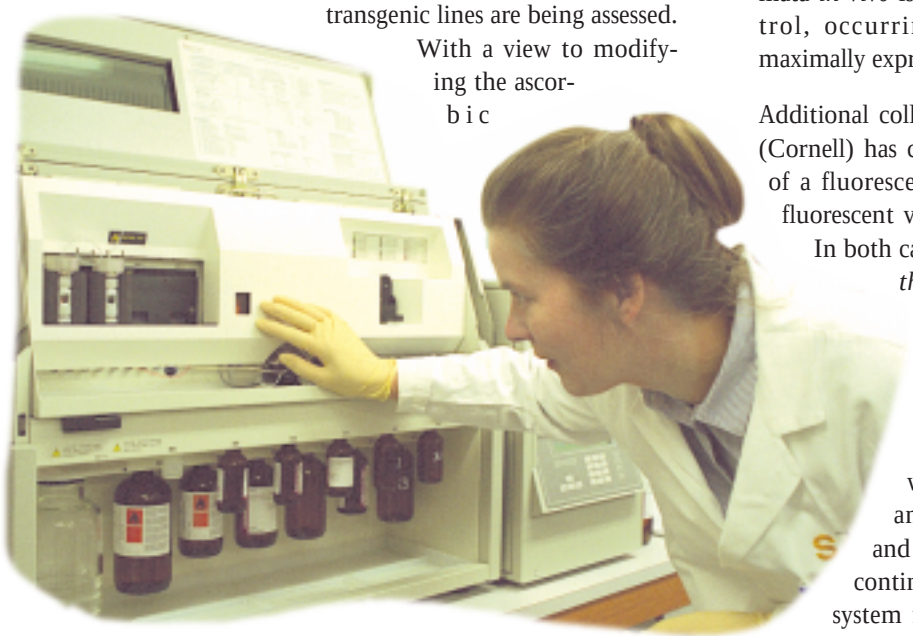
The Unit has also shown that transgenic potato plants in which fructokinase activity has been down-regulated, have a reduced starch content and a modified sugar balance. In related experiments, the factor causing interference during hexokinase enzyme assays has been identified. Potato tuber extracts can contain high apyrase activity. This hydrolyses NTP and NDP in the assay media with the production of metabolites which affect enzyme activity. Apyrase activity is higher in mature tubers compared with developing tubers and can lead to serious underestimations of catalytic rates. The enzyme is resistant to common denaturing treatment and no inhibitor of its activity has been described. *In vitro* redox regulation of the fructokinase activity has been demonstrated.

A BBSRC-funded research project on the use of stable isotopes for the study of metabolic processes in plants has been completed. Techniques for the isolation of plant products suitable for isotope analyses have been described. Evidence for isotopic-fractionation of intermediates during non-photosynthetic primary carbon metabolism, has been obtained. This makes the approach highly suitable for the quantitative study of carbon allocation within metabolic pathways. Furthermore, it appears suitable for comparative analyses of metabolic processes in wildtype and transgenic plants in undisturbed environments. When used in combination with the determination of intramolecular

distribution of stable isotope in plant products, it promises to represent an extremely powerful novel analytical approach for the study of plant metabolism.

Additional research on potato carbohydrate metabolism is to include a study of sink-to-source transition in potato tubers via an EU RTD programme funded recently. Whilst core activities on mechanisms of potato tuber formation have essentially ceased, work has continued through an EU-funded programme. A gene that is differentially expressed during the early stages of tuberisation has been isolated and characterised. In transgenic potato plants, in which the expression of this gene is down-regulated, there are drastic changes in tuber morphology. These changes suggest that the control of cell expansion and division is severely disrupted in the transgenic lines.

Fruit ripening and development research within the Unit is progressing well, and strong collaboration has been forged with the Department of Soft Fruit and Perennial Crops. The cDNA clones of several ripening-related genes have been isolated from raspberry fruit, including those encoding pectin methyl esterase, endo-polygalacturonase, and ACC synthase. The cDNA AFLP technique is being applied to raspberry fruit to identify a range of differentially expressed genes associated with the ripening processes, and initial results are encouraging. Studies on blackcurrant fruit-specific promoters continue and a patent has been filed on one of these sequences. Stable transformation of blackcurrant with a range of promoter-reporter gene constructs has been carried out, and the transgenic lines are being assessed. With a view to modifying the ascorbic acid (AsA) content of fruit, preliminary studies have been carried on AsA biosynthesis in developing potato tubers. Results are included in the review of ascorbate acid biosynthesis (p. 117).



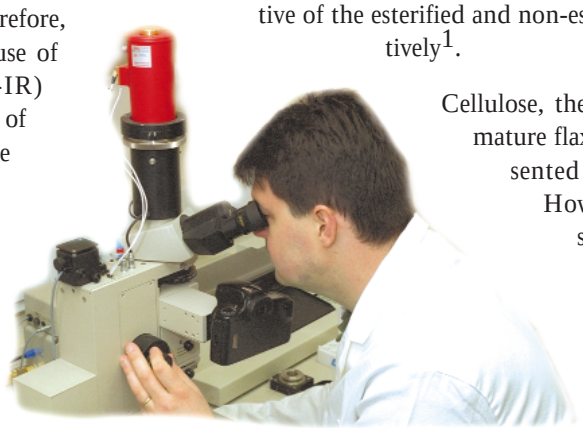
In the Unit of Plant Transport Processes, work is continuing on the ways in which viral movement proteins (MPs) modify plasmodesmata to permit the passage of viruses between higher plant cells. In this work, the use of green fluorescent protein (GFP) as a non-invasive marker for virus movement (*Ann. Rep. 1995, 78-82*), has been invaluable in unravelling the role of viral MPs in both targeting and 'gating' (increasing the molecular size exclusion limit) of plasmodesmata. To date, the viral vector potato virus X (PVX) has been used as a delivery system to release the putative MPs (fused to GFP) of a range of plant viruses in plant cells. MPs studied in this way include those of tobacco mosaic virus (TMV), cucumber mosaic virus (CMV; collaboration with Peter Palukaitis, Virology) and groundnut rosette virus (GRV; collaboration with Mikhael Taliansky, Virology). In all cases, the MP-GFP fusion proteins have been shown to successfully target plasmodesmata. In the case of TMV, collaborative work with Professor Roger Beachy (Scripps Institute, La Jola) has shown that 'gating' of plasmodesmata, as measured by microinjection of fluorescent dextrans, is restricted to the leading edge of expanding lesions of TMV expressing a GFP-MP fusion, and that cells in the centre of the lesion do not display plasmodesmatal gating. These results provide the first demonstration that viral modification of plasmodesmata *in vivo* is under both spatial and temporal control, occurring only where viral MP is being maximally expressed.

Additional collaborative work with Dr Bob Turgeon (Cornell) has compared the long distance movement of a fluorescent solute (carboxyfluorescein) with a fluorescent virus expressing a GFP OVERCOAT™. In both cases it was shown that in *Nicotiana benthemiana*, a host plant to several systemic viruses, class III veins (but not minor veins) regulate the unloading of solutes and viruses in developing leaves in a predictive manner. A model has been produced which links the behaviour of solutes and viruses in both source (exporting) and sink (importing) leaves. Work has also continued on *Arabidopsis* as an experimental system for studying the movement behaviour of xenobiotics in plants (see p. 112).

Application of Fourier-transform infrared microspectroscopy to plant science

D. Stewart, G.J. McDougall & I.M. Morrison

The traditional procedures used to determine the composition and structure of plant tissues are laborious, often using noxious chemicals and generally requiring microgram to milligram amounts of material which usually means that they are carried out on composite samples of tissues. Compositional and structural studies of the development and differentiation of individual cell types and their response to external stimuli are, therefore, extremely difficult. However, the use of Fourier-transform infrared (FT-IR) microspectroscopy permits the study of localised changes (≥8 µm sample diameter) in cell wall composition and structure of individual cells and the comparison of these with different and distant tissues.



The FT-IR microspectrometer has been used to follow changes in the composition and structure of flax cell walls during development. Flax stems were prepared for analysis using standard microscopic embedding and section procedure. Several anatomically distinct cell types were then scanned. The changes in the FT-IR spectra of the cell walls of the fibre bundles during development are shown in Figure 1. An obvious feature of the spectra is the dissimilarity of the spectrum of the 5-day sample relative to the others. This sample displays intense pectin-related absorbances (1680-1600 and, to a lesser extent, 1740 and 1260 cm^{-1}), which are absent from the spectra of the others. Additional pectin absorbances are evident at 1440 (and to a small degree 1740 cm^{-1}) and 955 cm^{-1}, representative of the esterified and non-esterified pectin, respectively[1].

Cellulose, the major constituent of mature flax fibre, is poorly represented in the 5-day sample. However, the older tissues show distinct cellulose absorbances at 1130, 1098 and 1050 cm^{-1}. The lack of pectic absorbances and the presence of a prominent ester absorbance at 1740 cm^{-1} suggests that acetylated non-cellulosic polysaccharides (NCPs) are closely associated with the cellulosic microfibrils. (These NCPs have since been shown to be acetylated glucomannans). It can be seen that from 7 days onwards,

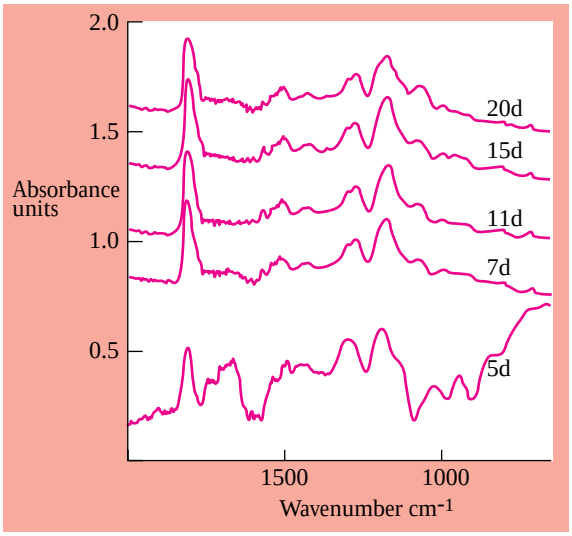


Figure 1 FT-IR spectra of flax fibre cell walls during development from 5-20 days.

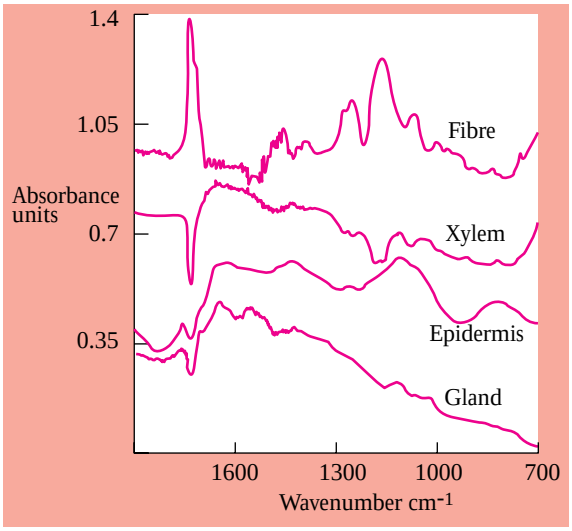


Figure 2 FT-IR spectra of 20 day-old flax fibre, xylem, epidermis and gland cell walls.

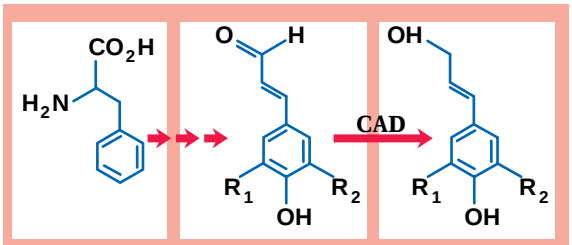


Figure 3 The conversion of cinnamaldehyde to cinnamyl alcohol, catalysed by cinnamyl alcohol dehydrogenase (CAD). *p*-Hydroxyphenyl alcohol, R_1=R_2=H: Feruloyl alcohol, R_1=H&R_2=OCH_3: Sinapyl alcohol, R_1=R_2=OCH_3.

the absorbances in the spectra of the older tissues are remarkably similar in resolution, maxima and intensity.

A comparison of spectra from four anatomically distinct cell types is shown in Figure 2. The differences between the cell walls are distinct. The spectra of the xylem, epidermal and gland cell walls have broadly similar line shapes but contain significant differences. For example, the spectrum of the xylem cell wall contains broad pectin and lignin (1510 & 1595 cm^{-1}) absorbances as well as small cellulose absorbances. The epidermal cell wall spectrum, in comparison, has increased lignin and hydrocarbon (1440 cm^{-1}) absorbances which, in conjunction, suggest the presence of suberin/cutin. This is consistent with the observed distribution of suberin/cutin in other plants where the suberin/cutin on the outer surface of the plant acts as a first line of defence against parasites and as a water-impermeable barrier.

The most obvious features in the spectrum of the gland cell wall are the absorbances at 1650 and 1540

Figure 4 A micrograph of a tobacco stem with cinnamyl alcohol dehydrogenase activity genetically down-regulated to approximately 7% of that in the wild-type. The striking red coloration is the site of active lignin formation.

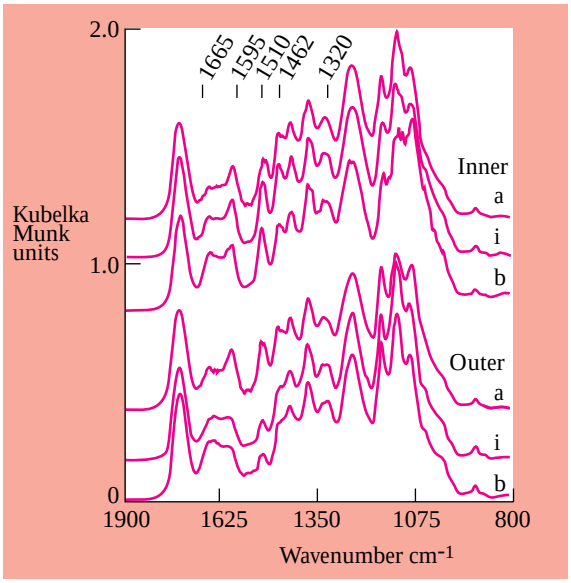


Figure 5 FT-IR spectra of the inner and outer surfaces of xylem strips dissected from wild-type tobacco plants. (a - apical, i - intermediate, b - basal). The absorbances at 1665 cm^{-1} are associated with conjugated carbonyl groups and the peaks at 1595 and 1510 cm^{-1} are lignin-related absorbances. The absorbances at 1462 and 1320 cm^{-1} are related to methoxyl groups and condensed guaiacyl rings respectively.

cm^{-1} which, due to their concomitant increase in intensity with age (not shown), suggest protein accretion. It is possible that these specialised epidermal cells play a role in defence or as storage organs.

Changes in the structure and composition of genetically manipulated lignin were addressed using FT-IR microspectroscopy to provide previously unobtainable information. In conjunction with Alain Boudet *et al.* (University of Paul Sabatier, France), FT-IR analyses were performed on tobacco plants which had been genetically manipulated, using antisense technology, to down-regulate Cinnamyl Alcohol Dehydrogenase, one of the key enzymes in the lignin biosynthetic pathway (Fig. 3).

The xylem of the manipulated plants with relatively low levels of CAD activity (≈7% c.f. wild-type) has a striking red coloration, particularly at the site of active lignin formation (Fig. 4). This may be due to the incorporation of cinnaldehydes into lignin thereby forming conjugated, chromophoric structures[2].

The xylem was isolated from the stems of wild-type and manipulated tobacco plants, and the FT-IR spectra of the inner and outer xylem surface were acquired (Figs 5 and 6, respectively). In general, the

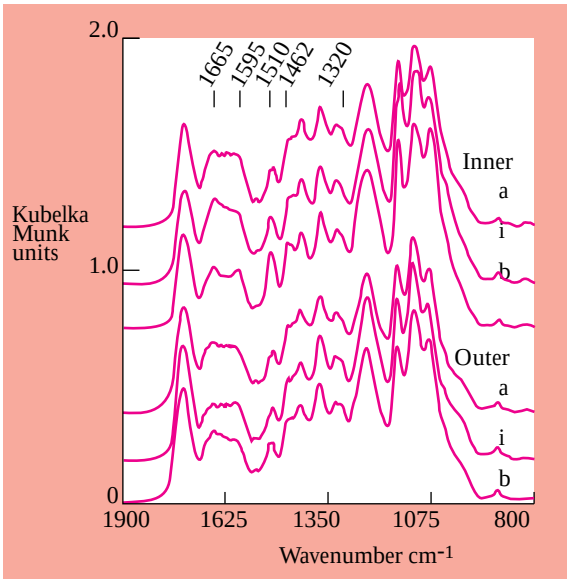


Figure 6 FT-IR spectra of the inner and outer surfaces of xylem strips dissected from antisense CAD tobacco plants (residual CAD activity of ≈7% c.f. wild-type). (a - apical, i -intermediate, b - basal). The effect of CAD down-regulation is evident by the change in the 1595:1510 cm^{-1} ratio. There is also an increase in the conjugated carbonyl absorbance at 1665 cm^{-1}.

spectra are very similar in both maxima intensity and absolute frequency. However, there are distinct differences when comparing the regions 1670-1420 cm^{-1}. All the spectra of xylem from the manipulated tissues have increased intensities at 1665 cm^{-1} (Fig. 6), the region of absorbance for conjugated aldehydes.

The spectra also display differences in the relative intensities of the lignin-related absorbances at 1510 and 1595 cm^{-1}. Overall the absorbances at 1510 cm^{-1} are less intense in the spectra of the manipulated tissues. Absorbances in this region are an indicator of cross-linked, or condensed, lignin. This suggests, therefore, that the macromolecular structure of the manipulated lignin has changed and has become relatively less condensed. This is in agreement with the reported increased alkali extractability of the lignins from anti-CAD plants[3].

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Predicting the phloem mobility of xenobiotics

K.M. Wright & K.J. Oparka

The mobility of molecules in plants is of considerable importance to the agrochemical industry, and has been the subject of investigation for many years. Each year, several new agrochemicals are synthesised with properties that enable their maximal penetration into the plant, and subsequent movement within the long-distance transport system of the phloem. By maximising the transport efficiency of agrochemicals to their target sites (often meristematic regions), theoretically it should be possible to minimise both the environmental impact of a given chemical and the economic costs involved in its application. It has frequently been assumed that since agrochemical molecules are foreign to the plant (termed xenobiotic), their behaviour will be governed solely by their physicochemical properties. However, this view does not consider key physiological aspects of chemical transport within plant cells. Indeed, there is increas-

ing evidence that plant cells can employ a variety of processes to both sequester and detoxify xenobiotic molecules and thus render them harmless to the enzymatic machinery of the cell.

This article describes a study which tests the assumption that physicochemical properties alone can be used to predict the uptake and phloem mobility of xenobiotics in plants. In order to visualise the movement of chemicals within the plant, we utilised a wide range of non-toxic, fluorescent probes with well-defined physicochemical properties, thus simulating the movement of xenobiotic molecules. This approach has the advantage that the potential phytotoxic properties of a given chemical can be detached from those properties that determine its mobility within the plant.

The experimental system The *Arabidopsis thaliana* seedling has a simple anatomy, and this plant has become a popular subject for genetic, developmental and physiological investigations. Seedlings between 5 and 10 days old, were used when the plants comprised two cotyledons and a single main root that was typically 20-30 mm long (Fig. 1). We investigated the movement of fluorescent xenobiotics out of the leaves, via the phloem, which in the *Arabidopsis* seedling forms two distinct poles running down to the root tip. Close to the meristematic region of the root is an unloading zone where solutes are delivered to the cells of the growing root tip. The phloem consists of sieve elements, joined to their companion cells to form a sieve element-companion cell (SE-CC) complex, and a few phloem parenchyma elements. All of these cell types are interconnected by membrane-lined pores known as plasmodesmata. At the root tip the phloem is connected, via plasmodesmata, to the adjacent cell layers of the pericycle, endodermis, cortex and epidermis.

Figure 1 *Arabidopsis thaliana* seedling labelled at the cotyledons with carboxyfluorescein diacetate and imaged using the confocal laser scanning microscope. Carboxyfluorescein can be seen in the two phloem poles running down the length of the root and moving out into the cells of the unloading zone near the root tip.

As with other members of the Brassicaceae family, the SE-CC complex in the leaves of *A. thaliana* is isolated from adjacent cells. Therefore, the sugars generated during photosynthesis, and also the fluorescent probes applied to the leaf, must cross the plasma membrane of the SE-CC complex before they can be transported to the root. When fluorescent probes are phloem-mobile they can be observed in real time, as they move down the root in the two phloem poles to the root tip, using a confocal laser scanning microscope[1]. Close to the root tip, they move from cell-to-cell via the plasmodesmata (i.e. symplastically), forming a characteristic 'tear drop' pattern (Fig. 1). Once in the cells of the unloading zone, the probes may be partitioned into either the cytoplasm or the acidic central vacuoles. The fluorescent xenobiotics used in this investigation were applied to the cotyledons as ester derivatives, as these are usually considered to be more membrane permeant than the non-ester forms. Once inside the cell, the ester moiety of the molecule is cleaved by esterase enzymes, releasing the fluorescent probe into the cytoplasm.

The model A structure-activity relations (SAR) model has been developed that attempts to describe the interaction of low-molecular weight xenobiotics within living cells. The model assumes that entry, accumulation and retention of xenobiotics within various cellular compartments is dependent on simple physicochemical properties. These include the solubility in water, the electric charge (and hence the acid base strength), hydrophilicity/lipophilicity, molecular size and protein binding. The lipophilicity of a molecule is its affinity for lipid as opposed to water (hydrophilicity), and is measured as the octan-1-ol/water partition coefficient (log *P*). The higher the log *P* value of a molecule, the greater its affinity for

Ester forms	Log *P* [b]	*CBN* [c]	*Z* [d]	*MW/IW* [e]	pK_a [f]
BCECF AM[a]	5.8	25	0	821	nx
Calcein AM	8.0	23	0	995	nx
Calcium green-1 AM	12.8	44	0	1291	nx
Calcium orange AM	4.3	46	0	1222	nx
Carboxyfluorescein diacetate	1.3,4.2	26,25	-1,0	459,560	6.4*
Carboxy SNAFL-1 diacetate	0.4,4.5	31,30	-1,0	509,510	-
Carboxy SNARF-1 AM	4.0	30	0	568	nx
Chloromethyl SNARF acetate	7.1	28	0	498	nx
Fluo-3 AM	13.5	37	0	1130	nx
Fluorescein diacetate	4.2	23	0	416	nx
HPTS acetate	-8.3	19	-3	497	nx
Rhod-2 AM	8.9	35	+1	1043	nx
Sulphofluorescein diacetate	1.2	23	-1	495	nx
De-esterified forms					
BCECF	-7.3,-3.2	29,28	-3,-2	517,518	7.3
Calcein	-5.7,-3.4	27,27	-4,-3	619,620	7*
Calcium green-1	-5.1,-2.7	48,48	-4,-3	915,916	-
Calcium orange	-4.9,-0.8	47,46	-2,-1	933,934	7*
Carboxyfluorescein	-1.9,0.9	29,28	-2,-1	374,373	6.3
Carboxy SNAFL-1	c.-0.5,2.5	35,34	-2,-1	424,425	7.8
Carboxy SNARF-1	-1.7,3.0	36,35	-2,-1	451,452	7.6,7.8
Chloromethyl SNARF	2.8,6.9	33,32	-1,0	456,457	-
Fluo-3	-2.2,0.2	37,37	-4,-3	766,767	7*
Fluorescein	-2.4,1.8	28,27	-2,-1	330,331	4.4,6.7
HPTS	-10.1	20	-3	455	7.3
Rhod-2	-2.6,-0.3	35,35	-2,-1	752,753	-
Sulphofluorescein	-7.5,-3.4	28,27	-3,-2	409,410	-

a AM indicates acetoxymethyl

b log *P* is the log of the octanol-water partition coefficient

c *CBN* is the conjugated bond number

d Electric charge : the most probable ionic forms at pH7 are given

e Molecular or ionic (i.e. adjusted for mass of counter ion/s) weights

f Literature values; * indicates an estimate; nx indicates no change in ionization expected around pH7; - indicates no data available

Table 1 Numerical parameters of fluorescent probes.

lipid, and thus its ability to pass through the lipid areas of plant membranes. However, high values indicate that the molecule is likely to be trapped within the lipid. The lipophilicity of a molecule is decreased if the molecule is electrically charged. Certain molecules dissociate, and become negatively charged, depending on the pH or acidity of the environment they encounter. This is measured by the ionisation constant (pK_a), which is the pH at which 50% of a molecule, or particular group on a molecule, is dissociated. The other properties used in this model are represented using the numerical parameters of electric charge (Z), molecular or ionic weight (MW or IW) and conjugated bond number (*CBN*).

The principal features of the SAR model may be summarised as follows:

•Living cells and organisms may be considered as built from collections of compartments, and of compartments within compartments.

•Movement of probes into and through such compartments is influenced by the permeability of compartment boundaries and the nature of compartment contents, as well as by biologically defined transit routes.

•Permeabilities of compartment boundaries vary markedly due to simple physicochemical factors, as well as due to complex biochemical and physiological mechanisms.

•The tendency of compartments to retain molecular probes varies, due to boundary permeability differences and also due to trapping of probes by compartment boundaries and compartment contents, which is again influenced by simple physicochemical factors.

Properties of fluorescent probes The fluorescent molecules used in this investigation are listed in Table 1 along with their physicochemical properties. The log *P* and *CBN* values have been calculated for the different ionic forms likely to be present at pH 7. The pK_a values are taken from the literature and do not necessarily relate to the ionic forms present at physiological pH. For example, the pK_a of 7.3 quoted for HPTS is the ionisation constant of the hydroxyl group, as opposed to the three sulphonate groups which result in an electric charge of -3. Similarly, the

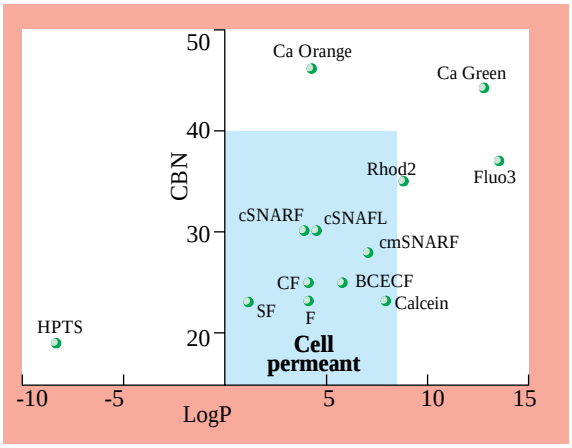

Figure 2 Graphical representation of esters predicted to be able to enter and move within plant cells. By plotting log *P* versus *CBN*, it is possible to identify the esters likely to enter plant cells (cell permeant) and those predicted to be excluded or restricted by trapping in the plasma membrane.

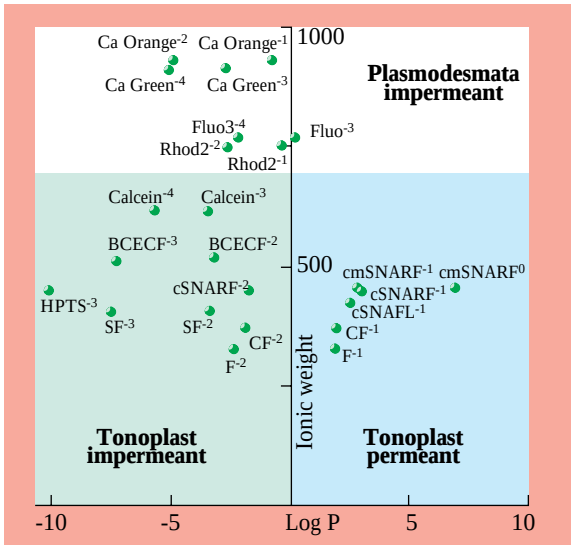

Figure 3 Graphical representation of SAR model predictions relating to symplastic movement and subcellular location. Certain probes are predicted to be unable to move symplastically due to their size (plasmodesmata impermeant). The subcellular location of the ionic species present at physiological pH can be predicted from the log P value as either cytoplasmic (tonoplast impermeant) or vacuolar (tonoplast permeant).

pK_a for carboxyfluorescein (6.3) represents the ionisation of the hydroxyl group, whereas independent measurements have indicated values of 3.3, 4.65 and 6.98 for the 5 (and 6) carboxyl, the 3 carboxyl and the phenolic hydroxyl group respectively.

Predictions of the SAR model The abilities of various probes to enter the phloem and be transported without diffusional loss to the unloading zone, and also the subsequent patterns of intracellular accumulation, were predicted using the SAR model. The predictions regarding the permeation of fluorescent probes and the distribution of molecules resulting from ester cleavage within the cell, are illustrated graphically in Figures 2 and 3. The following are specific predictions resulting from this application of the SAR model:

•Calcium green ester, fluo-3 ester and rhod-2 ester are superlipophilic molecules (log *P*>8), and will be trapped by the lipids of the plasma membrane (Fig. 2).

•The presence of large conjugated systems (*CBN*>40) will result in calcium orange ester and calcium green ester being trapped by the proteins of the plasma membrane (Fig. 2).

•The esters of the following probes have log *P* values between 0 and 8 and will enter cells by passive diffusion through the plasma membrane; BCECF, calcein, carboxyfluorescein, carboxy SNAFL, carboxy SNARF, fluorescein and sulphofluoresecin (Fig. 2). Once inside the cell, these molecules are assumed to be cleaved by esterase activity. Although chloromethyl SNARF ester will also enter cells by passive diffusion, it has long been known that such reactive chlorocarbons form covalent bonds with nucleophilic groups, and hence will be trapped by cell proteins.

•Certain molecules can pass from cell-to-cell via plasmodesmata, thus allowing their mobility in the phloem and subsequent symplastic unloading. Phloem mobility is also dependent on the retention of the probe within the SE-CC complex along the transport pathway. BCECF, calcein, carboxyfluorescein, carboxy SNAFL, carboxy SNARF, fluorescein and sulphofluorescein all fit into this category. Under the alkaline conditions within the SE-CC complex, the carboxylic substituents present on all these compounds will be ionised, and the polyanionic species formed (log *P*<0) will not be membrane permeant. Thus, they become 'trapped' within the phloem. The ionic weight of all these probes is under 700, allowing their symplastic exit from the SE-CC complex where plasmodesmata permit (Fig. 3).

•Certain probes will be able to permeate the tonoplast (vacuolar membrane) by passive diffusion, due to the presence of ions with log *P*>0 at the pH encountered in the cytoplasm. These are: carboxyfluorescin carboxy SNAFL, carboxy SNARF, and fluorescein (Fig. 3). This is contrary to the frequent assumption that ions are not able to permeate membranes. These

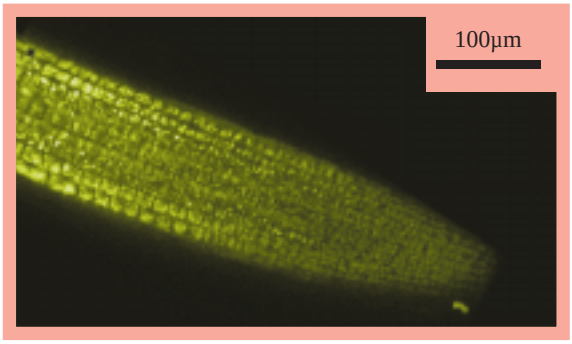

Figure 4 Root tip of *Arabidopsis* showing the distribution of calcein following unloading from the phloem. The calcein can be seen accumulating in the vacuoles of the endodermal, cortical and epidermal cells surrounding the unloading site.

probes could be accumulated within the vacuole by precipitation trapping, in which the acidic pH results in the formation of poorly soluble free acid forms of the probes.

•BCECF, calcein and sulphofluorescein will be tonoplast impermeant, since all the ionic forms likely to be present at the pH of the cytoplasm have a log P<0 (Fig. 3).

•HPTS acetate will not pass the plasma membrane by passive diffusion, as this ester is strongly hydrophilic (Fig. 2). Although the cleaved probe would be phloem mobile, it would be tonoplast impermeant, and would not be precipitation-trapped in acidic compartments (Fig. 3).

Observations of the intact *Arabidopsis* plant - testing the model *Arabidopsis* roots were observed, using the confocal laser scanning microscope, for evidence of dye movement from 0.5 to 3 h after applying fluorescent probe to the cotyledon. Five of the probes tested were not observed within the root phloem, or at the unloading zone, even after prolonged incubation. These were fluo-3, calcium green, calcium orange, rhod-2 and chloromethyl SNARF. In contrast, the following fluorochromes were observed to be phloem-mobile; BCECF, carboxyfluorescein (Fig. 1), calcein (Fig. 4), carboxy SNARF (Fig. 5), carboxy SNAFL, fluorescein, sulphofluorescein and HPTS. These probes were visible in the two phloem poles, later forming the characteristic 'tear drop' pattern around the unloading zone near the root tip. In this unloading zone, all the phloem-mobile probes ultimately accumulated in the vacuoles of the endodermal, cortical and epidermal cells.

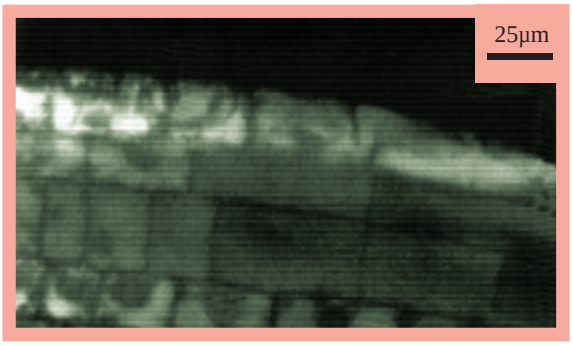

Figure 5 Image of carboxy SNARF following unloading at the root tip, showing accumulation in the cell vacuoles, the nuclei and cytoplasm remaining dark.

Conclusions The SAR model accurately predicts that calcium green, calcium orange, fluo-3, rhod-2 and chloromethyl SNARF are not phloem mobile. This lack of movement is probably due to their binding to either the lipid or protein of the plasma membrane and, in the case of chloromethyl SNARF, its binding to proteins in the cytoplasm. Although HPTS acetate is predicted to be membrane impermeant, and therefore should not enter the plant, it is phloem mobile. It remains to be determined how this probe enters the plant cells. As predicted, carboxyfluorescein, carboxy SNAFL, carboxy SNARF, and fluorescein are all phloem mobile and ultimately accumulate in the vacuoles of cells at the unloading zone. However, BCECF, calcein, HPTS and sulphofluorescein also accumulated in the vacuole, even though these molecules should not be able to permeate the surrounding membrane, the tonoplast. In the case of carboxyfluorescein and HPTS, this movement into the vacuole can be inhibited by a drug called probenecid, suggesting that this sequestration process is carrier-mediated. Therefore, although this model accurately predicted the phloem mobility of 12 out of 13 fluorescent xenobiotics, it does not take into account the carrier-mediated sequestration of xenobiotics into the plant vacuole. Since vacuolar sequestration may severely restrict the movement of xenobiotics from certain plant tissues, maximal phloem mobility of xenobiotics will only be achieved when this process is fully understood and minimised.

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Functions and metabolism of L-ascorbic acid

R. Viola

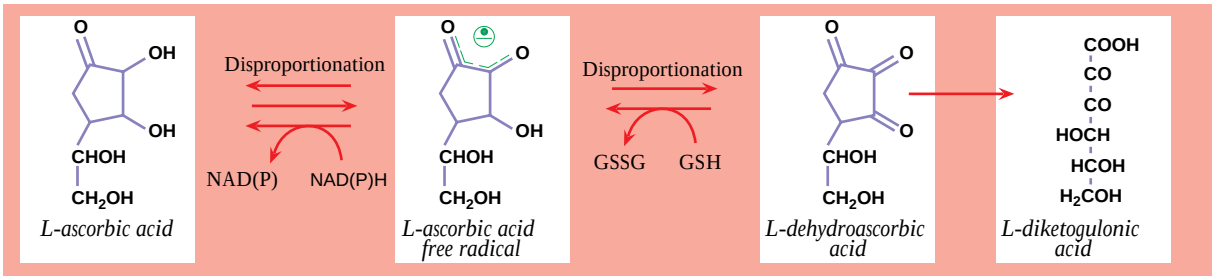

Figure 1 Oxidation of AsA to DHA occurs via the formation of a free radical (AFR) which spontaneously disproportionates to AsA and DHA. AFR and DHA are recycled to AsA via specific enzymes requiring NAD(P)H and reduced glutathione respectively. Unrecycled DHA is irreversibly converted into L-diketogulonic acid.

The molecule L-ascorbic acid (AsA) exists in a reduced or oxidised status; the ene-diol groups at the second and third carbon of the AsA molecule are sensitive to oxidation and can be easily converted into a diketo group, L-dehydroAsA (DHA) (Fig. 1). During this conversion, the ascorbate free radical (AFR) is generated. Both AFR and DHA can be converted back to AsA enzymatically using NAD(P)H and reduced glutathione (GSH) respectively, as a source of reducing power. The lactone ring of DHA readily hydrolyses to give an open chain acid, L-diketogulonic acid, which cannot be reduced back to AsA and undergoes further irreversible changes in solution.

The function AsA is an extremely effective antioxidant as it is a relatively poor electron donor in physiological conditions and acts primarily by transfer of single hydrogen atoms. AsA can react with and scavenge many types of reactive oxygen species (ROS) including singlet oxygen, superoxide anions and hydroxyl radicals. These ROS can participate in radical transfer propagation reactions which are greatly enhanced in the presence of transition metals and are responsible for the formation of aggressive organic peroxides. Certain ROS are extremely reactive in all organisms and severe oxidative stress can cause damage to DNA, proteins, carbohydrates and lipids, excessive rise of intracellular 'free' Ca^{++} and disruption of energy metabolism. In plants, AsA has a crucial role in the detoxification of peroxide, ozone and free radicals and it is essential for photosynthetic activity via the regeneration of membrane-soluble antioxidants (αdf-tocopherol) and zeaxanthin, and the pH-mediated modulation of PS II activity. In chloroplasts, high generation of superoxide anions and hydrogen peroxide oxidative damage is avoided by Cu,Zn-SOD, AsA peroxidase and glutathione reductase forming a detoxification sequence which finally oxidises NADPH at the expense of hydrogen peroxide (Fig. 2). This detoxification cycle prevents singlet oxygen formation in the absence of catalases.

AsA is also an important co-factor of certain metalloenzymes (oxygenases and dioxygenases) where it serves the purpose of reducing the prosthetic group should it become adventitiously oxidised during catalysis. In plants, AsA participates in the reactions catalysed by myrosinase, 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase and prolyl hydroxylase. AsA stimulation of cell division and cell expansion has been attributed to stimulation of prolyl hydroxylation of cell wall proteins which are deposited in large quantities during these processes. Humans and primates deprived of AsA develop a deficiency

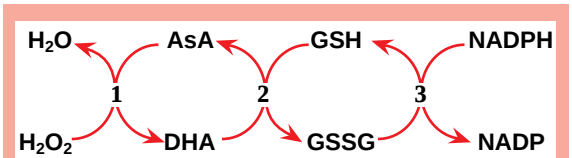

Figure 2 Removal of hydrogen peroxide in the chloroplast via the Halliwell-Asada cycle: (1) AsA peroxidase; (2) DHA reductase; (3) glutathione reductase.

disease (scurvy) which can be attributed to a reduction in prolyl and lysyl hydroxylases activities *in vivo*. The main symptoms of the disease, which is life threatening, are tissue and capillary fragility and are directly attributable to a defect in the hydroxylation steps during collagen biosynthesis and processing. In addition to collagen metabolism, AsA has been implicated in other important cellular processes involving dioxygenases, namely carnitine biosynthesis, dopamine hydroxylation from norepinephrine in catecholamine biosynthesis, α-amidation of peptide hormones and hormone-releasing proteins, and in the synthesis of homogenistic acid during tyrosine metabolism. Moreover, AsA prevention of many chronic diseases, including cancer and cardiovascular disease, has been attributed to its radical-quenching properties.

The synthesis AsA represents an essential nutrient for humans, primates, and a few other animals which can be described as *null* mutants with respect to the capacity for its biosynthesis. Given that plants are the major dietary source of AsA for mankind, the elucidation of the AsA biosynthetic pathway in plants, and of the mechanisms used to regulate its synthesis, are of considerable interest. Generally leaves contain high levels of AsA due to its high concentration (up to 50 mM) in the chloroplasts. However, there is a huge, unexplained, variability in the AsA content of non-green plant tissues and plant storage organs. For example, amongst fruits, the West Indian Cherry (*acerola*) contains 13 mg/gFW of AsA whilst the common cherry contains only 0.05-0.08 mg/gFW. The large variation in AsA content does not have a simple taxonomic explanation. As a further example, the family *Rosaceae* counts amongst its members rosehips and apples, with the first containing 200 times more AsA than the second. White and blackcurrants are two species of the genus *Ribes* but there is a large difference in their AsA content and the same applies even to genotypes of the same species of *Ribes* (*Ribes nigrum* L) (Fig. 3). Although selective breeding has been used with some success to increase the AsA content of fruits

Cultivar	Origin	AsA (mg/100 mL juice)
Ojebyn	Sweden	53
Ben Sarek	UK	87
Triton	Sweden	96
Ben More	UK	98
Titania	Sweden	100
Ben Alder	UK	116
Ben Tirran	UK	121
Baldwin	UK	124
Ben Lomond	UK	133
Blackdown	UK	204

Figure 3 AsA content of juice of berries of *Ribes nigrum* L. cultivars.

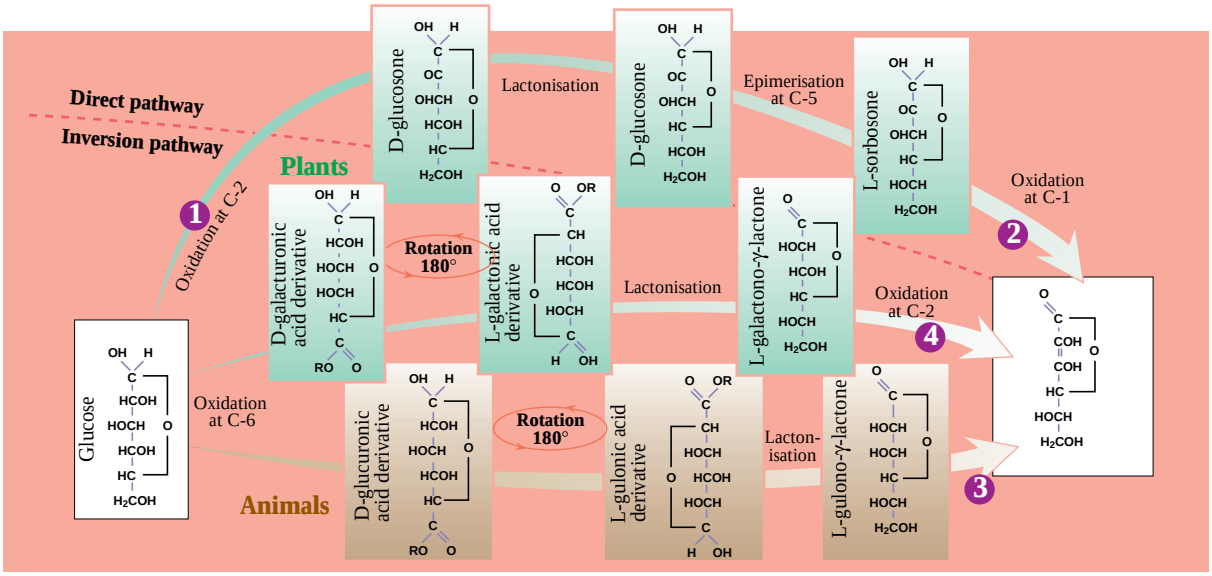

Figure 4 Pathways of glucose conversion into AsA in animals and (putatively) plants. Participation of intermediates of the uronate pools (glucuronate in animals and galacturonate in plants) occurs following oxidation of glucose at C-6 and results in inversion of the carbon chain of glucose. A 'direct' pathway from glucose to AsA involving an initial oxidation at C-2 is also shown. Key enzymes are indicated: (1) pyranose 2 oxidase; (2) sorbosone dehydrogenase; (3) gulono-γ-lactone oxidase; (4) galactone-γ-lactone dehydrogenase.

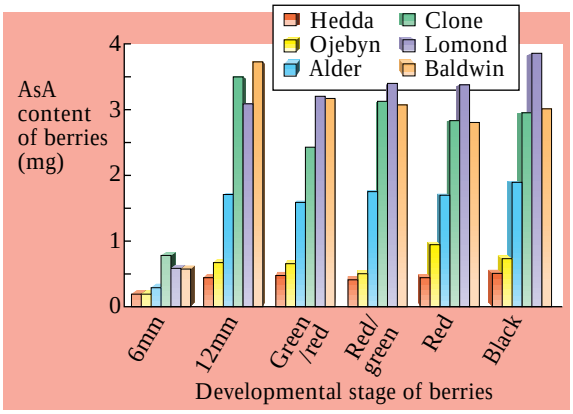

Figure 5 AsA content in blackcurrant berries of different cultivars at various developmental stages. In all cultivars, rapid accumulation of AsA occurs in the early stages of berry development.

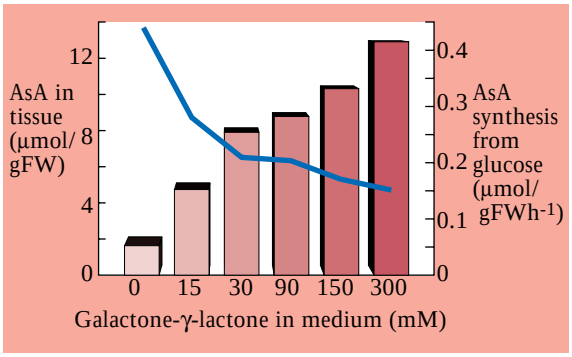

Figure 6 Incorporation of ^{14}C-glucose into AsA (line) by pea seedlings pre-incubated with increasing concentrations of galactone-γ-lactone (GL). A negative correlation is observed between the AsA synthesised during the pre-incubation with GL (bar) and the incorporation of radioactivity into AsA from ^{14}C-glucose.

(e.g. tomato, blackcurrants), the rapid development of techniques for genetic manipulation offers the real possibility to make rapid progress in the generation of plants engineered to contain elevated AsA levels. Arguably, a stable enhancement of AsA levels in crops would substantially improve the nutritional status of plant products in addition to improving tolerance to oxidative stress and, ultimately, increasing productivity. For this purpose, the pathway of AsA biosynthesis in plants needs to be established. In animals, glucose is converted via a sequence of reactions to D-glucuronic acid and finally to L-gulono-γ-lactone which is converted to AsA via a L-gulono-γ-lactone oxidase in the kidney or in liver (Fig. 4). This enzyme is not functional in humans and other animals which have lost the capacity for AsA biosynthesis. The reduction of D-glucuronic acid into L-gulono-γ-lactone is accompanied by a reversal of the numerical sequence of carbon atoms, hence the apparent 'inversion' of the carbon chain of the original substrate. In plants, a similar pathway has been envisaged but involving the derivatives of galactose galacturonic acid and galactone-γ-lactone followed by AsA synthesis catalysed by galactone-γ-lactone dehydrogenase, an enzyme associated with mitochondria and, at least *in vitro*, using cytochrome C as acceptor of electrons. However, experiments with radiotracers in plants (unlike with animals) have singularly failed to confirm that AsA biosynthesis proceeds with the inversion of the carbon chain of glucose. Indeed, on balance, the data collected from a number of investigations indicates that the original numerical sequence of glucose remains conserved, prompting the hypothesis of a 'direct' pathway from glucose to AsA. According to some authors, this conversion involves oxidation of glucose to the unusual osone, D-glucosone, followed by lactonisation and then epimerisation at C-5 to give L-sorbosone and oxidation at C-3 (Fig. 4). However, apart from the studies with tracers, the only evidence in favour of this pathway is the detection of a NADP-dependent sorbosone dehydrogenase in spinach and bean leaves. Direct conversion of glucose to D-glucosone has never been demonstrated in plants but some basidiomycetes contain a pyranose-2-oxidase which can carry out the reaction with generation of hydrogen peroxide. The controversy between 'direct' and 'indirect' pathway has surrounded research on AsA biosynthesis in plants for the past 40 years.

The pathway of AsA biosynthesis in plants is currently under investigation in the Plant Biochemistry Unit. In the past, research in this area has proved to be surprisingly difficult. Firstly, no AsA-free plant mutants are available (given the key role played by the compound on many key processes, these mutants may well be lethal). Secondly, tracer studies for identification of intermediates of AsA biosynthesis in plants have been made difficult by the very limited rates of AsA biosynthesis from simple substrates (sugars). However, progress has been made in these areas recently. An *Arabidopsis* mutant has been described which contains only 30% of the AsA compared with the wildtype[1]. Unsurprisingly, the mutant is hypersensitive to ozone, sulphur dioxide and ultraviolet B irradiation. Secondly, mutant strains of unicellular algae (*Chlorella*) producing 70-fold more AsA than the wildtype have been generated[2]. Access to these mutants may well prove invaluable to our research. Furthermore, evidence has now been provided that AsA biosynthesis is regulated by feedback mecha-

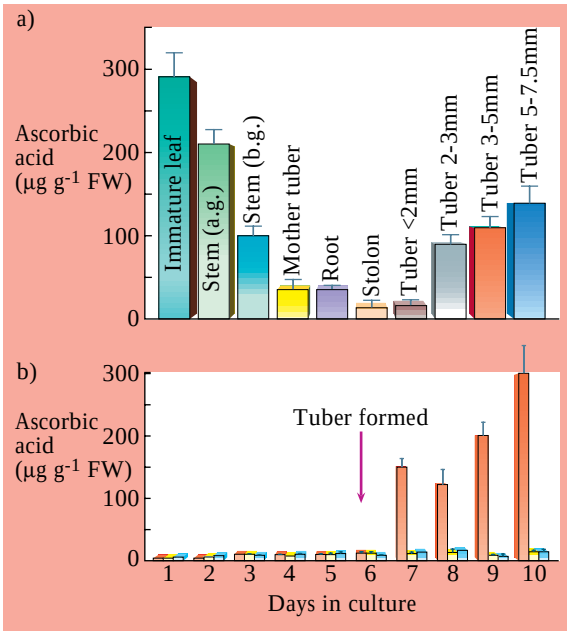

Figure 7 AsA content of (a) various parts of glasshouse grown potato plants cv. Desirée and (b) of single node potato cuttings cultured in the dark. Cuttings were cultured in a modified Murashige Skoog medium containing one-tenth of the standard concentration of nitrate salts and supplemented with 8% sucrose (■), 8% sucrose plus 0.5 mM gibberllin (■) or 1% sucrose (■). Tuber formed synchronously (arrow) after 5 days, only in cuttings grown under tuber inducing conditions (8% sucrose).

nisms, explaining the low level of AsA synthesis observed in many plant tissues. For example, pre-incubation of pea seedlings with the precursors L-galactone-γ-lactone to increase AsA content in the tissue, results in a corresponding decline in the conversion of exogenous ^{14}C-glucose into AsA (Fig. 5). This finding also explains the lack of conversion of ^{14}C sugars into AsA by mature blackcurrant berries which nevertheless contain up to 2 mg/gFW AsA. In these berries, rapid AsA accumulation occurs in the early developmental stages when genotypic differences are also established (Fig. 6). Subsequently, the AsA pool is maintained more or less at a steady state, presumably via DHA reductase and AFR reductase, which 'recycle' the products of AsA oxidation back into AsA (Fig. 2). The AsA pool size of a given tissue is therefore the net product of biosynthesis plus the capacity for recycling of oxidised products (AFR and DHA) versus the degradation of DHA. Very little information is available on the actual net rates of AsA synthesis and breakdown in plant tissues and on the impact of the AsA recycling system on the steady state AsA content. However, it is arguable that AsA steady state levels could be enhanced by stimulation of DHA and AFR recycling in plant tissues with very active rates of AsA utilisation. In other systems, AsA levels may increase substantially only as a result of increased AsA biosynthetic rates. It also remains to be established whether AsA is actually synthesised in storage organs and not translocated with the bulk of assimilates from photosynthetically active parts to the rest of the plant. For example, the AsA content of potato plants is highest in aerial parts (Fig. 7a) and very low levels are detected in roots, underground stems and non-swelling stolons. Following the onset of tuberisation, stolon tips begin to swell and their AsA content markedly increases (Fig. 7a). This raises the possibility that AsA is translocated from the leaves to the rapidly growing tubers together with the other assimilates. However, AsA accumulation is also observed in tubers produced by axillary buds of single node cuttings of potato plants cultured in the dark in a high sucrose-low nitrogen medium. In this system, shoots originating from the buds tuberised within days and a 10-fold accumulation of AsA occurred within 24 hours following the visible swelling. Moreover, no accumulation of AsA is observed when cuttings are cultured under conditions which do not permit tuberisation (Fig. 7b). These findings indicate that AsA accumulation in the tuber is independent of photosynthetic activity and occurs as a result of biosynthetic events *in situ*, presumably as a strictly developmentally-regulated process. Therefore, the *in vitro* tuberisation system described represents an excellent model system for the study of AsA biosynthesis in plant storage organs. The same system has already proved very useful for the investigation of biochemical and molecular events associated with the tuberisation process and the concomitant accumulation of starch in tuberising stolon tips[3].

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A combined theoretical and experimental approach to functional and genetic biodiversity

G.R. Squire & J.W. Crawford

It is widely believed that biodiversity is an important ecological concept, though it is still a poorly defined one. The most common assumption is that biodiversity is simply the total number of species present in a given community. However, this definition ignores the considerable phenotypic variation that occurs within species. Many organisms, moreover, cannot be assigned to species. In the soil ecosystem, for example, most of the different types of bacteria and fungi cannot be cultured *in vitro* and have to be defined by some attribute of general function or by one of several broad-spectrum molecular assays. Even among higher plants, many practical problems concerning biodiversity need to be examined at much finer levels than the species. The movement of genetic material in the agricultural ecosystem is one such instance. Certain crops, such as oilseed rape and sugarbeet, are able to exchange genes not just with a range of weedy and feral populations that originated from previous crops, but also to different degrees with various wild relatives of the crops. Each of the crop, weed, feral and wild populations has its distinct functional and genetic characteristics, yet each is potentially in genetic contact.

Perhaps the main limitation of the species, or any other homogeneous unit, as the primary unit of diversity is the tacit assumption that the dynamics of a population or ecosystem depends only on the 'mean' behaviour of the individuals within each species or unit, and not on any measure of the spread about that mean; and furthermore, that the 'mean' behaviour does not change with the number and corresponding trait distribution of the individuals within any species or unit. Since variation is central to adaptation, a workable definition of biodiversity must incorporate phenotypic variation and its genetic and environmental causes. The following is an indication of how theory and experiment on vegetation are being combined at SCRI to develop such a workable definition.

Theoretical The state of a complex system is defined in terms of the variables which uniquely determine its subsequent evolution. Such a definition can be used to identify the important factors which fix the state of the system, to determine the nature of the dynamics, and to predict the consequences of any change imposed on the system. If these variables are plotted against one another, it is possible to define a multi-dimensional state space, with any one point in the space representing a particular state of the system. The evolution of the system is then represented by a trajectory in this state space.

A state space in a biological system can be described by phenotypic variables and the number of individuals belonging to each phenotype. Therefore, any system can be represented by a frequency distribution across phenotype. In the following, we refer to such a distribution as a phenoscape. Moreover, the phenoscape is necessarily influenced by spatial interactions, since the same individuals can give rise to different community dynamics, depending on their relative spatial arrangement and the nature of the interactions between them. Individuals interact primarily through competition for resources and through the exchange of genetic information.

Because of the large numbers of individuals and phenotypic variables in a real ecosystem, the entire phenoscape can never be measured. However, it is not yet clear how much detail is actually required in order to make predictions regarding the system. In physical systems such as gases and fluids, the trajectories of individual particles in state space are highly complex and divergent. In part as a consequence of this complexity, the system assumes a higher level of organisation known as thermodynamic equilibrium, where its state and evolution can be uniquely defined in terms of a few bulk properties such as pressure, volume and temperature. These

In this simple model we assume a three-dimensional trait space and: (a) competition between individuals limits the number which reach the reproductive stage; the individuals which develop faster, use up the available nutrients, further disadvantaging the other individuals which develop slower; (b) the relative death rate of reproductives, a, and the fecundity, b, are constant in time but may depend on the rate of development to flowering,λ; (c) the rate of decline of progenitors is proportional to the rate of development to the reproductive stage, and the constant of proportionality, c, allows for a loss of viability in the progenitors. The system dynamics are therefore governed by,

1a

$$\dot{f}(\lambda,a,b) = \frac{\lambda s(\lambda,a,b)}{K + \int_{\lambda}^{\lambda_C} s(\lambda,a,b)d\lambda} - af(\lambda),$$

1b

$$\dot{s}(\lambda,a,b) = bf(\lambda,a,b) - c\lambda s(\lambda,a,b) \qquad c \geq 1,$$

where $f(\lambda,a,b)$ is the number of reproductive individuals with traits λ, a and b; $s(\lambda,a,b)$ is the number of progenitors with traits λ, a and b; and λ_C is the maximum rate of development. We denote partial differentiation with respect to time with a dot-accent i.e. $\dot{f}(\lambda,a,b)$ denotes the rate of change of $f(\lambda,a,b)$ with time.

The solution to the above shows that co-existence of individuals with a broad range in traits requires that a and b depend on the development rate constant λ. The nature of that dependency can be characterised mathematically. The stability of that distribution further constrains the interdependency between the traits, and shows that only a small subset of the trait space can be occupied by the population. Perturbation outside that subset as a consequence e.g. of mismanagement, disease or pest attack, will lead to a rapid narrowing of the distribution corresponding to a decline in diversity. Inclusion of genetic coupling shows, not surprisingly, that the nature of gene exchange can have a significant influence on the stability as well as the shape of the distribution across the trait space.

Figure 1 Simple theoretical construct.

properties can be related directly to the distribution of individuals across the state space and so the details relating to individual particles are not required. Although the concepts underlying thermodynamics may not be applicable directly to ecosystems at the population level, it might nevertheless be possible to derive population-level descriptions which are directly measurable and uniquely define the state of the system.

These ideas are being explored by using mathematical approaches to determine how far it is possible to reduce the complexity of ecosystems yet still capture their essential behaviour. The main aim of initial work is to derive a method for relating the trajectory of the individual to the dynamics of the population of which it is part. As a first step, we have examined the relative roles of individual variation and gene flow on the stability and dynamics of a community in a spatially homogeneous environment. In the simplest case examined (Fig. 1), the genetic coupling between individuals is assumed to be insignificant, and therefore individuals self-propagate. The frequency of a specific trait therefore depends on its frequency in the previous generation and a multiplicative factor depending on competition and fecundity. We also began by defining the population in terms of three traits (time to flower, fecundity and death rate) since development to a greater number of traits is an algebraic, rather than a conceptual, inconvenience.

With this simple approach, we are able to demonstrate that diversity is maintained through interdependencies and trade-offs between traits. Indeed, the state space can be reduced to a single trait, to which the other two are functionally related. This supports our hypothesis, stated earlier, that community level descriptions may be derivable in terms of only a few variables. These approaches are being extended (with colleagues) in order to introduce to the models some spatial heterogeneity and various degrees of genetic coupling between generations.

Experimental The experimental parts of the programme are linked tightly to the theoretical parts and use a series of natural and artificial vegetation systems. A common approach is used, based on following individuals through their life cycle and building knowledge of the behaviour of the whole population by combining the trajectories of the individuals. One of the most instructive examples of this approach derives from a set of developmental data supplied by the University of Nottingham for the African subsistence legume, bambara groundnut (*Vigna subterranea).* The development of variation in a simple vegetative trait,

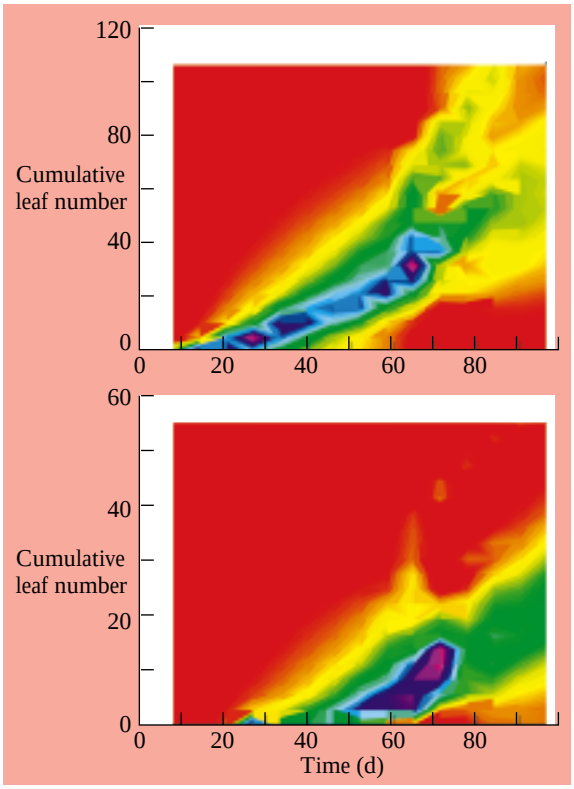

Figure 2 Leaf number (a) and flower number (b) phenoscapes for a population of bambara groundnut in Sierra Leone. Each shows the frequency of character-time states estimated from measurements every 3 days on a population of 48 plants. In the colour scale, orange-red is zero and purple a frequency of about 25% in the population.

such as leaf number, was first visualised by combining leaf-time curves for all individuals into a phenoscape showing the fraction of the population occupying any particular leaf state at any time (Fig. 2a). A similar procedure for flowers gives a corresponding map of flower-time states (Fig. 2b). These phenoscapes - each for one variable on time - show that the ridge of high occupancy around the mean during the few weeks after sowing invariably dissolves over time into a much broader distribution of states. It is a characteristic of many populations that, even when the mean stabilises or starts to fall as more leaves die than are produced, the variation between individuals continues to increase. This is indicated by a continued rise of the variance/mean and of the 'entropy' of the leafing states after the mean has stabilised.

The two parts in Figure 2 each show cross-sections through two-parameter state space. However, the trajectories of individuals through the three-parameter space (leaf, flower, time) are not simply obtained by overlaying one cross-section on the other. Plants with the fastest leaf production are not necessarily those with the fastest flower production and *vice versa*, i.e. there are trade-offs between traits as discussed earlier. The ranking of individuals, and the correlation of early leaf and later flower states, changes considerably from emergence to maturity, such that the mean or modal group in early vegetative growth is not the same as that during flowering. In common with theoretical aspects of the research (Fig. 1), one of the main aims of work with real plants is to examine the complicated linkages between vegetative (resource capturing) and reproductive states. In this instance, both the theory and experiment also have practical relevance, since a proportion of the cultivated populations (in common with subsistence species generally) do not yield under stress even though they capture resources. To explain yield-limiting processes in the dry tropics, therefore, knowledge is required of the individual rather than just the bulk stand.

Several new experimental and observational systems were established in 1995 and 1996 in order to examine phenoscapes in detail both spatially, and within and between generations. A main spatial experiment was established at the Invergowrie trial site in 1995 and is designed to run initially for 4 years. It will examine the way phenoscapes in three common, wild species, differing in developmental habit, are influenced by spatial configuration. The design of a two-species plot (Fig. 3) employs a precision-sown,

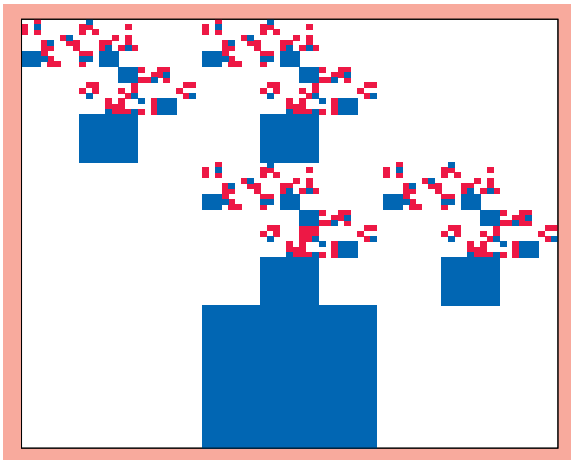

Figure 3 Field plan of a two-species plot in the spatial experiment at the High Pilmore field at SCRI. The plot has a side length of 8.1 m and is divided into 10 cm squares, some of which were sown with one species to form a series of block structures (blue) and another species to form an interaction front (red).

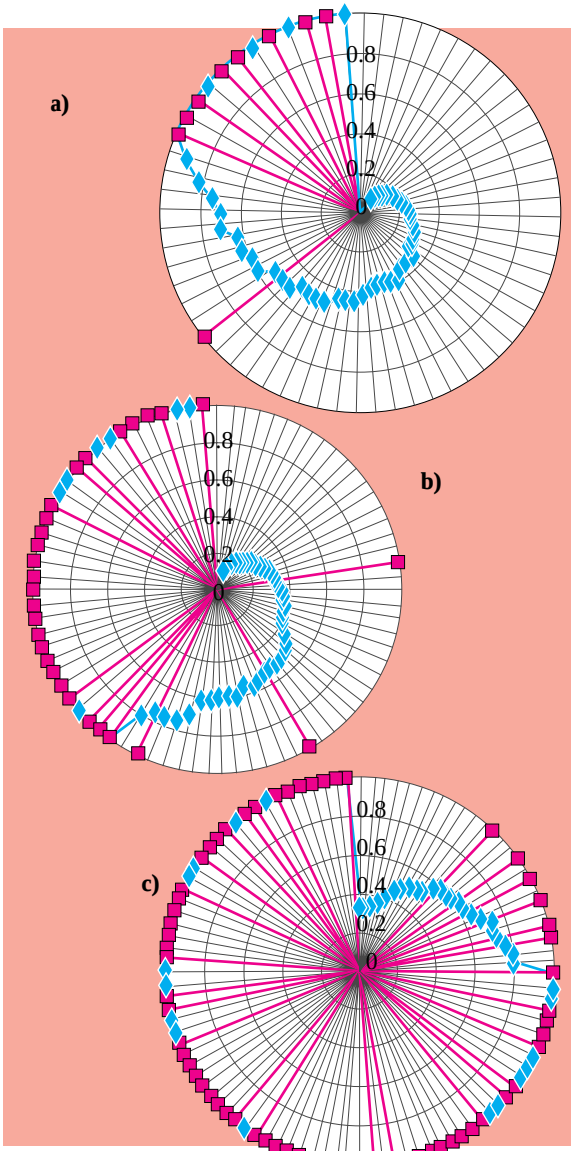

Figure 4 Examples of circular ranking diagrams for populations of *Thlaspi arvense* derived from measurements on the same day in June in the spatial experiment at High Pilmore (Fig. 3). Ranking of the individuals within a defined area is by vegetative state (blue diamond) expressed in terms of arbitrary progress of leaf node number to a maximum; largest plants at the top left, mid-point at the bottom. The corresponding reproductive state of each plant is shown as either 'on' (pink squares) or 'off' (no symbol). (a) in the 'front' structure with strong interactor, *Anchusa arvensis;* (b) in the block structure with weak interactor, young *Holcus lanatus;* (c) with no interaction, isolated plants outside the design in Figure 3.

recursive fractal lattice, that allows a wide range of interaction distances and groupings to be examined in a small area of land. The primary effects on plant performance are likely to be caused by the immediate neighbourhood, which can be defined by the plants and spaces surrounding any individual. The trajectories of individuals are followed to examine the effect of spatial scale on resource capture and reproduction. Typical effects of spatial position are illustrated by the circular ranking diagrams in Figure 4, which show at a particular date both the vegetative and reproductive states (the latter on or off). The shapes of these diagrams are indicative of a cross-section in time through a simple phenoscape. The concepts developed from estimating the interaction strengths over distance are being used to refine the spatial models referred to above.

This theoretical and experimental approach is being applied to ecological problems in the Tayside area and more widely. Specific systems (which also represent case studies of different complexity) include oilseed rape as a crop, weed and feral plant; wild raspberry populations; and species-rich grassland. The links between phenoscapes and the genome are being examined through collaborative work on DNA markers with the Crop Genetics Department (oilseed rape), the Soft Fruit and Perennial Crops Department (wild raspberry) and with other SOAEFD organisations, SAC, MLURI and BioSS (grassland species). Within the Unit of Soil-Plant Dynamics, research on plants runs parallel to studies on soil structure and biota. The combined aim is a rigorous and quantifiable description of biodiversity that can be applied to a wide range of fundamental and applied ecological problems.

We are grateful to Dr S.N. Azam Ali and Dr S. Collinson at Nottingham University (Sutton Bonington) and Dr Abu Sesay (previously Njala University College, Sierra Leone) for the data on bambara groundnut. Colleagues in the Unit, Dr Bruce Marshall, Gladys Wright, Dr Eric Grist and Gordon Dunlop, are collaborating on spatial experimentation and spatial modelling.

The potential impact of terrestrial planarians to Scottish agriculture and wildlife

B. Boag & R. Neilson

The New Zealand flatworm (*Artioposthia triangulata*) was first reported from Scotland in 1965. It was found to be an obligate predator of lumbricid earthworms, and in the late 1980s it was demonstrated that it could reduce earthworm numbers to below detectable levels under agricultural conditions[1]. In 1991, the Scottish Office Agriculture, Envivonment and Fisheries Department (SOAEFD) commissioned the Scottish Crop Research Institute to undertake a survey to determine the distribution and status of the New Zealand flatworm in Scotland. Examination of samples from 348 domestic gardens indicated that it was distributed throughout most of mainland Scotland plus some of the islands e.g. Bute, Gigha, Orkney and Skye[2]. It also found that 56 of the 217 botanic gardens, nurseries and garden centres visited were positive for *A. triangulata*. Although only one of the 600 randomly selected farms was found to have the New Zealand flatworm, it is known to be present in a total of 13, the majority of which are in the west of Scotland, especially around Dunoon. Analysis of the data obtained from the questionnaires suggested that the New Zealand flatworm probably first spread between botanic gardens, nurseries and garden centres and then became established in domestic gardens via the trade in containerised plants. Because of the lack of exchange of soil from domestic gardens to agricultural land, only a few farms have been infected. Three of these were known only because fishermen could not find worms to use as bait and had informed the farmers.

In 1992 a second report of *A. triangulata* was obtained from England[3], with another three in 1993 and a further 20 in 1994. A survey organised by Dr H. Jones, Manchester University was undertaken by the television programme *'Tomorrow's World'* in 1995, which again added new reports of *A. triangulata* in England and Scotland[4]. Because of numerous television, radio and press releases, over 100 new reports of flatworms in Scotland were received annually between 1993 and 1996. These data showed that the distribution of *A. triangulata* extended to all the major islands, and confirmed Edinburgh as the epicentre of the infection with over 100 records from the 10 km^2 which contain the Botanic Gardens. A retrospective analysis of the rate of spread of the New Zealand flatworm in Scotland can be seen in Figure 1.

Probably the most important finding resulting from the *Tomorrow's World* survey was the rapid spread of a second flatworm species, the Australian flatworm (*Australoplana sanguinea* var. *alba*). This species, which was first recorded from the Scilly Islands in 1981, had spread throughout south-west England, extending up through the midlands and Wales to Dumfries in Scotland. It was also found in the Isle of Man and Ireland[4].

Interest in the rate of spread and distribution of the New Zealand flatworm has not been confined to the British Isles since it was found to have become established in the Faroe Islands. Investigations into the distribution of *A. triangulata* in New Zealand indicated that it was limited to the centre and south of the South Island[5]. The application of the CLIMEX model using meteorological data from New Zealand and Britain, was used to predict the potential spread

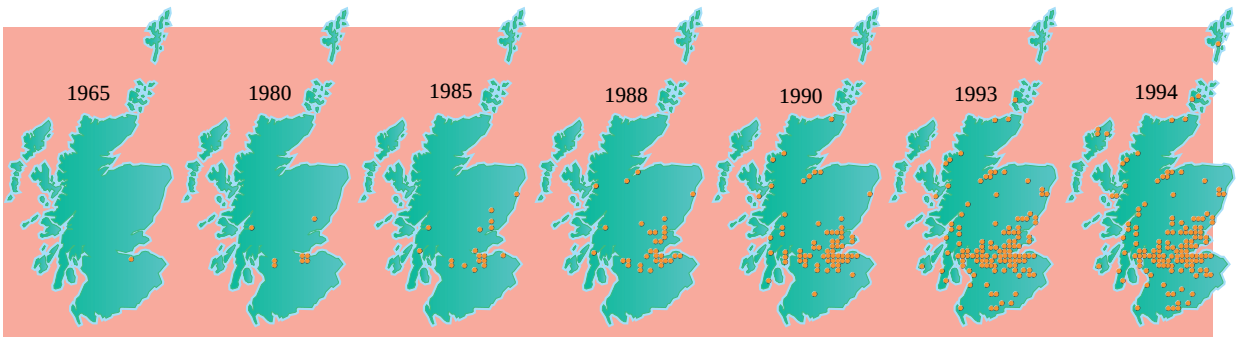

Figure 1 Spread of New Zealand flatworm in Scotland.

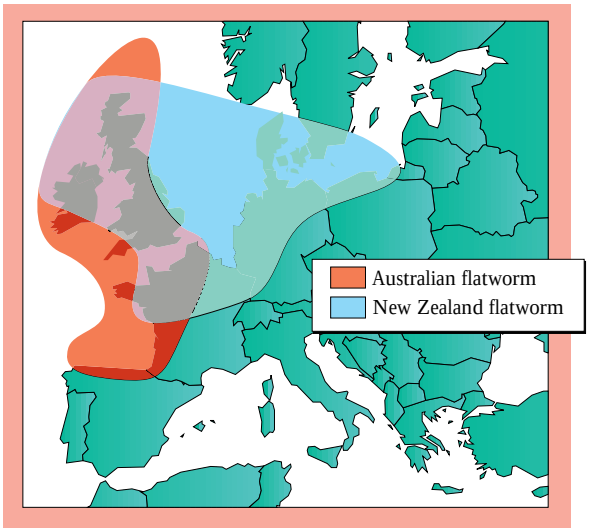

Figure 2 Potential spread of the New Zealand flatworm and Australian flatworm to continental Europe.

of *Artioposthia triangulata* and *Australoplana sanguinea* throughout the world and to continental Europe in particular[6,7]. Results indicated that New Zealand flatworm has the potential to become established in north-western Europe as far south as the middle of France, while the Australian flatworm has potentially a more southerly distribution which included the north of Spain (Fig. 2). However, while the New Zealand flatworm may become established in an area, it does not necessarily mean that it will have a significant impact on earthworm populations and hence agriculture and wildlife. Observations in Scotland have shown that in the drier east of the country, earthworms have co-existed with flatworms for many years and it is only in the damper west that earthworm numbers have been decreased to below detectable levels in agricultural land.

The prediction, made in 1993, that even more species of predatory terrestrial planarians could become established in the British Isles was unfortunately proved to be true when another species was found in the summer of 1996 in Edinburgh (Fig. 3c). Experiments undertaken in New Zealand in 1996 by the senior author have confirmed the predatory behaviour of other planarians, some from the North Island e.g. *Artioposthia testacea* (Fig. 3d), which are probably better adapted for warmer/drier conditions and which could become an even greater threat to agricultural production and wildlife if they become established in the British Isles and continental Europe. Since there is no known approved chemical or biological control agent for these alien planarians, it is imperative that strict hygiene is observed both between retail outlets and domestic gardeners to slow down its spread within the British Isles, and by wholesale traders who import and export containerised material to stop their spread to continental Europe from either the British Isles, Faroes or New Zealand.

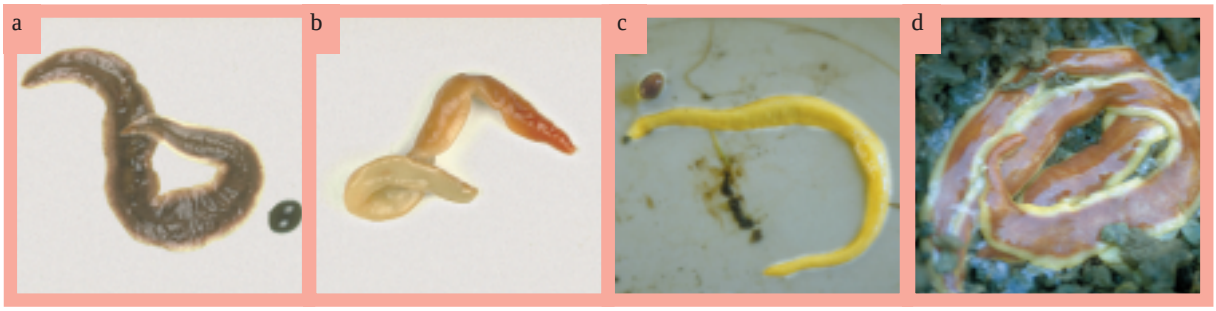

Figure 3 (a) *Artioposthia triangulata*. (b) *Australoplana sanguinea*. (c) The new flatworm, Edinburgh 1996. (d) *Artioposthia testacea*.

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Prions, plants and transmissible spongiform encephalopathies

J.M.S. Forrest, T.M.A. Wilson & J.R. Hillman

An article with such a title may appear out of place in the 1996-1997 Annual Report of the Scottish Crop Research Institute; after all, prion genes have not been found in plants. A product isolated from barley DNA by PCR, with selected primer sequences from the ovine prion gene, was in fact highly homologous with part of the retrotransposon-like, dispersed repeat from the R173 family in rye[1]. So, have the research programmes at SCRI and other plant-based Institutes escaped the ravages of Bovine Spongiform Encephalopathy (BSE)? Certainly not. Real and perceived damage has been done to previously exemplary UK policies, regulatory and monitoring procedures. The BSE episode has further eroded public credibility and acceptability of science and scientists, although it was inadequate regulation of an industrial process plus an admitted lack of scientific research and knowledge on prions, which caused the problem: in essence, a manifestation of ignorance. Remedial spending on BSE from the public purse is expected to be approximately 1.9 billion ECUs between 1996-1999, a figure which does not include the cost of intervention purchases, the selective cull of cattle over 30 months old (abattoirs were paid between £41 and £87.50 per animal slaughtered in 1996), or the impact on employment and commerce in the UK and elsewhere. At some point, the cost of BSE will far outweigh any accumulated past savings from feeding supplementary animal protein to herbivores. There will be no such thing as a cheap lunch for cattle!

Cattle are primarily herbivores (although a newly calved cow will eat its own afterbirth, presumably recycling nutrients), and one role of SCRI has always been to improve the crops which nourish our domesticated livestock. For economic reasons and convenience, intensively reared cattle require artificial diets with protein supplements to compensate for the lack of time and space for free-range grazing. Here, the roles of forage crops and the scientists who breed and develop them, have gradually been usurped by a policy to include animal protein in the herbivore diet, to reduce production costs by increasing 'efficiency', while disposing of undesirable animal waste. Further economies, through modification of the rendering process by which animal carcasses are converted to meal and tallow, are blamed for the recent unforeseen and devastating consequences to the beef industry. Moreover, the full environmental penalty has yet to be paid for depleting global fish (and sand eel) stocks to obtain supplementary fish meal for animal feedstuffs.

At a different level, the urgent need for information about BSE, and its transmissibility to livestock, pets, the natural fauna, and particularly to humans from an array of livestock species, will grossly distort the science budget for the non-medical life sciences. It is doubly unfortunate that forage crop plant science should suffer in this way.

Stephen Dorrell's statement in the House of Commons in March 1996, linking BSE with the human equivalent, Creutzfeldt-Jakob Disease (CJD), initiated an enormously adverse chain of events on the beef and beef-based food industries, and on the agricultural supply industry. Across the UK, exports to the order of £520 million per annum have been banned by EU edict. The necessity for disposal of specified risk material (SRM) has added an estimated cost of £6 (1991 prices) for every cow slaughtered and rendered. A broad swathe has also been cut through the profitability of diverse businesses such as butchers, manufacturers and suppliers of feedstuffs, cooked meats, flavourings, stock cubes, and edible, cosmetic or pharmaceutical products containing gelatine or collagen. In May 1997, it was announced that the export ban on UK gelatine would continue. Lifting of the ban on beef itself is even more remote. Around £1.2 billion of the Scottish economic output is beef-related, and agriculture, slaughtering and meat processing are closely interde-

pendent. The loss of 0.5 % of the Scottish Gross Domestic Product and up to 8,000 jobs has been predicted[2], yet the 7,950 cases of BSE recorded in Scotland since 1988 represent only about 4.7% of the UK total to-date. In June 1997, strict procedures for dealing with sheep carcasses were announced by the new Government, but costs to the industry have yet to be revealed.

Whatever the economic reasons, and wherever blame is apportioned in the UK, it is notable that Switzerland, the country next most affected by BSE after the UK, apparently embarked on the inclusion of animal protein in foodstuffs for different reasons *viz.* to discourage African countries from exporting soya meal for animal feed while the indigenous population went hungry. No matter how laudable the drive to amend feedstock practices, we ignore the application of sound scientific knowledge and fundamental research at our peril. It must be clear, retrospectively, that plant breeders and their co-workers hold the most natural and sustainable solution to food production for both humans and livestock, through the continued development of high-yielding crops with pest resistance and improved nutritional status. The scale of remedial expenditure envisaged for BSE would have sustained all our accelerated and conventional plant breeding programmes and their associated research over many decades.

Public perceptions of food safety, heightened by media attention over the years to 'Chernobyl' lamb, *Salmonella* in eggs, *Listeria* in cheese, BSE in beef, and, most recently, *Escherichia coli* O157 in meat products, may even lead to a permanent shift from omnivory towards vegetarianism by a sizeable portion of the population. This, in turn, could create a market for new edible vegetable products. Fears and misconceptions about the safety and nature of genetically engineered crops, such as soybean, still have to be assuaged, but pale into insignificance alongside the panic caused by the present BSE crisis. The biotechnological manipulation of crops may be deemed by some to be 'unnatural', but the feeding of waste animal products to herbivores must be far less ethically acceptable to those concerned with animal welfare.

Public opinion, however, should recognise the fact that renderers carry out the essential function of processing very large quantities of animal waste. Another product currently under scrutiny is animal tallow, a traditional feedstock for the production of fatty acids. The 'splitting process' is conducted under such prolonged high temperatures and pressures that it is difficult to envisage the survival of any infectious agent, including prions. Nevertheless, customers are demanding the use of imported tallow or alternative feedstocks, such as erucic acid, derived from plants. Perhaps what is required is Government action both to publicise methods of processing which destroy prions and to stimulate research into new products in less sensitive areas than animal feed. British renderers can also do much to protect their share of the market by imposing the strictest quality controls on all stages of tallow production.

Prions (proteinaceous infectious particles) are thought to consist mainly of an abnormal form of the prion protein, designated PrP^{Sc}. The presence of PrP^{Sc} is a diagnostic feature of the group of diseases of humans and animals (Table 1), collectively known as the Transmissible Spongiform Encephalopathies (TSEs).

The normal prion protein, PrP^{C}, occurs naturally in all mammals and is found on the surface of many cell

	Disease	Transmission
Animal		
Sheep, Goats	Scrapie	Sporadic inherited infectious
Cattle	Bovine spongiform encephalopathy (BSE)	Infectious
Cats	Feline spongiform encephalopathy (FSE)	Infectious
Mink, Ferrets	Transmissible mink encephalopathy (TME)	Infectious
Mule deer, Elk	Chronic wasting disease (CWD)	Infectious
Antelope, Kudu, Nyala, Oryx	Exotic ungulate encephalopathy (EUE)	Infectious
Human		
	Kuru	Infectious
	Creutzfeldt-Jakob disease (CJD)	Sporadic inherited infectious
	Gerstmann-Sträussler-Scheinker syndrome (GSS)	Inherited
	Fatal familial insomnia (FFI)	Inherited

Table 1 Transmissible spongiform encephalopathies.

types, but at especially high concentrations on neurons. Its role is uncertain. Transgenic mice devoid of PrP^C are viable and resistant to scrapie infection, although some behavioural abnormalities reminiscent of scrapie become apparent with age. These mice lack Purkinje neurons which are thought to control balance[3] and regulation of circadian rhythms and one possible role of PrP^C is the long term survival of these cells. Although PrP^C is also absent from the Purkinje cells of transgenic mice with half genomic constructs, it is abundantly present in the hippocampus and cerebellum of the same mice, suggesting that control elements essential for specific expression in Purkinje cells are missing from these constructs.

Both isoforms of the protein (PrP^C and PrP^{Sc}) are encoded by the same gene, and are believed to differ only in their three-dimensional folding or so-called 'conformation', which can be modified after synthesis. Current theories suggest that once PrP^{Sc} is introduced, either as a contaminant, or by a rare spontaneous change in conformation, it may convert further PrP^C molecules to the new isoform. This may involve dimerisation, unfolding and refolding in the abnormal conformation - a sort of chain reaction leading to loss of PrP^C and an increase in PrP^{Sc}. The conformational change is sufficient to alter the properties of the new isoform, PrP^{Sc}, and the way in which it is processed within cells. Thus, PrP^{Sc} is insoluble, partially resistant to degradation by proteases, and tends to accumulate in the cytoplasm rather than at the cell surface. There is considerable debate as to whether the diseases are caused by the absence of functional PrP^C or the presence of PrP^{Sc}, or indeed if these are by-products of another (related) series of faulty signalling interactions. 'Protein splicing' is a further post-translational process involving intermediate steps which could cause conformational change. No evidence has yet been found for the occurrence of this process in prions[4], but it could produce new conformations or even hybrid molecules from those proteins closely associated with PrP itself - something which might help to explain the variety associated with 'strains' of prion. There is no shortage of dimerising proteins such as the 14.3.3 group, which participate in signal transduction pathways and cell cycle regulation and occur in the cerebrospinal fluid of patients presenting with dementia. So far they have attracted attention mainly because of their potential for confirming the diagnosis of CJD. However, it is also interesting to note that in plants, highly homologous proteins of the 14.3.3 group may have a role in signal transduction in response to pathogens.

One peptide from the prion protein is toxic to neurons in the presence of other brain cells known as microglia. Affected brains are usually characterised by vacuolation of the grey matter in the medulla oblongata close to the obex. Enhanced cell death and degradation may account for the vacuolation and deposits of PrP^{Sc}. Proteins regulating apoptosis (programmed cell death) and longevity are likely to prove fruitful subjects for prion research. A considerable number of researchers still continue to challenge the 'protein only' theory[5]. The presence of PrP^{Sc} in the nucleus of mouse neuroblastoma cells[6] and association of prion protein with Carbohydrate Binding Protein (CBP35) in ribonucleoprotein complexes as well as in purified infectious prion particles, has led to suggestions that PrP may be involved in regulating its own mRNA at the post-transcriptional level[7].

BSE is a fatal, neurodegenerative disease, which probably exists at a low level throughout Europe, but has reached epidemic proportions in Britain, leading to the slaughter of more than 167,000 cattle between 1986-1995. Numbers of infected cattle which have entered the human food supply over this period, are estimated to be very much higher, at around half a million[8]. Speculation that a new variant of CJD (vCJD), which has recently appeared in people less than 40 years of age, may be acquired by eating SRM or beef contaminated with BSE PrP^{Sc}, caused a greater than 30% fall in beef consumption throughout Europe in 1996. Furthermore, the demonstration that the prion protein derived from vCJD has similar strain characteristics to BSE transmitted to mice, cats and macaques, provides supportive evidence for such a link[9]. While consumption of PrP^{Sc}-contaminated beef seems the most likely source of the problem, it is not the only possibility. For many years, eyes of freshly slaughtered cattle and sheep have been dissected by secondary school children studying mammalian biology. Were our young people unwittingly placed at risk? Kuru is thought to have been transmitted by conjunctival nasal and skin contamination with infectious tissue. Many modern viral vaccines and therapeutic proteins are grown in animal cell cultures. Vaccines have been prepared with the use of bovine products (e.g. foetal calf serum) in culture media, and it is not inconceivable that cattle or humans could have been co-inoculated with bovine prions. Similar dangers may assail the new industries which use transgenic animals to obtain human proteins or whole organs for transplantation. If there is evidence of a TSE epidemic, it would be preferable on grounds of

safety and economics to produce some of these proteins in plants[10,11,12]. Finally, it is also evident that large numbers of people have received blood and organs from asymptomatic donors already infected with CJD itself[13]. This possible route of transmission is currently disputed through lack of evidence, but it could be the source of the new variant and may act to increase the incidence of CJD quite independently of BSE. In contrast, scrapie and the human consumption of lamb has never been associated with the transmission of TSEs over the past 200 years. Now, the effects of consuming abnormal prions from different species must be considered. BSE has been transmitted to sheep by feeding infected cattle brain, and so sheep may have become naturally infected by contaminated feed supplements. Sliced neck of lamb, a traditional stewing cut which contains vertebrae and sections of spinal chord, ceased to be available on supermarket shelves during the second half of 1996. This sign of concern was recently confirmed by the Government order enforcing the removal of SRM from sheep and goats over one year old. The position occupied by meat from omnivores such as pigs and chickens as potential sources of abnormal prions, has still to be established. PrP^{C} was also reported for the first time in the brains of salmon.

One controversial condition imposed by the European Commission which must be fulfilled prior to the resumption of British beef exports, is the compulsory cull of cattle aged over 30 months. Since the BSE epidemic now appears to be in a phase of rapid decline, and is predicted to be close to eradication by the year 2001[8], it has been argued that only culling very large numbers of cattle will substantially increase the current rate of decline. The most cost-effective cull could be achieved by targeting herds with a high incidence of BSE, and the progeny of previously affected dams within those herds. This prediction assumes that no new infections from contaminated feed occurred after 1994, and that the only mode of transmission will be (infrequently) from dam to calf. Maternal transmission is still a contentious issue. Late in January 1997, following the alleged birth in Germany of a BSE-infected calf to a Galloway cow, a proposal was made to slaughter all 5,000 cattle imported from the UK and Switzerland. The calf was subsequently proved to have been born in the UK, but this did not reverse the slaughter policy.

Even if vCJD can be contracted by eating PrP^{Sc}-contaminated beef and other products, it may remain a relatively rare disease with a frequency of less than one in 10^{6} per annum. Virtually all victims of sporadic CJD share the same genetic trait *viz.* homozygosity at codon 129 of the prion gene for either methionine or valine. This is also true of vCJD and of iatrogenic (acquired) CJD, which developed in children treated with growth hormone derived from infected pituitary glands. Among children treated before 1985, 1.5 % have already succumbed to the disease[14]. This is a considerable increase over the natural incidence of sporadic CJD. Homozygosity for codon 129 is present in approximately 50% of European caucasians. We do not know how many people have received critical doses of prions or if further predisposing factors will act synergistically, with catastrophic results. Every effort must be made to identify the source of the problem and provide a cure. Only when this has been achieved can we say with certainty that there is no danger to our population from BSE. An important part of the CHABOS Science Strategy 1997-2000 has therefore been devoted to addressing the BSE/CJD problem.

A few examples illustrate the extremely robust nature of PrP^{Sc} which has been the cause of the BSE problem. Destruction is only guaranteed by prolonged autoclaving at higher temperatures than are required to kill other pathogens. Prions will therefore survive cooking and many standard sterilisation procedures. Remarkably, scrapie prions have also been shown to retain their infectivity after burial of infected carcasses for up to two years, and animals from scrapie-free flocks, grazed on farms which have been without sheep for three years, still contract the disease. The previous UK Government wisely banned the use of meat and bone meal as food and fertiliser, but there are more obscure sources of potential infection. Cattle with BSE which have been buried either officially or clandestinely to avoid detection (JCB disease!), may in turn act as sources of prions. There may be further dissemination by routine cultivation and carrion feeders such as blow flies. It is significant that 'hay mites' which consist of a mixture of phytophagous and parasitic species, have been reported to harbour and perhaps multiply prions[15], but these results await confirmation. Furthermore, it has now been established in the USA that wild as well as captive mule deer and elk are affected by the TSE known as 'Chronic Wasting Disease', and it remains to be seen whether there is a link between this disease and BSE or Transmissible Mink Encephalopathy. Meanwhile, in Australia it has been pointed out that scrapie does not occur naturally, although there are

genetically susceptible sheep present, implying the absence of some factor essential for the propagation of the disease.

In the UK the possibility that wild mammals, especially small insectivores, may act as reservoirs of prions must also be considered, and the influence of parasites such as ticks which may feed on a range of hosts, should be borne in mind. Finally, there is the possibility of importing infected livestock or livestock products. Although sperm and embryos have not yet been shown to carry infection, doubts about the nature of maternal transmission may yet lead to an embargo on exports of cattle semen from the UK.

The scenario described above may 'ring a bell' with plant scientists, conjuring up familiar images of phytosanitary schemes to produce various important crops, e.g. soft fruit, *Narcissus* and healthy potato seed for ware production; the eradication of viroids from true potato seed; tissue culture of potato meristems to eliminate viral infections; propagation of virus-free stem cuttings protected from aphid vectors; planting of certified seed tubers of known origin in areas where virus vectors are sparse, and in fields which are cropped with potatoes only once in 10 years; and production on farms which have been sampled and found free from persistent soil-borne pests such as potato cyst nematode. It is likely that a similar path will be trodden to establish the BSE-free nucleus of the national beef herd, each member of which can be traced to its healthy origin, and which will be reared in a BSE-free environment to become the first fruit of a sophisticated quality assurance scheme which guarantees the wholesomeness of the product to the consumer. A leading UK supermarket chain has taken the initiative to establish something similar to this scheme with owners of BSE-free herds in North East Scotland.

As domestic demand for beef products starts to return to normal, the present Government has correctly drawn attention to the folly of importing beef from countries where controls over SRM are now much less stringent than our own, and likely to remain so for some time. Indeed, the original source of BSE could have been infected cattle, beef or other material imported from countries where veterinary pathology has been inadequate.

The single most important achievement to contain and solve the BSE problem will be the establishment of practical diagnostic tests which can be used to detect pre-clinical disease in live animals or fresh carcasses. This will enable the identification and removal of infected breeding stock as well as carcasses from the human food supply, at abattoirs or even in transit between member states. A blood test would be the most suitable for live animals and there is already evidence that PrP^{Sc} is present in the blood components of some forms of familial CJD as well as in sheep with scrapie[16,17]. Lymphoid tissues of slaughtered animals may be more easily obtained for examination than brain, and pre-clinical scrapie has been diagnosed from the tonsils of sheep a year before the development of the disease. It remains to be seen if lymphoid tissue is a suitable target in cattle, but there are other accessible organs such as eyes. At present, detection of PrP^{Sc} relies on treating tissue with Proteinase K to degrade PrP^{C} completely, and PrP^{Sc} to a residual fragment called PrP27-30 which can then be identified serologically. Currently, there is no antibody which can distinguish between native PrP^{C} and PrP^{Sc}, but this is a target of present research which should generate new and powerful probes.

There is still much unfinished business which can be exploited by plant breeders for the benefit of livestock. Swedes have traditionally been used to fatten cattle and lambs, and overwinter ewes. They supply metabolisable energy at about half the cost of that in silage or barley. Kale, forage rape and stubble turnips have played a part respectively in the feeding of dairy cattle and store lambs. The yield, utilisation, palatability and nutritive value of these brassica crops remain to be improved further.

Faba beans are grown on a relatively small scale in the UK, but most of the crop is used as a protein-rich

Data supplied by N. Offer, SAC.

	Metabolisable energy Megajoules/kg Dry matter	Crude protein g/kg dry matter	Degradability †	Relative * feed value £
Barley	13.0	125	0.85	105.00
Faba bean meal	12.8	300	0.89	143.00
Rape meal	12.0	400	0.80	160.00
Soya bean meal	13.3	520	0.70	196.60
Meat and bone meal	17.5	666	0.40	270.40
White fish meal	14.2	693	0.45	250.00

† Proportion of crude protein broken down in rumen.
* Relative value of each feed in supplying unit energy and protein, using barley and rape meal as reference feeds

Table 2 A comparison of some cattle feed components.

component of animal feed, replacing products such as fish meal and meat and bone meal. Faba bean has 20-30% protein and 41% starch (see Table 2 for a comparison of some current and former components of cattle feed). Constraints on the use of faba beans could be lifted by breeding both to reduce anti-nutritional factors, to improve pest/disease resistance and to increase yield. Maize is already grown as cattle fodder in the mildest parts of Scotland. A variety tolerant of late frosts would extend its range of cultivation considerably. Improved barley cultivars, produced specially for livestock, could make a more subtle contribution to stock feed than in the days of 'barley beef'. These programmes of plant breeding and their underpinning research must be addressed urgently. The development and use of the products will represent the return of a traditional role to the plant-based research Institutes.

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Chemistry

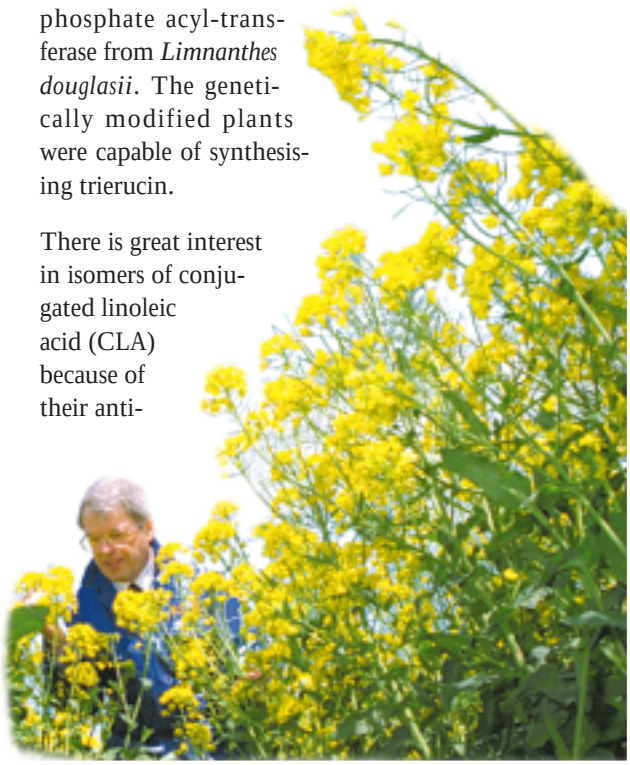
W.W. Christie

The progress achieved through the chemical expertise of the Institute is reviewed. It encompasses the investigations of the Chemistry Department, which includes the Lipid and Magnetic Resonance Units. Some topics are covered directly by the Institute's commissioned remit; others are supported financially by outside agencies.

In order to investigate the mechanism of silver ion chromatography, the retention properties of various benzyl, phenacyl, and normal- and branched-chain alkyl esters of 6-18:1, 9-18:1 and 11-18:1 fatty acids were studied. The weak electron-donating effect of the alkyl substituents on the carbonyl oxygen in the aliphatic alkyl esters ensured base-line resolution of *iso*propyl and *tert*.-butyl esters, but not of *n*-alkyl esters. By comparing benzyl and phenacyl derivatives, it was confirmed that the silver ion interacted simultaneously with the double bond of the fatty acid and the carbonyl oxygen of the phenacyl moiety. Thus, introduction of electron-withdrawing or electron-donating substituents in the *p*-position in the benzene ring decreased or increased retention, respectively. It is the dual interaction with the silver ion and the two centres, carbonyl oxygen and double bond, in the fatty acid derivative that enables the separation of positional isomers.

In collaborative work with Professor Slabas of Durham University, it was demonstrated that the erucic acid content at the *sn*-2 position of triacylglycerols in oil seed rape was affected by the introduction of 1-acyl-*sn*-glycerol-3-phosphate acyl-transferase from *Limnanthes douglasii*. The genetically modified plants were capable of synthesising trierucin.

There is great interest in isomers of conjugated linoleic acid (CLA) because of their anti-



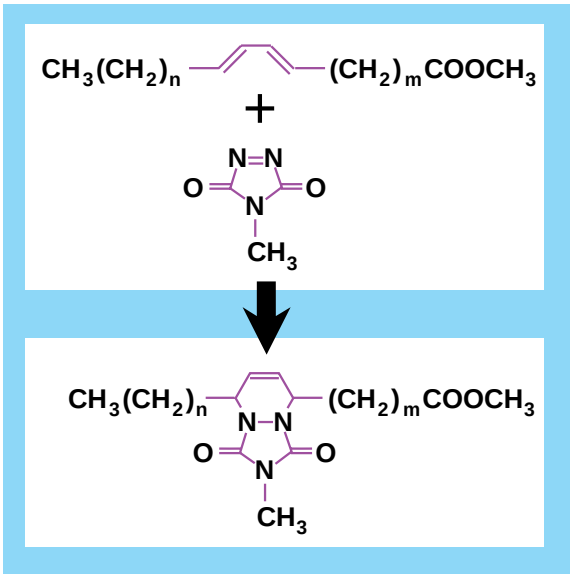


Figure 1 Reaction of 2-methyl-1,2,4-triazoline-3,5-dione with a fatty acid containing a conjugated diene moiety.

carcinogenic properties. The dienophile, 2-methyl-1,2,4-triazoline- 3,5-dione, was shown to react specifically with conjugated fatty acid methyl esters to form Diels-Alder cycloaddition products. The dienes gave single adducts (Figure 1), whereas the triene formed four products corresponding to two stereoisomers of the adducts of the 9,11-diene system and two of the 11,13-diene system. The mass spectra of such adducts are simple and informative, allowing the positions of the double bonds to be determined. A new procedure for synthesizing CLA from ricinoleic acid (from castor oil) has been developed.

Analytical methods developed for the determination of the concentration of pyrimidinone glucosides were utilised in a study to determine the distribution of vicine and convicine in developing seedlings of *Vicia faba* and *V. narbonensis*. In both species, the roots and stems contained, at the seedling stage, quantifiable amounts of both pyrimidinone glucosides. The total amount of convicine in the roots of the *V. narbonensis* seedlings was greater than could be accounted for by either translocation or by conversion of the vicine present in the seed. This would suggest that the young plants from this species have the capacity to synthesise convicine. Further studies using a mutant faba bean line with low pyrimidinone glucoside concentrations in the seed demonstrated that similar biosynthetic capabilities existed within *V. faba*. Therefore, it appears that selection for faba beans with reduced concentrations of vicine and convicine in the seed, and consequently improved nutritional value, could be achieved without losing any possible plant defence capabilities endowed by the presence of these compounds in young vulnerable seedlings.

Considerable progress has been made in extending continuous-flow analysis of hydrogen and oxygen stable isotopes to organic compounds. The pyrolysis method for water isotopes, described in the last report, has been modified to make it suitable for volatile compounds such as hydrocarbons, alcohols and ketones. The principal modification was to the packing in the pyrolysis reactor, replacing nickelised-carbon with nickel wire onto which carbon is deposited *in situ*. Volatile organic compounds can now be analysed with adequate precision for natural abundance applications, using both hydrogen and oxygen isotopes. This technique is currently restricted to pure compounds when using hydrogen, but ongoing research is aimed at achieving such analyses from chromatographically separated components of complex mixtures. Fractionation during chromatography is much more pronounced with compounds containing hydrogen isotopes, and this cannot be ignored as it is with heavier elements. Current research is aimed at characterising and correcting for time-dependent fractionation during chromatography.

Detailed chemical analysis of raspberry epicuticular waxes has revealed the complexity of these substances. Over 230 different compounds have been identified and quantified by GC-MS. These include alkanes (C_{15}-C_{35}; C_{27}, C_{29}, C_{31} mainly), saturated and unsaturated carboxylic acids (C_{14}-C_{32}; C_{16} and C_{18} mainly), primary alcohols (C_{10}-C_{38}; C_{22}, C_{24}, C_{26}, C_{28} mainly), secondary alcohols (C_{29}), ketones (C_{29}, C_{31}, C_{33}), triterpenoids (squalene, cycloartenol, cholesterol, ß-sitosterol, campestrol, stigmasterol, α- and ß- amyrin and γ-tocopherol) and wax esters (C_{28}-C_{50}, C_{42}, C_{44}, C_{46} mainly). There are over 150 individual long-chain wax esters, a number of which have unsaturated sites within their acid moieties, but which lack the complex branched structures found for the brassica species previously studied at SCRI. A further difference between the waxes from these different species is the presence in raspberry waxes of many more low molecular weight components in the range C_{10}-C_{20}. Differences in the distribution of the low molecular weight components within raspberry waxes, particularly within the primary alcohol fraction, may be associated with the resistance or susceptibility to attack by aphids. Differences in the distribution of the triterpenoid components of the wax may also be significant factors.

A major step forward in instrumental technique this year has been the development of a linked gas chromatography-electroantennogram system (GC-EAD), whose key feature requires the simultaneous electronic measurement of insect physiological response to an applied chemical stimulus, together with the chromatographic separation and detection of the chemical component eliciting that response. Preliminary work with the system linked to a mass spectrometer showed that vacuum/pumping effects from the mass spectrometer prevented the GC effluent from reaching the biological preparation. This was subsequently overcome by using a flame ionisation detector at atmospheric pressure. Early experiments employing the raspberry beetle (*Byturus tomentosus*) and a series of test compounds and flower volatile profiles have improved sensitivity and proved the viability of the system.

EPR-based methodology has been developed to investigate the high temperature decomposition of vegetable oils. The objective of this work was two-fold, firstly, to provide industry with a simple accelerated shelf-life test; and secondly, to provide a non-invasive probe of the chemistry of such oils at frying temperatures. A European inter-laboratory comparison is currently underway to evaluate the methodology developed at SCRI.

The aldehydic lipid peroxidation products, malondialdehyde and 4-hydroxy-2-nonenal, have been detected in callus cultures of *Daucus carota*. Both compounds have known cytotoxic and genotoxic properties in animal cells, but their study in plant cells is limited. The relative distribution of these two aldehydes may be involved in free radical-mediated stress in ageing *in vitro*. A role for 4-hydroxy-2-nonenal in cellular differentiation has been postulated.

NMR microscopy was used to visualise, in real time, the spread of tissue damage in entire grape berries caused by the fungal pathogen *Botrytis cinerea*. Studies were also made of the damage to imported grapes, which is thought to be caused by over-exposure to SO_2 during post-harvest fumigation.

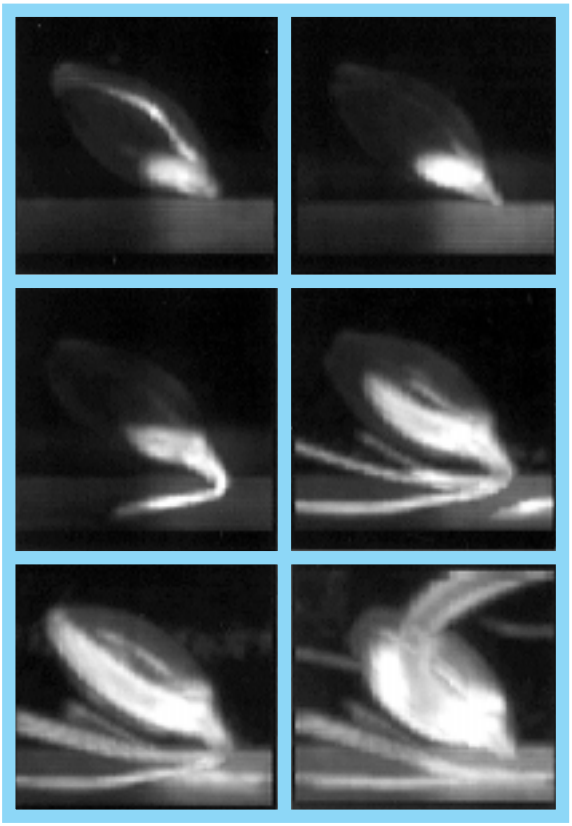


Figure 2 Frames from time-lapse sequence of germinating barley. The images shown were acquired at intervals of 24 hours.

Complete 3D-reconstructions of bean and barley seeds, germinating inside the magnet, have been made into time-lapse films, revealing non-invasively the internal water movement as the roots and shoot emerge.

Collaborative research with BioSS concerned the analysis of NMR images obtained under different spectroscopic conditions. Other collaborative research with Wageningen Agricultural University (The Netherlands), under an EU initiative, involved a comparison of images of raspberry fruits obtained at high magnetic field (7.2T; 300MHz) at SCRI with those of fruits of the same cultivar observed at low field strength (0.48T; 20MHz) in Wageningen.

Laboratory accreditation

T. Shepherd

As competition for access to research funding increases, organisations such as SCRI must place greater emphasis on providing objective evidence of the quality of their work programmes. In the SCRI context, quality can be defined as meeting the needs of our customers (sponsors of our work), using appropriate work methods and facilities ('fitness for purpose'), providing value for money, and 'getting it right first time' to avoid unnecessary, expensive and time consuming repetition. In practice, quality is a trade-off between the need to reduce cost, the desire to produce work of the highest standard, and the need to work to a particular timescale. Evidence of quality can include the scientific reputation of staff, as shown by the ability to obtain work in the face of strong competition; the qualifications, skills and attitudes of staff; work practices (appropriate, effective, up-to-date); customer service; achievement of performance targets; and audits, reviews and accreditation (objective assessment of the quality of all aspects of work, not just the final product). Such evidence of the delivery of quality, together with the culture, attitudes and systems which must function within an organisation to ensure delivery of quality, are the primary elements of Quality Assurance (QA).

As an organisation, we are already familiar with several forms of audit and review, conducted both internally and by third parties. These include annual staff appraisal; the various formal systems for obtaining funding and reporting the outcome of funded work, including the ROAME system; internal and external peer review of scientific publications; and the comprehensive, in-depth review of our work programmes by externally appointed visiting groups. Extensive and important though these processes are, they are no longer considered to provide sufficient evidence of quality in themselves, but should be integral parts of a co-ordinated quality management system covering all aspects of work.

Quality Management Systems For organisations like SCRI, where activities are generally laboratory based but which also have a significant support and service function, a number of different approaches to quality management are available. These are listed in Figure 1. ISO 9000, NAMAS and GLP are formal systems based on internationally accepted standards with third party certification (accreditation). Visiting groups have international representation, but are

	Generic quality standard	BS EN ISO 9000	ISO 25 (NAMAS)	GLP	Visiting groups
International Standard	None	Flexible	Strict	Strict	None
Creative Thought	-	-	-	-	✔✔
Research Design	✔✔	✔✔	-	✔	✔✔
Experimentation	✔✔	✔✔	✔✔✔	✔✔✔	✔✔
Interpretation and Reporting	✔	✔	✔	✔	✔✔
Technical and Project Management	✔✔	✔✔	✔✔	✔✔	✔✔

Figure 1 Alternative approaches to Quality Assurance. BS: British Standard; EN: European Standard; ISO: Intenational Organisation for Standardisation; NAMAS: National Accreditation of Measurement And Sampling; GLP: Good Laboratory Practice.

infrequent and are not conducted to an accepted international standard. Generic systems may include many elements of the others but are not based on a specific standard, and are not third-party certified. Of the three formal systems, ISO 9000 is the most versatile and widely applicable within SCRI. NAMAS is too specialised in scope for general application and GLP is only available to organisations producing regulatory information. None of the accreditation standards include the process of creative thought within their remits.

The practical difference between a formal system such as ISO 9000, and the generic approach, is just one of degree. The formal system takes things further to meet the requirements of the standard. When implementing a quality system, it is usual practice to first develop a generic system and then to modify this to meet the needs of formal accreditation if necessary, and this is the approach adopted within SCRI.

There may be a specific need for accreditation, arising from a requirement of the customer/sponsor, from a legal requirement (e.g. information for regulatory purposes), from a requirement for legal credibility (e.g. forensic, expert evidence), and from a need to provide product assurance. The benefits of accreditation are usually held to include retention of existing customers/sponsors and attraction of new customers, improved efficiency and reduced operating costs, increased management confidence, improved in-house status and recognition for participating parts of the organisation, and improved quality of work with wider acceptance of the products of the work.

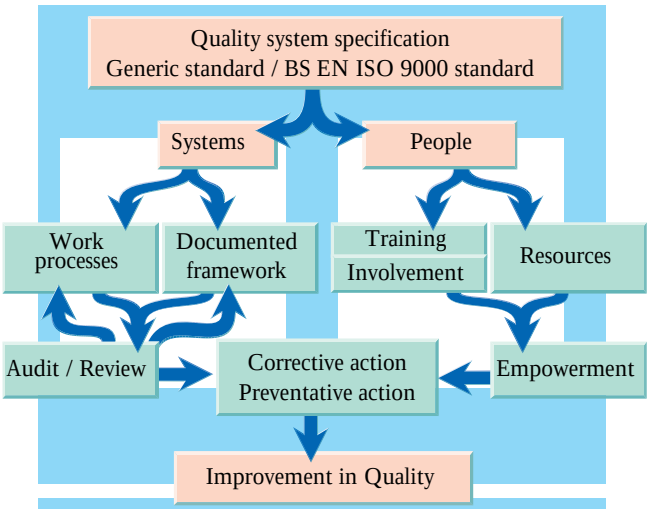


Figure 2 Major elements of BS EN ISO 9000 based Quality Systems.

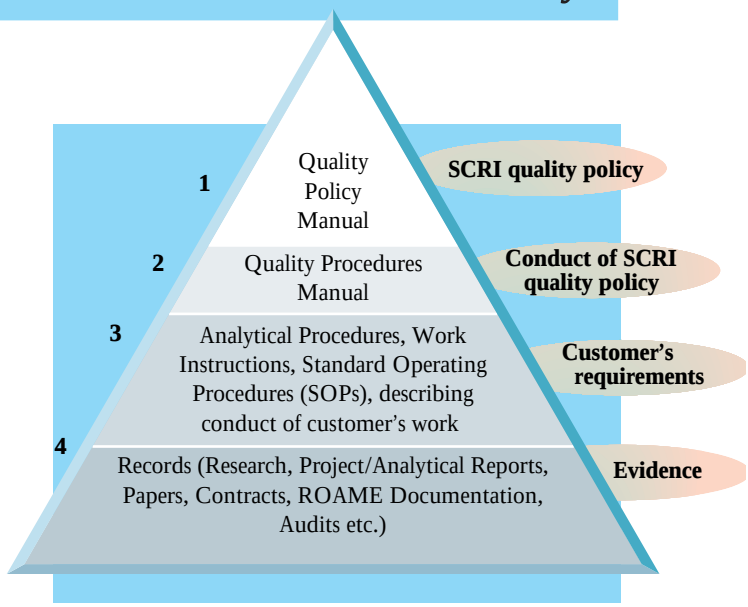


Figure 3 BS EN ISO 9000 Quality System Documentation.

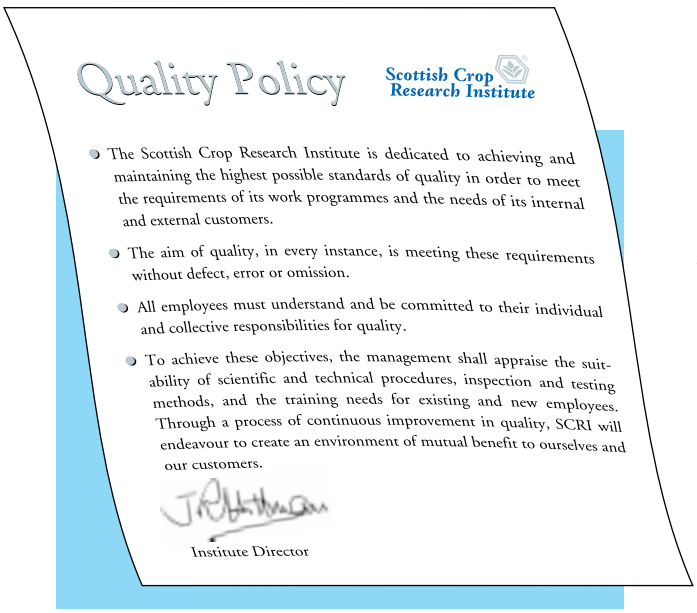
ISO 9000 Quality System for SCRI We have chosen to implement ISO 9000 selectively within SCRI, starting initially with the Chemistry Department, and we have been assisted in this by QA consultants from Inveresk Research International. ISO 9000 requires the development, operation and maintenance of a quality system which meets the appropriate specification published by the British Standards Institution[1]. This defines the elements the quality system has to encompass. However, the design and implementation of the quality system is influenced by the specific requirements of SCRI, its objectives, products, services supplied, and the processes and practices used. The system developed for SCRI should be applicable to any activity, allowing for variation in detail to account for differing circumstances. It is particularly important that staff are fully involved in development of the quality system as applied to their areas of work. This helps to foster understanding of what the quality system is about, how it functions and also helps to allay any existing misconceptions about what may be involved. Ideally staff should feel that they have 'ownership' of their quality system.

The major elements of the quality management system are shown in Figure 2. The organisational structure must be such that staff are suitably trained, have defined authority and responsibility and have access to the appropriate facilities and resources for conducting their work. There must be appropriate processes for control of the work (e.g. use of appropriate validated methods for experimental work, quality control and equipment calibration) and there must also be a documented framework to describe how the work is done

and how results are communicated. Through a process of in-house and external audits, compliance with the system is assessed, and staff are empowered to take such corrective and preventative action as is necessary.

Quality system documentation There are four levels of documentation, as shown in Figure 3. *Level 1* consists of a Quality Policy Manual[2], which describes the quality system in general, organisational structures, definitions and terms of reference, and includes a Quality Policy Statement which is reproduced in Figure 4. *Level 2* consists of a Quality Procedures Manual[3]. *Level 3* consists of written procedures generally referred to as Standard Operating Procedures (SOPs) for work activities. *Level 4* consists of all records generated in the conduct of these activities. Most of this documentation, including SOPs, experimental methods and the quality manuals, are written to standard formats for layout and content. Copies of the quality manual are held in all areas to which the quality system applies, and a copy is retained in the library for reference. SOPs are held at the locations where they are used. ISO 9000 requires that production, issue and copying of documentation is strictly controlled, although this may be more relaxed in a less formal system.

Quality records and record traceability Creation and management of records is the backbone of the quality system. Effective management of records gives confidence in the quality of work, whereas lack of management leads to chaos. During the course of any work,



Quality Policy

Scottish Crop Research Institute

- The Scottish Crop Research Institute is dedicated to achieving and maintaining the highest possible standards of quality in order to meet the requirements of its work programmes and the needs of its internal and external customers.
- The aim of quality, in every instance, is meeting these requirements without defect, error or omission.
- All employees must understand and be committed to their individual and collective responsibilities for quality.
- To achieve these objectives, the management shall appraise the suitability of scientific and technical procedures, inspection and testing methods, and the training needs for existing and new employees. Through a process of continuous improvement in quality, SCRI will endeavour to create an environment of mutual benefit to ourselves and our customers.

Institute Director

Figure 4 SCRI Quality Policy Statement.

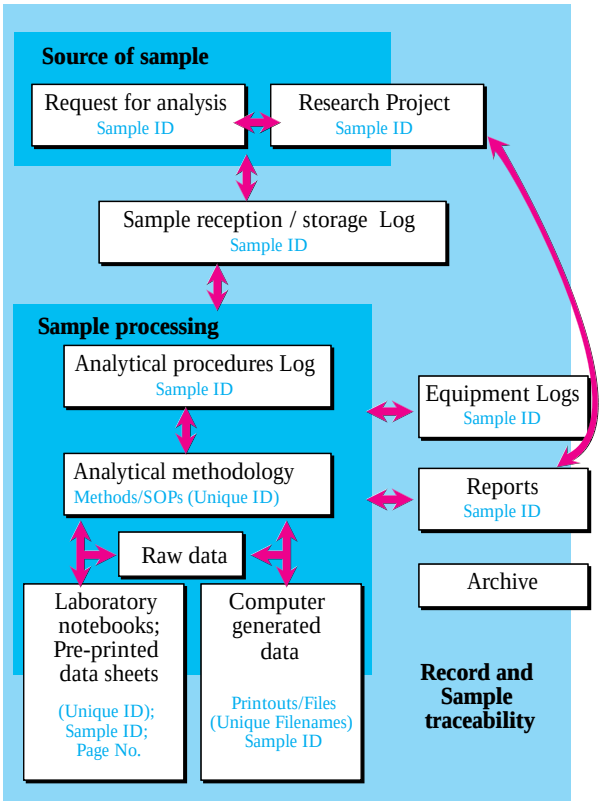


Figure 5 Traceability of records.

many different types of record may be generated, and it is a fundamental element of record management that all such records relating to a specific piece of work should be interlinked and traceable. This can be done by inclusion of certain items of common information, such as a sample or material identification code, a computer file name, or some other unique identifier, and by creating a master record which identifies each of these records by type and location, e.g. in a laboratory notebook. Figure 5 is a schematic representation of how various types of record can be linked and traceable. The need for traceability arises from the requirement that the status of the sample/material within the work area must be identifiable at any given time, and any record must be readily identified and retrievable on demand. Under ISO 9000, records are archived for a predetermined period.

Certification process Conformance with ISO 9000 is assessed by a certification body accredited by the United Kingdom Accreditation Service (UKAS) to carry out third party assessments of organisations such as SCRI. Before application is made for certification, the quality system must function for at least 12 weeks, during which time the appropriate records must be created and maintained. The certification process

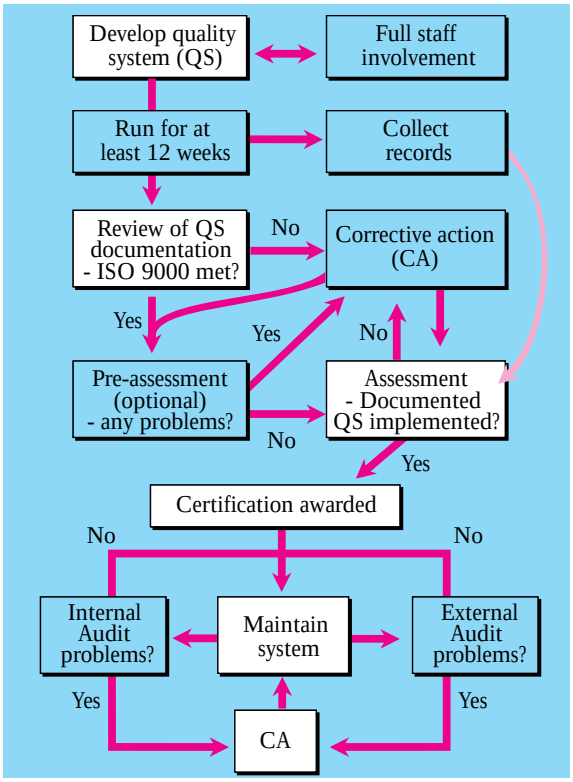


Figure 6 BS EN ISO9000 certification process.

(Fig. 6) involves a review of the quality manuals and quality procedures against the ISO 9000 specification, followed by on-site assessment of the specific activities to be covered by certification. If at any stage there are problems with the system, appropriate corrective action must be taken before proceeding further. If the assessment is satisfactory, recommendation is made for certification, which follows once the assessment findings have been formally approved. Subsequently, compliance with the quality system must be maintained, and this is checked by regular internal audits, and external audits conducted by the certification body, both at roughly 6-month intervals. Deficiencies are addressed by the appropriate corrective action. If serious deficiencies are not corrected to the satisfaction of the certification body, certification can be withdrawn.

ISO 9000 Certification at SCRI ISO 9000 consists of a family of 'nested' standards, of which ISO 9001 and ISO 9002 apply to the work remits of SCRI and MRS Ltd. The former standard includes an additional design element, relevant to R&D, but in all other respects, the standards are identical. Following the development of our quality system over the period 1994-1996, application was made to SGS Yarsely International Certification Services Ltd, for certification to ISO 9002 for 'the provision of an analytical chemistry service within SCRI and to MRS Ltd', in July 1996. Following the initial review of our documentation, and a pre-assessment in October, the full assessment was held in November and certification was awarded in December 1996. The scope of certification currently applies to the Stable Isotopes Facility and the MRS Lipid Analysis Unit. In the near future, we intend to widen the range of application to include mass spectrometry, NMR and EPR spectroscopy, lipid chemistry and phytochemical analysis, and to upgrade the standard to ISO 9001 to cover research activities. We further intend to extend coverage to MRS Ltd and to the Media Kitchen. Extension of the system can be made at six monthly intervals to coincide with planned surveillance visits by the certification body.

A simplified version of the quality system will be introduced within other parts of SCRI. This will be based on a code of good work/research practice, based in part on the ISO 9000 model and in part on the codes of practice of scientific professional bodies such as the Royal Society of Chemistry. If wider application of ISO 9000 becomes necessary, or if certification to the NAMAS or GLP standards is required, the quality system can be upgraded to the required standard with relative ease.

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1 (a) BS EN ISO 9001: 1994, Quality systems - Model for quality assurance in design, development, production installation and servicing. (b) BS EN ISO 9002: 1994, Quality Systems - Model for quality assurance in production, installation and servicing. British Standards Institution.

2 Shepherd, T. 1996. *Scottish Crop Research Institute Quality Manual Volume 1, Quality Policy*. Scottish Crop Research Institute, Dundee, 57pp.

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Molecular recognition: the role of mass spectrometry

N. Deighton

In recent Annual Reports, the techniques of nuclear magnetic resonance (NMR) solution spectroscopy and electron paramagnetic resonance have been discussed and the role of these techniques in the context of SCRI research considered. A major application to date has been in specific aspects of molecular characterisation. In a previous article[1], the process of electrospray ionisation mass spectrometry (ESI-MS) was introduced and several areas where this technique was likely to have a major impact were indicated. Many reports of the use of ESI-MS in these applications are now appearing in press. Of the areas previously considered, the only one that has been relatively slow-moving is that of molecular recognition.

Molecular recognition can be considered as the significant non-covalent interaction of two or more biomolecules in aqueous solution. An example could be the interaction between antigen and antibody, or within a multi-subunit protein. In the laboratory, other examples include those complexes that allow for the use of immunoassay and affinity chromatography. Such complexes can be binary or ternary in nature, are invariably large and thermodynamically unstable, preventing their isolation.

The forces that hold such complexes together (electrostatic, hydrogen bonding, hydrophobic interaction) are weak and prone to disruption by salt, pH effects and detergents. In appropriate solution, however, these complexes are long-lived and amenable to study. In the case of protein complexes, circular dichroism has given information for several decades on the α-helix and ß-sheet content of proteins in solution. Conformational changes upon addition of substrates, inhibitors and cofactors have all been studied extensively. NMR spectroscopy is increasingly used to probe solution structures as an alternative to the 'snap-shot' view afforded by X-ray crystallography. At present, the most complete picture of the true structure of a protein is a poorly-defined compromise between data obtained by these two techniques.

A number of studies in recent years have demonstrated the use of ESI-MS in the characterisation of supramolecular complexes of biopolymers, as well as specific (non-covalent) complexes with low molecular weight species. Increasingly it is becoming accepted that the observation of complexes in the gas phase (mass spectrometer) is dependent upon the existence of those complexes in solution. There is perhaps no more striking demonstration of this than the recent report of electrosprayed solutions of rice yellow mottle virus and tobacco mosaic virus which were then trapped by a physical collector in the mass spectrometer. Transmission electron microscopy of the trapped material demonstrated that the viral capsids (which are stabilised by non-covalent interaction between protein sub-units) had survived intact. Thus direct characterisation of non-covalent complexation provides another analytical tool in understanding supramolecular chemistry. An example of the direct observation of heme complexation with apohemoglobin is presented in Figure 1. Apart from the mere observation of the non-covalent complex, ESI-MS is often able to provide information upon the stoichiometry of the interaction (Table 1).

The case of lysozyme and N-acetylglucosamine is an example of enzyme-inhibitor interaction. Several other studies have chosen this interaction, presumably because of the high affinity of the inhibitor for the enzyme, and the resultant stability of the complex.

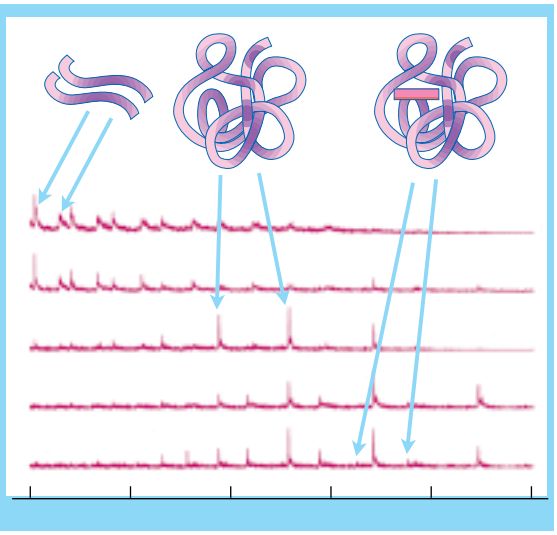

Figure 1 ESI-MS spectra depicting the non-covalent complexation of heme to apohemoglobin. Both α- and β-subunit complexes are observed.

Protein	Mr	Ligand	Stoichiometry
Ferredoxin	10,828	$Fe^{III}S$	1:4
Lysozyme	14,305	NAG_6*	1:1
Myoglobin	16,951	Fe-heme	1:1
HIV-1-protease	10,747	JG-365¶	2:1

*NAG = N-acetylglucosamine , ¶JG-365 = HIV-1-protease inhibitor

Table 1 Protein:ligand complexes determined by ESI-MS.

ESI-MS of bovine carbonic anhydrase has been used to screen various *p*-benzenesulfonamide inhibitors. It was found to be possible to determine relative binding constants and structural information for a mixture of inhibitors in a single experiment. Binding constants, in solution, as low as 1.7×10^6 M^{-1}, gave ESI mass spectra of complexes. It is concluded that this method is relevant to the study of drug leads and may find use in screening libraries for tight-binding compounds. In this context, work is currently underway at SCRI to study the competition between monosaccharides and peptide mimetics binding to lectins.

At present, examples of the direct observation of complexes between two or more large biopolymers are rare. Leucine zipper proteins have attracted considerable recent interest because of their significance in transcriptional regulation as well as their structure and specificity in DNA recognition. Leucine zipper structural elements, consisting of regular heptad repeats of leucine residues next to basic DNA-binding units have been identified in a number of transcriptional activator proteins (Fig. 2). The most extensively studied of these, GCN4 (from yeast), consists of a double stranded α-helical coil with a 4-3 heptad sequence of hydrophobic and polar residues. ESI-MS of GCN4 produced specific odd-charge $[M+5H]^{5+}$ and $[M+7H]^{7+}$ ions of dimers, arising from hydrophobic interactions. These dimers were readily disrupted through the application of collision-induced dissociation voltages, confirming their non-covalent nature. The direct observation of the dimer:duplex DNA complex has not yet been reported.

ESI-MS detection of double-stranded oligonucleotides with conventional quadrupole mass spectrometers has also been reported. Duplex-specific ions for self-complementary oligonucleotides, as well as for sequences of different base composition, have been observed by ESI-MS. Of particular interest here is the observation of a quadruplex ion from the guanine-rich oligonucleotide d(CGCGGGGCG) formed by quartet pairing of the central guanine bases, alternative to conventional Watson-Crick base-pairing.

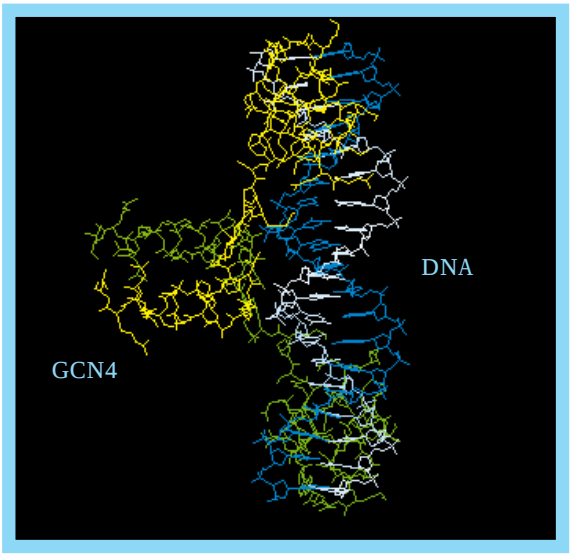

Figure 2 DNA:GCN4 (transcriptional activator protein) interaction.

In early reports of ESI-MS studies of specific protein complexes with DNA and RNA sequences, highly charged ions were not detected and as such the complexes were not observed. As a consequence, it was felt that these complexes were only likely to be observed in studies of the ions of high mass-to-charge ratio, necessitating further development of high mass analysers. One recent report indicates that this is not the case[2]. Dissociation constants for the non-covalent complex of bovine serum albumin and a 20-mer phosphorothioate oligonucleotide have been determined by ESI-MS. Stoichiometry was a mixture of 1:1 and 1:2. Variation of the dissociation constant with buffer concentration for the 1:1 complex indicated that the principle mode of interaction was electrostatic. The mass of the 1:1 complex was determined as 72,870Da. The methodology described paves the way for similar studies on macromolecular complexes up to this total mass.

The possibility that ESI-MS can provide information on receptor-ligand, antibody-antigen, enzyme-substrate, protein-cofactor and other significant associations in general enables the technique to become broadly applicable to many specific non-covalent interactions.

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Genetic and environmental effects on the glycoalkaloid content of potato (*Solanum tuberosum*) tubers

D.W. Griffiths, M.F.B. Dale & H. Bain

Potato tubers represent an excellent source of dietary carbohydrates, high quality protein and vitamins, providing up to 25-30% of our daily vitamin C requirement, given an average potato consumption. However, in common with many other domesticated crops, potatoes have the capacity to synthesise potentially toxic compounds, in particular, the steroidal glycoalkaloids α-solanine and α-chaconine. These structurally similar compounds (Fig. 1), which represent differently glycosylated forms of the steroidal aglycone, solanidine, account for approximately 95% of the total glycoalkaloids (TGA) present in potato tubers. Glycoalkaloids in potatoes probably have a defence role against various pathogens. Resistance to Colorado beetles and potato leafhoppers has been attributed in part to TGAs and correlations between TGAs and tuber resistance to wireworm and some fungi have been identified.

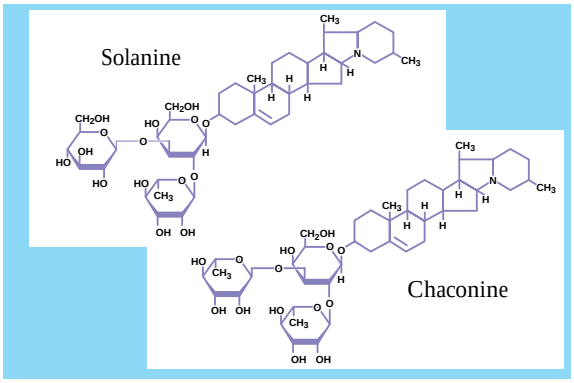

Figure 1 The structures of the major glycoalkaloids in potato *(Solanum tuberosum)* tubers.

Both compounds are heat-stable and consequently are not destroyed during normal cooking processes. However, since they tend to accumulate in the periderm and cortex, the concentration consumed may be significantly reduced by peeling. The presence of glycoalkaloids at low concentrations may have a beneficial effect on potato flavour, but at levels exceeding 15 mg 100 g^{-1} fresh weight (FWt), they tend to impart a bitter flavour. Steroidal glycoalkaloids have been described as some of the most toxic components of the human diet and indeed, some 30 human fatalities have been attributed directly to their consumption. Although both mild and severe cases of glycoalkaloid poisonings may often be diagnosed as gastro-enteritis, over 2,000 cases of poisoning related to potato consumption have been reported. In view of this, a maximum level of 20 mg 100 g^{-1} FWt has been recommended as an upper acceptable limit in tubers of new potato varieties in the UK. Glycoalkaloid levels in cultivated potatoes have been reduced directly or indirectly by selection over many centuries, with evidence that selection for reduced glycoalkaloid levels was made during the original domestication of the potato.

Although the biosynthetic pathway of the steroidal glycoalkaloids has not been fully elucidated, they are known to be a product of the mevalonic acid pathway and to be derived from cholesterol. Many factors, both post- and pre-harvest, may affect the final TGA content of a given tuber, and the objective of this work has been to determine the relative importance of genetic and environmental effects on TGA content.

Effect of storage temperature There is an increasing awareness of environmental and food issues within Europe. Increasingly, new cultivars of potatoes for consumption, either fresh or more particularly for processing, can be stored at low temperatures (*c.* 4-6°C). This provides an alternative to using sprout suppressant chemicals in order to prevent dormancy break, minimises losses due to respiration which are detrimental to tuber quality, and reduces the incidence of storage diseases. While storage at lower temperature avoids the use of chemicals, it may have an effect on TGA levels.

In order to examine the effects of temperature on tuber glycoalkaloid content, tubers from five cultivars (Brodick, Eden, Estima, Pentland Dell and Record) were stored at 10°C, 7°C and 4°C, and a further subsample stored at 10°C for 9 weeks and subsequently moved to a 4°C store for the remainder of the experiment. The tubers were analysed for glycoalkaloid

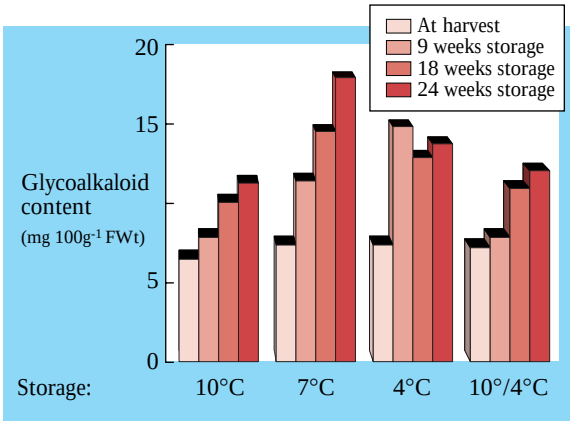

Figure 2 Effect of storage conditions on the glycoalkaloid content of potato tubers from the cultivar, Brodick.

content using high-performance liquid chromatography at harvest and after 9, 18 and 24 weeks storage.

Storage at 10°C did not result in any major increase in the glycoalkaloid content of the tubers. The small increases observed in, for example, Brodick, which increased from an initial value of 7.5 mg 100 g^{-1} FWt to 11.4 mg 100 g^{-1} FWt after 24 weeks storage, could be accounted for largely by the expected changes in dry matter content.

Storage at 7°C resulted in consistently higher TGA content than found in tubers stored at 10°C, but with the exception of Brodick and Record, these differences were not statistically significant. As shown in Figure 2, the concentration of TGA in Brodick tubers stored at 7°C increased linearly with time, reaching a value of over 18 mg 100 g^{-1} FWt after 24 weeks storage. For both Record and Brodick, a significant correlation was found between storage time and TGA content, and from the regression equations, it can be calculated that tubers from the latter cultivar accumulated TGA almost five times faster than those of Record.

Storage at 4°C also resulted in a significant increase in the TGA content of Brodick. This appeared to occur in the first 9 weeks of storage, during which time the TGA content of the tubers almost doubled to reach a mean value of 15 mg 100 g^{-1} FWt, but in contrast to the effect of storage at 7°C, TGA contents then remained stable. In the four other cultivars studied, accumulation of TGA was not significantly affected by low temperature storage.

Tubers stored for 9 weeks at 10°C and moved subsequently to a 4°C store behaved in a similar manner to those stored continually at 10°C, suggesting that following the onset of true tuber dormancy, cold stress did not result in TGA accumulation even in the most sensitive cultivar, Brodick[1]. Further studies are currently being undertaken to confirm this and to study the effects of storage temperature on subsequent TGA accumulation in response to light exposure.

Effect of light Exposure of potato tubers to light is one of the most important environmental factors which initiate the synthesis of glycoalkaloids. This can occur at many of the potato production stages, including during the growing season, at harvest, packing, in storage, and eventually in the processing or retailing outlet.

To examine light-induced TGA synthesis, the tubers of five cultivars (Ailsa, Brodick, P. Dell, Eden and Torridon) were cut in half longitudinally with one half placed in moist light conditions and the other in control moist dark conditions at 20°C. The light-exposed and dark (control) tuber halves were analysed after 8, 16, 24, 48, 72, 96 and 168 hours.

Significant differences ($P<0.001$) were observed between the TGA contents of the five cultivars in response to light. The results (Figs 3a & 3b) indicated a good genotype-specific relationship between the rates of chlorophyll synthesis, i.e. greening, and increases in glycoalkaloid content (mg/100 g freeze dried matter (FDM)) within the individual cultivars[2].

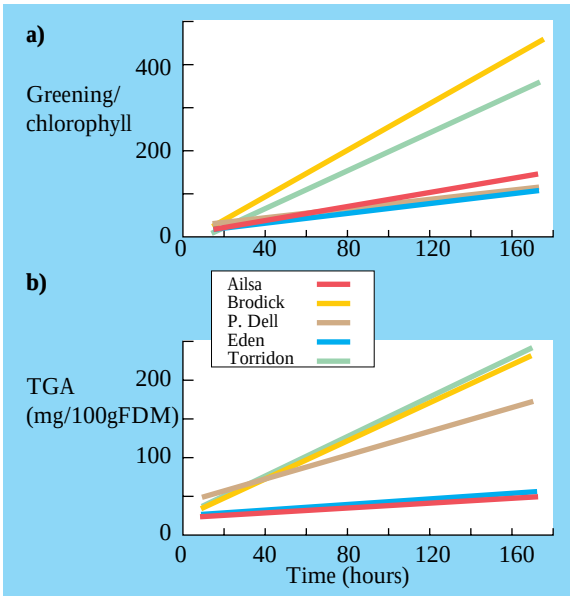

Figure 3 a) Increase in greening (colorimetric estimation) and b) increase in total glycoalkaloid content (mg/100gFDM) of five cultivars plotted against time when exposed to light.

Effect of damage Previous studies of potatoes have indicated that damaged potatoes can produce high levels of TGAs, especially at the point of injury. Tubers from five potato cultivars (Ailsa, Brodick, Eden, P. Dell and Torridon) were selected, based on previous research, for their low, intermediate or high rates of TGA synthesis in response to other environmental influences such as light-exposure or cold-storage temperatures.

Forty tubers of each cultivar were halved, and one half uniformly damaged using a 100 gm bolt to inflict four equally-spaced blows. After 8 weeks, the damaged half-tubers were analysed and compared with the undamaged controls. The results summarised in Table 1 indicate that there are inherent differences in TGA levels within these cultivars (undamaged half-tubers) and that they respond differently when damaged. The cultivars Ailsa and Eden had consistently low TGA levels, while cultivars Brodick and Torridon were comparatively higher. In response to damage, the cultivar Pentland Dell exhibited moderately high TGA levels, though its rate of TGA synthesis in response to damage and light exposure was moderately low. The cultivar Torridon, with comparatively high inherent TGA levels, did not exhibit increased rates of TGA synthesis (Table 1) in response to damage, as might have been expected. This is, in part, explained by the observed response of severe cell death and localised tissue necrosis in a relatively large area surrounding the point of damage, preventing TGA synthesis[3].

Evidence from recent studies has also indicated that the rate of TGA synthesis is influenced by the physiological state of tubers when exposed to stress[1,4]. It is evident from the research that there are a number of important factors affecting the final TGA content of potato tubers. The first is the inherent TGA content, and the second, and perhaps the more important factor, is the rate at which individual cultivars synthesize TGA in response to various environmental factors, notably light exposure, cold temperature in store and damage. Studies are progressing to identify and ultimately locate critical genes and gene products associated with synthesis (e.g. solanidine glucosyl transferase) and breakdown of these potentially toxic compounds. Efforts are also directed through conventional breeding to select germplasm for both low inherent TGA levels and low rates of TGA synthesis.

Cultivar	Undamaged half	Damaged half
Ailsa	24.9	31.6
Eden	20.7	33.9
Pentland Dell	67.5	78.1
Brodick	42.7	85.1
Torridon	40.9	41.9
LSD (P<0.05)		5.46

Table 1 Increase in TGA content (mg/100gFDM) of five potato cultivars in response to damage.

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Fungal & bacterial diseases

James M. Duncan

In March 1996, a meeting was held in Lima, Peru under the auspices of the International Potato Centre (CIP). Its purpose was to establish the major scientific and developmental tasks and goals that would form the core of the Global Initiative on Late Blight (GILB). GILB will shape the nature of potato late blight research throughout the world, but especially the developing countries, for the next ten years. Three phases of work were identified at the Lima meeting: Phases I and II, which cover years 1-6, are mostly research based - I is devoted to the basic biology underlying resistance and pathogen variation whereas II is directed towards the development of disease-resistant germplasm. Phase III is much more concerned with development and uptake of late blight-resistant material by farmers.

GILB has been favourably received by international sponsors of research. Research programmes supported by GILB will start formally later this year. Identified as a high priority of Phase I is a GxE experiment to study the effect of location on the expression of late blight resistance. This experiment springs directly from earlier work done at SCRI on the effects of such factors as temperature and light on the expression of resistance (Lowe & Harrison, SCRI Ann. Rep. 1995). In fact, the GxE experiment had begun informally in 1996 with the collaboration of a CIP scientist in Ecuador and others in the USA, Canada, The Netherlands, France and SCRI. Germplasm in the experiment included CIP material, old European varieties thought to have some resistance, the field resistance standards and, depending on site, some new resistant clones and cultivars from SCRI. Without the efforts of SCRI staff, particularly Robert Lowe and Helen Stewart, aided by BioSS, it is doubtful if the experiment ever would have started. They arranged for the virus-free production and distribution of much of the germplasm material at SCRI, and the collection, collation and processing of the results. No world-wide experiment ever runs without a hitch and this one did not either, but enough has come from it to suggest that location, especially latitude, is an important factor in the expression of resistance. Of equal importance in the initial results was the strong suggestion that the resistance of some cultivars is environmentally robust, *i.e.* does not alter much from site to site, thus indicating that breeding for high levels of durable resistance is feasible. The experiment will

Progress of late blight in a plot of a susceptible potato cultivar unpotected with fungicide. The two pictures were taken 15 days apart.

continue in 1997 as an integral part of GILB and with a larger number of participants in more countries.

Also identified as a priority in GILB was the need for rapid molecular techniques for fingerprinting strains of *Phytophthora infestans*. Shifts in populations due to immigration of new races into an area, or the emergence of new strains as a result of sexual or other forms of recombination, are difficult to follow, partly because there are few suitable phenotypic characters available to discriminate among races/strains, and some characters, such as virulence, are often variable in all but the most experienced hands. Knowing the origin of new strains and races is important for determining the contribution that sexual reproduction makes to the generation of variation within the fungus and hence the possible role of oospores in survival in soil. The Fungal and Bacterial Plant Pathology Department, through a SOAEFD flexible fund award in collaboration with the Scottish Agricultural Science Agency (SASA), has been collecting isolates of *P. infestans* throughout Scotland from seed and ware crops and from gardens and allotments. The purpose of the project is to determine the hazard that sexual reproduction and oospores might pose to potato crops in Scotland, especially seed which is sold throughout the world and which, in theory, could carry oospores and hence both mating types with it. Since 1995, over two hundred isolates from a total of 58 samples of seed and ware crops from commercial fields, gardens and allotments have been characterised for mating type, phenylamide resistance and virulence. In this survey, A1 mating type was prevalent but A2 was not uncommon (25% and 10% of all isolates in 1995 and 1996 respectively). Mixtures of A1 and A2 in the same sample were less common but they did occur, although only in samples from gardens or allotments. However, phenotypic and limited molecular characterisation of isolates using the RG57 probe and mitochondrial DNA, indicated that very little, if any, sexual crossing was occurring between A1 and A2 strains, even where both were found together. New molecular techniques, in particular amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs) are well advanced. In the coming year, these will be used on the collections from the past two years, and on isolates from more intensive sampling from sites where both mating types have been found together. The aim is to determine allele frequency and gene exchange, thereby providing some estimate of sexual recombination. This work has been done in close collaboration with a group at Bangor in N. Wales who are carrying out a similar study in England & Wales. They hope to include samples from outbreaks in crops grown from Scottish seed and thus gauge the importance of seed in distributing the fungus.

Although generally viewed as a pathogen with a narrow host range, principally potato, tomato and aubergine, *P. infestans* in fact has been recorded on 28 genera of plants. A minority of these records, such as its occurrence on *Acer*, may well be the result of misidentification of the fungus, but most of the others involve genera within the Solanaceae and are probably correct. Nevertheless, it can still be viewed as a pathogen with a narrow host range, especially when compared with species such as *P. cryptogea*. Even allowing for recent molecular studies in FBPP that suggest that many isolates assigned to *P. cryptogea* actually belong to other *Phytophthora* species (see below and Cooke p. 151), its host range is enormous at over 23 families of plants. *P. infestans* and *P. cryptogea* differ in the amounts and types of relatively small proteins, called elicitins, that they produce. Elicitins have been implicated in determining host range in some species. Working in collaboration with INRA, Antibes in France, and using a transformation system developed at SCRI, genes encoding particular elicitins in *P. cryptogea* have been introduced into *P. infestans*. The expression of these genes by the modified strains *in vitro* and on tobacco plants is reported on by Birch (p.148).

Symptoms of blackleg on plants in the field

In general, *P. infestans* is viewed as a 'good' species, but although its morphology and physiology are well characterised relative to many other species, it would be true to say that it is most often identified through its close association with its principal hosts, potato and tomato. Its nearest neighbours within the genus are *P. mirabilis* and *P. phaseoli* and while the latter is clearly distinguishable from *P. infestans* because it is self-fertile, the former might well have gone unrecognised as a separate taxa from *P. infestans* had it been isolated from potato or tomato and not from a Mexican weed, *Mirabilis jalapa* L. Despite being invalid as a taxonomic character, host range is clearly important in identifying plant pathogenic fungi, especially *Phytophthora.*

With *P. cryptogea*, the problem is much greater. Many isolates come from plant species which economically are relatively unimportant compared to potato. Such isolates have probably been studied less intensively than if they had been found on crop plants. Further complication arises through possible conspecificity of *P. cryptogea* with *P. drechsleri:* they are distinguished from each other largely by the inability of the former and the ability of the latter to grow at high temperatures. Protein profiles and more recently mitochondrial DNA have hinted that *P. cryptogea* as presently described is polyphyletic.

Molecular techniques may be one way by which some of the problems surrounding species identification can be resolved. In many organisms, sequences from specific regions of the genomic ribosomal gene repeat (rDNA), in particular the internally transcribed spacers, ITS1 and ITS2, have proved useful in distinguishing among taxa. This is true also for *Phytophthora*. ITS sequences from isolates that have been carefully identified by morphology, physiology and protein profiles by Dr Clive Brasier of the Forestry Commission, a leading authority on *Phytophthora* taxonomy, have given a coherent phylogeny for over 40 species. Nearly all isolates, identified by the more traditional techniques as truly belonging to *P. cryptogea* or *P. drechsleri*, clustered into one group by molecular methods, whereas others that did not fully fit the description of these species clustered into other groups. The same proved true for another notoriously complex species, *P. megasperma.* Moreover, most of the groups resulting from the molecular analysis closely matched groupings of isolates on the basis of the host from which they were originally isolated. Host range is clearly a, if not the, major selection pressure in the evolution of *Phytophthora* and probably all other species of plant pathogenic fungi. This is hardly a surprising conclusion, but one that should affect the range of characters to be considered when describing taxa. This work, which is described by Cooke (p. 151), was also able to provide a preliminary analysis for the first time of some downy mildews and their relationship to *Phytophthora.*

Similar problems of taxonomic assignment exist in plant pathogenic bacteria. *Erwinia* probably comprises relatively unrelated species assembled on the simple basis that they are pathogenic to plants. Methods used by plant pathologists for identifying them 'are markedly different from those listed for other *Enterobacteriaceae*' and consequently 'A meaningful comparison with other .... *Enterobacteriaceae* has not been adequately done' (Bergey's Manual of Determinative Bacteriology, Ninth Edition). Below the species and even sub-species level, new technology, some traditional and some molecular, is needed to classify isolates and strains. Using a combination of serotype, fermentation patterns, molecular markers and phage susceptibility patterns, Toth & Hyman (p. 154) have been able to fingerprint strains and isolates of the important blackleg pathogen *E. carotovora* subsp. *atroseptica.* In combination with laboratory and glasshouse studies with strains marked with the green fluorescent protein, this work heralds a new generation of epidemiological studies in which routes of infection of seed and ware and subsequent spread will be more clearly identified.

Heterologous expression of a basic elicitin gene from *Phytophthora cryptogea* in *P. infestans*

P.R.J. Birch, S. Unkles, I. Lacourt & J.M. Duncan

The genus *Phytophthora* comprises a diverse group of plant pathogenic oomycetes which differ greatly in modes of infection and host-range, providing a number of models for the study of pathogenicity and specificity in plant-fungus interactions. At one extreme is the potato pathogen *P. infestans*, which has a narrow host-range, and an air-borne, hemibiotrophic mode of infection, and at the other extreme are pathogens such as *P. cryptogea*, which have a broad host-range, and a soil-borne, necrotrophic mode of infection. As yet, it remains unclear what the molecular bases for such differences are, although we are now entering a period in which an increasing number of genes are being isolated which may have a rôle in pathogenicity and host-specificity.

A key process in the characterisation of such genes involves their transfer between representative species of *Phytophthora* to see whether they alter a particular plant- fungus interaction. For this, systems of DNA-mediated transformation are required. So far, transformation has been well documented only for *P. infestans*, although at low efficiency. Nevertheless, it has been used successfully for antisense inhibition of a bacterial transgene and for co-transformation. There has been no report, to date, of the expression of a foreign *Phytophthora* gene, complete with 5' and 3' UTRs, in *P. infestans*.

To date, only one family of genes from *Phytophthora*, that which encodes elicitins, has been well characterised. All *Phytophthora* species studied secrete these small, homologous holoproteins which elicit an hypersensitive response (HR)-like necrosis and systemic acquired resistance (SAR) in tobacco[1]. Only isolates of *P. nicotianae* which are highly virulent to tobacco, causing the disease black shank, do not produce elicitins. Elicitins thus act as avirulence factors in tobacco-*Phytophthora* interactions. However, their role in other plant-*Phytophthora* interactions remains unclear.

Elicitins are highly conserved but nevertheless may exhibit limited amino acid substitutions which result in large net charge differences, and this has been shown to effect the degree to which they induce tobacco leaf necrosis. Hence, two distinct classes of elicitin have been identified and defined as either basic, causing strong necrosis, as are produced by *P. cryptogea*, or acidic, causing little or no necrosis, as are produced by *P. infestans*. Furthermore, 10 to 50-fold higher concentrations of purified acidic elicitin are required to induce levels of resistance in tobacco similar to those observed with basic elicitins[2].

These phenotypically characteristic interactions between tobacco and specific elicitins offer an unique opportunity to assay the heterologous expression of genes encoding such proteins following their transfer from one *Phytophthora* species to another, by comparing the level of tobacco leaf necrosis induced by transformants with that of the untransformed fungus.

Co-transformation of *P. infestans* with a plasmid containing a hygromycin resistance gene and a plasmid containing a basic elicitin gene from *P. cryptogea* Recently, elicitin genes from *P. cryptogea* have been isolated, characterised and found to be clustered[3]. One region of DNA was shown to contain two elicitin genes, one of which is transcriptionally inactive and the other (*crypB)* codes for the major basic elicitin produced by this organism. We introduced a clone containing this region of DNA (pBG31) into *P. infestans* to see whether the alien basic elicitin gene would be expressed and, if so, alter the phenotypic interaction of its host with tobacco.

Transformation of *P. infestans* has previously been achieved with a plasmid vector containing the bacterial *hyg*^{R} gene (pTH210), which conveys resistance to the drug hygromycin and thus acts as a selectable marker. In order to introduce the *crypB* gene, spheroplasts of *P. infestans* were co-transformed with both pTH210 and pBG31, each digested with restriction enzyme *Hin*dIII. It has been shown previously that plasmids digested in this way and introduced simultaneously into *P. infestans* can ligate in the host cell to form a single DNA molecule carrying both selectable marker and gene of interest[4]. All transformants were initially selected on medium containing 25 µg/ml of hygromycin (a dose which kills the untransformed fungus). Ten hygromycin resistant colonies were selected, two of which proved to be unstable and reverted to drug sensitivity when further plated on selective medium. An additional two strains lost their resistance to hygromycin after several consecutive inoculations, leaving just six drug resistant strains for further investigation.

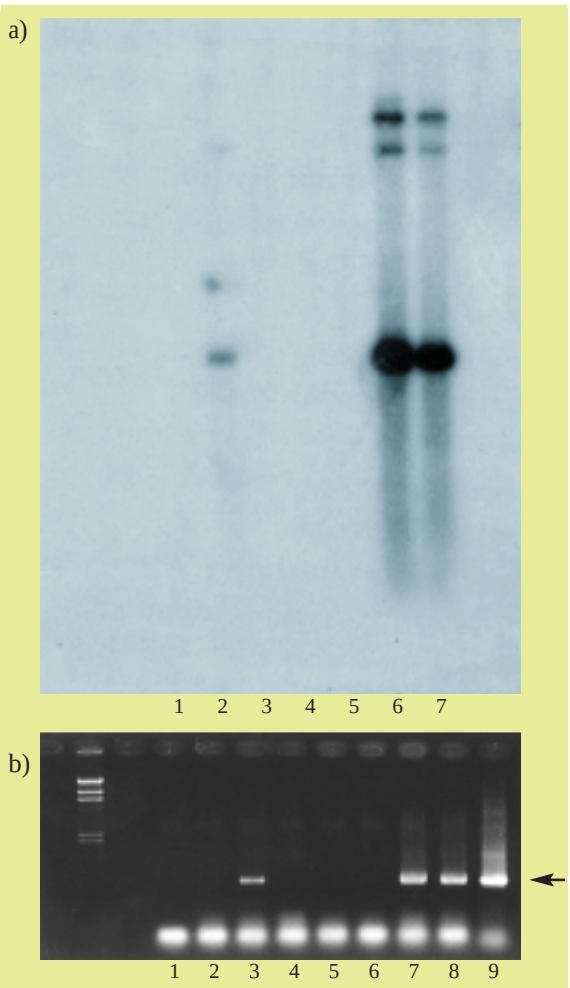

Figure 1 a) Southern analysis of genomic DNAs digested with *Sal* I from untransformed *P. infestans* (lane 1) and hygromycin resistant transformants H1 (lane 2), H2 (lane 3), H6 (lane 4), H7 (lane 5), H8 (lane 6) and H9 (lane 7), using a region of the *crypB* gene 3'UTR as a radiolabelled probe. b) PCR using primers which anneal specifically to regions of the *crypB* sequence. Template DNA was genomic DNAs from untransformed *P. infestans* (lane 2), and hygromycin resistant transformants H1 (lane 3), H2 (lane 4), H6 (lane 5), H7 (lane 6), H8 (lane 7) and H9 (lane 8). As a negative control, no template DNA was included in the reaction shown in lane 1 and pBG31 was amplified (lane 9) as a positive control. The arrow indicates the amplification products.

Southern analysis was used to investigate whether the hygromycin-resistant strains had acquired the *crypB* sequence. A region of the 3' UTR from this gene, which shows no homology to the equivalent region of the endogenous infestin gene, was used to probe digested DNAs from each of the hygromycin resistant strains and from the untransformed fungus. Hybridisation was observed only to DNA from three of the drug-resistant strains, indicating that these had acquired the alien sequence (Fig. 1a). Furthermore, the strength of hybridisation varied between these three DNA populations, suggesting that the copy numbers of the introduced sequences differed.

PCR was used to further demonstrate the presence of the entire *crypB* sequence in these strains. Two primers were designed to anneal, respectively, to specific regions of the 5' and 3' UTRs flanking the normal origin and termination of transcription. When PCR reactions were performed with the DNAs used for Southern analysis, amplification of a fragment of expected size was obtained only from the three DNA populations which previously hybridised to the *crypB* DNA probe (Fig. 1b). We hence deduced that the entire *crypB* sequence and flanking regions were present in these strains.

The alien *crypB* gene is transcribed in *P. infestans* co-transformants RT-PCR was used to test whether the *cryB* gene was being transcribed in transformants containing this sequence. Two oligonucleotide primers were designed; one to anneal specifically to the 3' UTR of this sequence, and another to anneal to the poly A tail of cDNA. The six transformants and the untransformed strain were grown for one week in pea broth medium, mRNA was extracted, and from this cDNA was synthesised. Using the above primers, a DNA fragment of expected size was amplified by PCR only from cDNA populations derived from the three

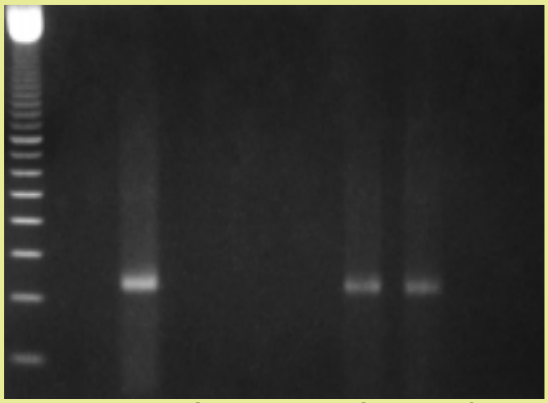

Figure 2 RT-PCR analysis using a primer which anneals to a specific region of the *crypB* sequence and one which anneals to the polyA tail of cDNA. Templates for the reactions were cDNA populations from untransformed *P. infestans* (lane 1), and hygromycin resistant transformants H1 (lane 2), H2 (lane 3), H6 (lane 4), H7 (lane 5), H8 (lane 6) and H9 (lane 7). In the case of lane 2, no template was included in the reaction. The arrow indicates the amplification products.

transformants containing the *crypB* DNA sequence (Fig. 2). These bands were excised from the gel, cloned and sequenced; the sequences confirmed them to be cDNAs derived from the *crypB* gene.

Transformants containing the *crypB* gene secrete active cryptogein To assess whether mRNAs encoding cryptogein were being translated and the protein secreted, all six drug-resistant strains and the untransformed fungus were grown in liquid culture and the extracellular medium was analysed using HPLC. In all cases, a single peak corresponding to the endogenous acidic infestin was observed. In transformants expressing the *crypB* gene, an additional peak was seen at the expected position for cryptogein. Figure 3 shows a histogram of the combined elicitin secreted in relation to mycelial growth.

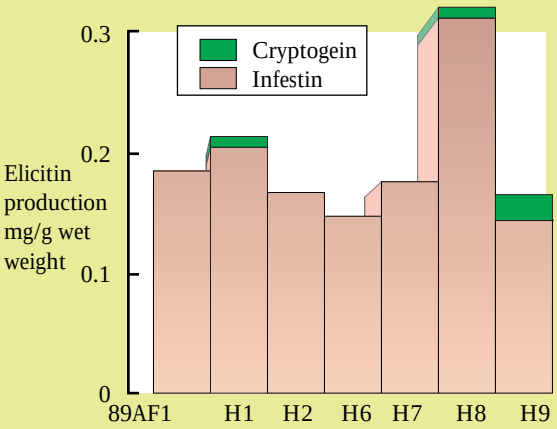

Figure 3 Histogram of the combined extracellular production of infestin and cryptogein from each of the untransformed *P. infestans* (89-AF1) and transformants H1, H2, H6, H7, H8 and H9 against mycelial growth, measured as wet weight of mycelium harvested.

To test whether the secretion of cryptogein effected the interaction between the co-transformants and tobacco, gel plugs containing the cryptogein-producing transformant H9, the non-cryptogein-producing transformant H6, untransformed *P. infestans* (89AF1), and, as an additional control, a strain of *P. cryptogea* (52) were inoculated onto decapitated 6-week old tobacco plants. After 3 days, the level of necrosis was investigated. In the case of transformant H6 and untransformed *P. infestans*, no necrosis was observed after this time. However, necrosis was observed on the leaves of plants inoculated with either *P. cryptogea* or the cryptogein-producing transformant H9 (Fig. 4).

Figure 4 Necrosis on wild-type tobacco plants three days after inoculation of decapitated stems with, as a control, *P. cryptogea* (a), transformant H9 (b), untransformed *P. infestans* (c), and non-cryptogein producing transformant H6 (d). Duplicate leaves are shown in each case.

Conclusion The results presented report the first demonstration of heterologous expression in *P. infestans* of a gene from another *Phytophthora* species. The *crypB* gene, encoding a basic elicitin from *P. cryptogea*, is transcribed, translated and the protein secreted from the transformants. Moreover, the secreted cryptogein elicits a strong necrosis of tobacco

leaves, suggesting that it has been correctly folded, as this is required for such activity.

Recently, Keller et al.[5] used a transgenic tobacco plant expressing the bacterial *nahG* gene, which catabolises salicylic acid (SA) to catechol, to uncouple the phenomena of necrosis and SAR. Accumulation of SA was shown to be necessary for elicitin-induced SAR in tobacco but was not required for necrosis. In addition to the results presented above, preliminary work with such transgenic tobacco plants has indicated that the presence of the *crypB* gene in *P. infestans* transformants increases not only its ability to cause leaf necrosis but also to induce systemic acquired resistance. We thus conclude that the transfer of a single elicitin gene from one *Phytophthora* species to another can dramatically alter the interaction of the transformed species with tobacco.

Acknowledgements

This work was done in collaboration with F. Panabieres, M. Ponchet, H. Keller and P. Ricci at INRA, Station de Pathologie Vegetale, Antibes cedex, France.

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The uses of ITS regions in *Phytophthora* species

D. Cooke, N. Williams & J.M. Duncan

Anton de Bary first named the genus *Phytophthora* (aptly named from the Greek 'Plant-destroyer') in 1876 when describing the species *Phytophthora infestans* as the causal agent of potato late blight. In the ensuing 121 years, many new species have been added; the 1990 key lists 67 species, and there have been several more recent additions. *Phytophthora* can grow on agar in the laboratory but is not competitive in nature unless in association with susceptible plant material in which colonisation and sporulation occur. It is this specialised ability to infect plants that has resulted in the global importance of *Phytophthora* as a devastating group of plant pathogens. They commonly cause root, crown and stem rots but also infect leaves and pods. It is likely that many species occur in natural communities and only those causing serious plant disease or widespread ecological damage are recorded by man.

As reported previously[1], the study of *Phyto-phthora* species has proved challenging. Compared to foliar cereal diseases, such as rust and powdery mildew, their identification in the field is difficult. Although there are some distinctive leaf blights (late blight of potato), in most cases a 'trained eye' is needed to spot the cryptic, often secondary, symptoms (e.g. wilting) which result from their tendency to attack stem bases and roots. Once isolated from field material, identification is the next difficulty. There is a paucity of distinct morphological characters and many vary markedly within a species (e.g. sporangial shape). To date, the definitive key to their identification subdivides the genus into six groups, on the basis of the type of papillum on the beak of the sporangia and the morphology of the developing sexual oospore[2]. The need for an objective 'natural' classification of the genus has long been

recognised. A classification based on evolutionary lineage (i.e. phylogenetic analysis) rather than clustering on the basis of shared morphology (i.e. pattern cladistics), will allow a rational examination of the biology, pathology and molecular biology of the genus.

In realisation of the need for more marker systems, a range of molecular methods has been applied. Initially isozymes and total protein profiles and, more recently, DNA-based methods such as RFLPs, RAPDs, CAPS and DNA sequencing of nuclear and mitochondrial DNA, have been used to unravel the complexity within defined groups of taxa. Perhaps the most useful region of the genome for a global examination of the genus is the ribosomal RNA gene repeat, with its highly conserved regions coding for ribosomal genes interspersed with variable spacer regions. The internal transcribed spacer (ITS) regions show sufficient sequence variation to discriminate at the species level in *Phytophthora*[1,3]. In this article, we report on progress at SCRI on their use in phylogenetic analysis, species identification and detection.

Phylogenetic analysis In a preliminary study, primarily of *Phytophthora* species important to the soft fruit industry, the 900 base pair (bp) ITS regions were amplified and the ITS1 and ITS2 regions sequenced and aligned to each other. Since these regions have been shown to accumulate neutral mutations at approximately the rate of speciation processes, then a measure of similarity in DNA sequence gives an indication of species relationships. The computer programme PHYLIP is used to make pairwise comparisons of all the 17 sequences. This similarity matrix is analysed to create a phylogenetic tree which not only clusters the most similar species but also identifies ancestral differences and reconstructs the evolution of a species. Those in the same branch of the phylogenetic tree can be said to share a common ancestor (represented by the node where two branches meet).

The ITS-based tree represented in Figure 1 yields new insights into the *Phytophthora* genus. A re-examination of the morphological characters in light of this tree allows us to assess the significance of each. The form of the sporangium appears to be an important indicator with a divergence into two lineages, one of which consists of the non-papillate forms and the other the papillate or semi-papillate forms combined.

In *Phytophthora*, it is the asexual sporangia which are responsible for rapid epidemic development. They

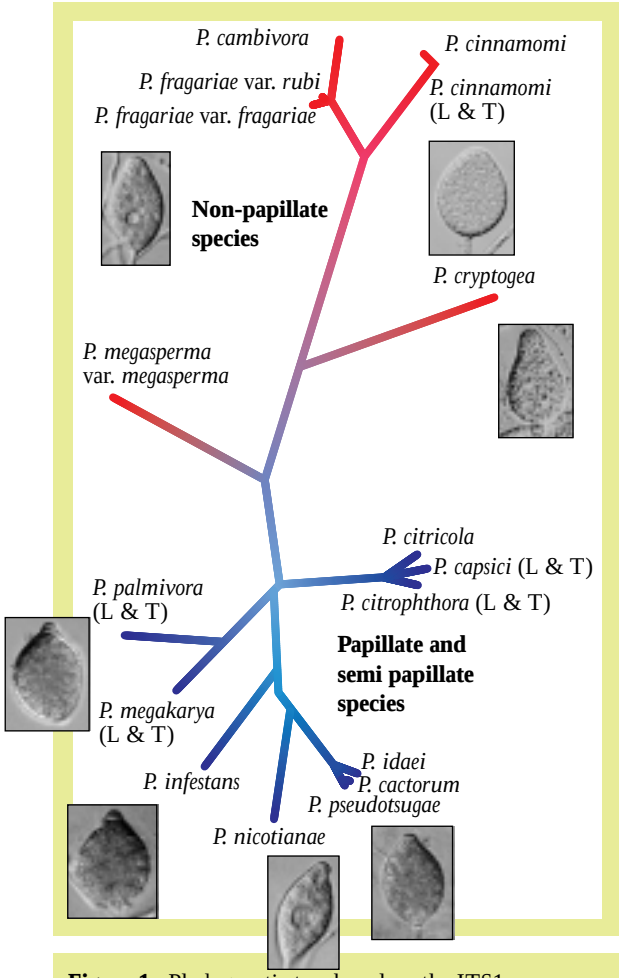

Figure 1 Phylogenetic tree based on the ITS1 sequence of 17 species of *Phytophthora* illustrating the division into two lineages (papillate/semi-papillate and non-papillate). Examples of the typical sporangial form for each lineage are shown. All species sequenced at SCRI unless marked L & T[5].

release motile zoospores which subsequently encyst, germinate and infect plant material. The mode of spread of these pathogenic propagules is associated with the form of the sporangia. Non-papillate species release zoospores from a wide exit pore in a sporangium which remains attached to the hyphae (termed non-caducous). After zoospore release, a bud forms inside the empty sporangium which develops into a new secondary sporangium (i.e. sporangial proliferation). Since the sporangia are non-caducous, spread is limited to mass flow of water and these species are primarily soil-borne. In the case of papillate and semi-papillate species, the sporangia can be detached from the hyphae on which they are borne (termed caducous) and the flexibility of airborne (via wind or rain-splash) or soil-borne dispersal introduced. Sporangia from papillate species (e.g. *P. infes-*

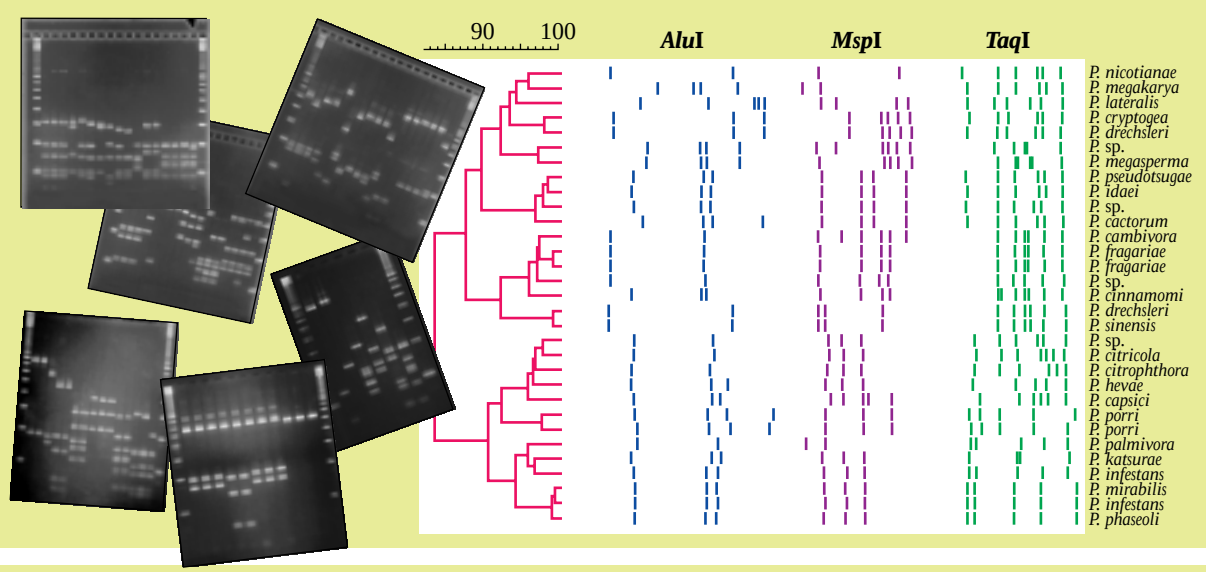

Figure 2 Typical banding patterns of digested ITS products as raw data on agarose gels (left) and after processing on the computer package GelCompar (right). The *Alu*I, *Msp*I and *Taq*I digests are all combined in a single gelstrip and after cluster analysis the patterns of the unknown isolates (labelled *P.* sp.) are aligned with reference isolates to aid species identification. The dendrogram represents the clustering of banding patterns and is not a phylogenetic tree.

tans) also have been shown to infect the plant directly by producing a germ tube and indirectly via zoospores depending on the environmental conditions. Analysis shows that other species conform to this pattern and indeed, some downy mildew species (a biotrophic genus in the same family, primarily spread *via* airborne spores) show closer ties to the papillate *Phytophthora* species than non-papillate ones.

This natural classification of the genus sheds light on species concepts pondered by *Phytophthora* workers for decades. It allows a clear vision of the evolution of the species that plague us today, and insights into ongoing speciation processes, allowing us to assess future threats. It also rationalises genetic analysis of the genus, providing a framework upon which further work can be based. It should be recognised that this tree is a 'gene' tree of ITS evolution and other genes (both rapidly and slowly evolving) should be examined to corroborate this data.

Species identification Through collaboration with the International Mycological Institute (Geoff Hall) and The Forestry Authority (Professor Clive Brasier), our culture collection has expanded to include over 400 cultures with representatives of over 35 *Phytophthora* species. This puts us in a unique position to develop a species identification system. On the basis of the ITS differences seen in the above phylogenetic analysis, the ITS region clearly has value in species identification. A rapid method for characterising the ITS regions is to digest with frequent-cutting restriction enzymes (4 base pair recognition sites) such as *Taq*I, *Msp*I and *Alu*I, and run the resultant fragments on a high resolution NuSieve agarose gel. We have now examined over 140 isolates with each of the three enzymes and have an extensive collection of banding patterns of reference *Phytophthora* species and unknown taxa. Because of the size of the database and the problem of gel-to-gel variation, we are using the GelCompar computer programme for analysis. Gel images are digitised, each lane defined and converted into a 'gelstrip' before normalisation against the standard DNA marker ladder on each gel. The software then allows comparisons of lanes from different gels, and a 'virtual gel' can be reconstructed in which similar banding patterns are clustered together (Fig. 2). In some cases a single enzyme may not distinguish a species, so we also combine the three digest profiles end to end to form a species profile. Once the database is established in this way, identification becomes a relatively simple task. The ITS regions are amplified from freeze-thawed mycelium, digested and entered into the database, and the identity, or nearest affinity, determined.

Whilst this system cannot replace the skills of an experienced mycologist, it is making routine identification considerably easier, which means more isolates can be examined, for example in survey work. With increasing amounts of movement of plant material and changing climatic conditions, we must be aware of

outbreaks of new *Phytophthora* diseases and such a rapid and objective system will assist in this.

Disease detection The sequence variation noted in ITS regions has been exploited in the design of PCR primers specific for individual species[4]. As discussed in the SCRI Annual Report for 1994, the rationale for this research programme was the detection and diagnosis of *Phytophthora* disease in plant tissues, water samples and even soils. We now have primers which allow specific and sensitive detection of *P. fragariae* var. *rubi*, *P. fragariae* var. *fragariae*, *P. cambivora*, *P. cactorum*, *P. cinnamomi*, *P. cryptogea*, and *P. nicotianae*. In combination with a first-round primer, specific to *Pythium*, *Phytophthora* and downy mildew species, these primers are proving valuable for routine disease detection and diagnosis work[4]. The sensitivity of the nested approach is sufficient to detect as few as five zoospores, making the primers useful in testing for contamination in water samples (e.g. field irrigation water or recirculating glasshouse irrigation systems). We have also demonstrated their use in the detection of *Phytophthora* diseases in plant material, which is proving invaluable in the testing of vegetatively propagated plant material such as soft fruit and hardy ornamentals.

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Typing strains of *Erwinia carotovora* subspecies *atroseptica* for epidemiological analyses

I.K. Toth & L.J. Hyman

Blackleg is a seed-borne bacterial disease of potato plants. Although the name blackleg is often associated with a typical black stem rot in the field, the disease can manifest itself in several other forms including; bacterial wilt and aerial stem rot of the plant in the field; blanking, stolon end rot and progeny tuber soft rot of tubers in the field; and soft rot of tubers in store. The disease is mainly caused by one of three soft rot erwinias, *Erwinia carotovora* subspecies *carotovora* (*Ecc*), *Erwinia carotovora* subspecies *atroseptica* (*Eca*) and *Erwinia chrysanthemi* (*Ech*), their distribution worldwide depending on both host range and climatic distribution. *Ecc* and *Ech* infect a large number of crops, mainly in tropical and sub-tropical areas. *Eca*, on the other hand, is restricted almost exclusively to potato in temperate regions. Although contamination of potato stocks by *Ecc* is common in temperate regions, and low temperature forms of *Ech* are becoming responsible for

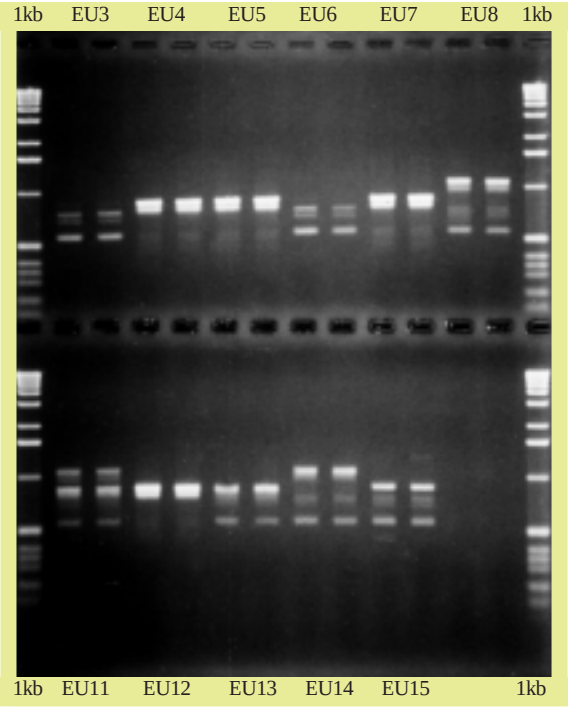

Figure 1 RAPD patterns produced by *Eca* strains EU3-8 and EU11-15 carried out in duplicate.

the disease in a small number of cases in England and mainland Europe, *Eca* is the main pathogen[1].

In Britain, blackleg and soft rot diseases, caused predominantly by *Eca*, lead to large crop losses and are of major economic importance. Since blackleg is a seed-borne disease, the most effective method of disease control is the production of clean seed, by avoiding or reducing *Eca* contamination. This can be achieved in several ways including early harvesting to reduce spread of the pathogen from rotting mother tubers to daughter tubers, the use of micropropagated stocks, the removal of rotting tubers during harvesting, reducing mechanical damage, and suitable storage conditions. In addition to controlling levels of the pathogen on tubers, monitoring these levels on seed stocks using selective media, immunological or molecular detection methods, have led to improvements in seed quality[2].

Although much of the ecology of *Eca* is known, some important questions remain unanswered, for example; how diverse are *Eca* strains in Scotland / Britain?; do *Eca* populations on contaminated stocks change during the bulking process?; at what stage during the bulking process and from what source does 'clean' seed become contaminated with *Eca*?

Answers to these questions would aid in developing the understanding of the disease and, therefore, devising ways to combat it. In order to answer these questions, individual strains of the pathogen must be easily identified (strain typing) using rapid, reliable methods. Traditionally, strain typing has been carried out using serology, where antibodies with different specificities have been produced to differences in cell surface structure[3]. Unfortunately, whereas *Ecc* and *Ech* are serologically diverse, *Eca* is limited to just a few serogroups, one of which predominates (serogroup I). In Scotland, the predominance of serogroup I is believed to be as high as 95 %, and in The Netherlands 100 %, making a serological approach, for tracking individual strains of *Eca*, not possible[2].

At SCRI, we have a long history of work on the ecology of the soft rot erwinias[1]. We, therefore, wanted to develop new methods of *Eca* strain typing, to allow us to study new areas of their ecology. To date, three methods of *Eca* typing have been developed at SCRI. These include Random Amplified Polymorphic DNA (RAPD) analysis, phage typing, and carbon source utilisation (BIOLOG).

Random Amplified Polymorphic DNA (RAPD) The polymerase chain reaction (PCR), first described in the mid 1980s, has revolutionised molecular biology. This technique, which uses primers of predefined sequence, allows a selected region of DNA to be amplified millions of times. This product can then be visualised on an agarose gel. A variation on this method, RAPD, using arbitrarily defined primers, allows the detection of polymorphisms (variations in DNA sequence) leading to the amplification of a number of PCR products of differing length. Again, these products can then be visualised on an agarose gel. Using this technique, polymorphisms in the genome of different bacterial strains can be identified and compared. At SCRI, the genomes from a number of *Eca* strains have been extracted and tested against several RAPD primers. Primers identifying clear polymorphic differences between strains have been used to group these strains (Fig. 1).

Phage typing Bacteriophages (or phages) are viruses that infect bacteria. These phages have different bacterial hosts, their specificity depending on their own genetic and structural make up and on that of their host(s). Using *Eca* strain SCRI 1043, we have isolated 20 *Eca* phages from sewage (Fig. 2)[4]. These phages are specific to *Eca* strains only, infecting no other bacterial strains tested (including a number of *Ecc*, *Ech* and

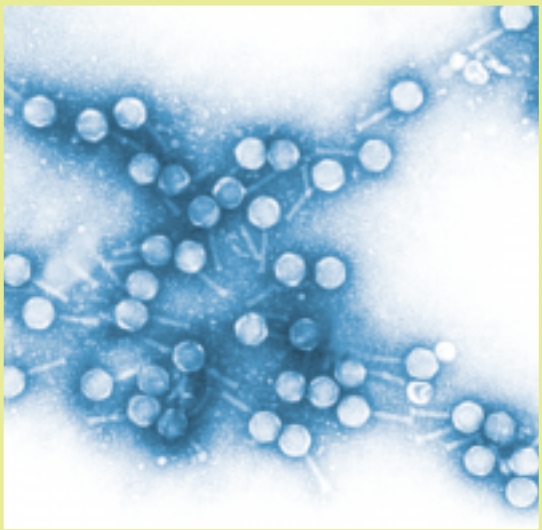

Figure 2 One of the phages (family Myoviridae) used in phage typing of *Eca*.

other *Erwinia* species). Each phage exhibits a unique host range profile, allowing us to identify individual *Eca* strains (Fig. 3). At present all 20 phages are being used in a phage typing system, but recent data suggests that a reduction in the number of phages used does not significantly affect the grouping of the isolates tested. Since this reduction in phage number simplifies the procedure, the time and cost of the procedure can also be reduced. In addition to this work, new methods of phage typing are being devised to increase the speed and accuracy of strain typing.

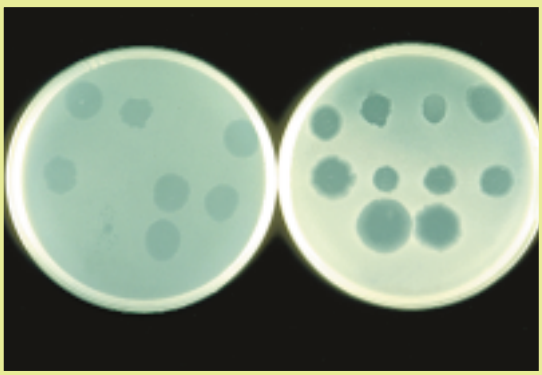

Figure 3 Phage typing of *Eca* strains SCRI 1043 and SCRI 84 using 10 different phages.

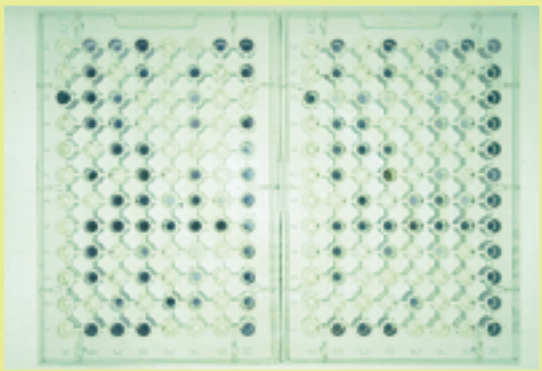

Figure 4 Biolog plates showing a typical pattern produced by an *Eca* strain.

Carbon source utilization The Biolog system, developed by Biolog, Inc., Hayward, U.S., was devised for the identification of bacterial species by comparing differences in their carbon source utilisation. Two plate types for bacterial identification are currently available, GN plates for Gram-negative bacteria and GP plates for Gram-positive bacteria. We have recently found, at SCRI, that by combining these two plate types, the accuracy of the system is sufficiently increased to differentiate between individual strains of *Eca*. Using this system, it has been possible to differentiate between many strains which exhibit different carbon source utilisation profiles (Fig. 4). In addition to the work on *Eca*, carbon source utilisation has been used to distinguish between strains of *Ecc*, *Ech* and a number of other species.

Conclusions As the development of the typing techniques reaches completion at SCRI, work is underway using these techniques to answer questions about the nature of blackleg disease. For the first time details are emerging about *Eca* strain diversity and its role in blackleg development, leading to an increased understanding of the disease and possible ways to combat it.

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Potato brown rot in temperate regions - a review

I. K. Toth, J. R. Wood[1] and J. M. Duncan

The organism Brown rot is a disease of potato caused by the bacterium *Ralstonia solanacearum* (Smith) Smith, formerly called *Pseudomonas solanacearum*. The bacterium has a wide distribution in the tropics, sub-tropics and warm temperate regions where it is one of the main factors limiting potato production.

R. solanacearum is a strictly aerobic, non-spore forming, Gram-negative organism, with a wide and diverse host range affecting several hundred plant species from 44 families, including the *Solanaceae*, *Compositae* and *Leguminosae*. Host plants of economic importance include potato, tomato, tobacco, pepper, eggplant, groundnut and banana. In addition, several ornamental plants and weeds can act as host reservoirs of infection. *R. solanacearum* has been divided into five races, based on host range, and five biovars based on biochemical tests. Of the five races, 1 and 3 cause symptoms on potato, with major yield losses from rotting tubers (brown rot) (Fig. 1) and wilting with subsequent death of the plant (bacterial wilt) (Fig. 2 and 3)[1].

Race 1 has a wide host range, infecting many species of plants in the tropics and sub-tropics; race 3, equivalent to biovar II, has a narrower host range, infecting potato, tomato, eggplant and some solanaceous weeds such as bittersweet (*Solanum dulcamara*) and black nightshade (*S. nigrum*). Race 3 is adapted to pathogenesis at lower ambient temperatures and is believed to have originated in the temperate highlands of Peru and Bolivia. It is closely associated with the potato, and is responsible for the present brown rot outbreaks in Europe and North Africa, and is the focus for this review[2].

Molecular analyses [Random Fragment Length Polymorphism (RFLP) and pulse-field gel electrophoresis of genomic DNA digested with rare-cutting restriction enzymes (RC-PFGE)] of race 3 isolates from around the world, have indicated that genetic diversity is greatest among isolates within central and western South America[3,4].

Recent investigations using four molecular techniques [Amplified Fragment Length Polymorphism (AFLP), Enterobacterial Repetitive Intergenic Consensus (ERIC), Repetitive Extragenic Palindromic - Polymerase Chain Reaction (REP-PCR), and (RC-PFGE)], on different isolates from contaminated potato material from different European countries, have indicated that several different clonal lines of race 3 are present in Europe[5]. Genetic variation, however, was small compared with those both within race 1 and between races 1 and 3. The importance of this variation among race 3 strains in terms of ecological fitness is still to be established.

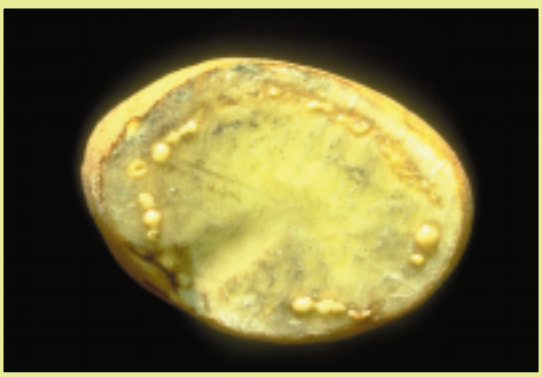

Figure 1 Potato tuber showing brown rot symptoms caused by *R. solanacearum*. Bacterial ooze is seen exuding from the vascular ring.

Figure 2 Potato plant showing bacterial wilt symptoms caused by *R. solanacearum*.

[1]Scottish Agricultural Science Agency, East Craigs, Edinburgh, EH12 8NJ

Figure 3 Potato field in Peru showing symptoms of bacterial wilt caused by *R. solanacearum*.

The disease Infection commonly occurs *via* the soil, where bacteria enter the root system of the plant at root emergence points, at wound sites, e.g. caused by nematode activity or soil particle abrasion, or *via* infected mother tubers. The pathogen enters the vascular system and under favourable conditions cell numbers increase and spread up the stem and to daughter tubers. As infection continues, pathogen numbers increase further and large amounts of extracellular polysaccharide (EPS) are produced. The EPS helps to prevent bacterial recognition and mobilisation of plant defence components, and also aggregates bacterial cells causing occlusion of transport vessels within the plant stem. In warmer regions, where transpiration rates are high, the disease usually manifests itself as a general wilting of the stem (bacterial wilt) (Fig. 2 and 3). In cooler regions, wilting may be less evident or absent. Where symptoms develop, brown staining is seen in the vascular tissues of cut tubers, caused by cell-wall-degrading enzymes produced by the bacteria (Fig. 1). Bacterial ooze may also exude from the vascular tissue and, in severe cases, from the eyes resulting in soil sticking to the exterior of the tubers. As disease progresses, a general rot may develop. Tubers may also harbour latent infections[1].

Race 3 has caused up to 50% loss of potato crops in Burundi and up to 75% in Florida. Crop loss is only part of the overall economic loss due to the disease. As it is a quarantine organism, there can be large costs due to disease testing and administration of seed production to control the disease.

Resistance, or more accurately tolerance, to the disease has been observed in potato. A major source of resistance to *R. solanacearum* has been from *S. phureja*, but resistance genes from other potato species, including *S. tuberosum*, have been identified and used. Tolerance in cultivars that harbour relatively large populations of *R. solanacearum*, in the absence of disease symptoms, increases the risk of spread through trading infected tubers[1].

Spread and survival The most important means of both short- and long-distance dispersal of *R. solanacearum* race 3 is through the movement and growing of infected seed potatoes. The disease can also be spread by trade in ware potatoes, *via* irrigation water, plant debris, mechanical transmission, insects, root-to-root transmission, wind and rain, wounding during cultivation practices and by nematodes.

The survival of *R. solanacearum* race 3 in the environment is not well understood. However, protecting the organism from desiccation and antagonism by other microorganisms can prolong this survival. *R. solanacearum* tends to persist longer in wet but well-drained soil, in the deeper soil layers (>75 cm), or in the presence of alternative crops, weed hosts or groundkeepers. Soil survival is reduced by extreme cold and the presence of antagonistic microorganisms. Under temperate conditions in Australia, England, Kenya and Sweden, evidence suggests that race 3 cannot be detected in soil two years after harvest of infected potatoes. This suggests that long term persistence in soil in temperate regions may not be a major factor in establishment of the pathogen[6].

R. solanacearum infects alternative hosts including solanaceous weeds, which increases the likelihood of survival. Although disease symptoms may not develop in these hosts, bacteria continue to multiply and may become a source for re-infection of potatoes. Race 3 has been shown to survive and multiply in the roots of the secondary hosts bittersweet (*S. dulcamara*) and black nightshade (*S. nigrum*) growing in rivers in a number of European countries[6]. This survival in secondary hosts, as well as in groundkeepers, could make crop rotation ineffective.

Brown rot in Europe European Community (EC) Plant Health legislation classifies potato brown rot as a quarantine organism, with consequent restrictions to prevent its spread. In the European and Mediterranean area, outbreaks of brown rot have been previously reported in Portugal, Italy, Greece, Spain, Egypt, and Cyprus. More recent occurrences have been recorded in Sweden in 1972; in Belgium, The Netherlands and England in 1992; and in Belgium, France, Italy, Austria, Portugal, Turkey, England and Spain between 1995 and 1996. Brown rot has never been found in Scotland.

The 1995 outbreak of brown rot in The Netherlands, a major producer of seed potatoes, resulted in the introduction of emergency EC legislation (95/506/EC), aimed at preventing the spread of the disease. These measures required The Netherlands to prevent movement or export of any seed potatoes from infected farms, and ensure that all other consignments of seed potatoes were tested for latent infection.

During 1995, the Dutch Plant Protection Service tested 60,000 seed stocks for *R. solanacearum* at a cost to the industry of £3 million. Extensive surveillance performed in 1995 and 1996 has made good progress in establishing the source and extent of the infections. Measures were introduced to control potato production in infested zones to prevent spread of the organism[7].

Under the 95/506/EC legislation, other Member States were permitted to monitor and test imports for the presence of the disease. The Agriculture Departments in Great Britain, the Ministry of Agriculture Fisheries and Food (MAFF) in England and Wales, and the Scottish Office Agriculture, Environment and Fisheries Department (SOAEFD) in Scotland, introduced measures whereby all consignments of potatoes, grown in the Netherlands in 1995 and imported into Great Britain had to be notified prior to arrival. These arrangements continued for the 1996 season.

In 1997/98, a Brown Rot Directive will be introduced throughout the EC which will specify that surveys must be performed to monitor potato production for the disease, and the measures that will apply if an outbreak is detected. Once infected fields are identified, measures will require that land remains free from potato cultivation for four to five years, with at least the first three years under bare fallow, grasses or cereals (non-host rotation crops) and groundkeepers will have to be controlled. Measures will be specified for potato production in adjacent fields and in infested zones.

Testing for *R. solanacearum* In the EC, stocks are sampled at a rate of 200 tubers per stock or 25 tonne lot for *R. solanacearum* testing, giving an 87% probability of detecting a 1% level of infection (see box). Cores containing vascular tissue are removed from the stolon end of each tuber, combined, and an extract prepared by maceration and centrifugation.

Several sensitive methods have been developed for detection, including growth of bacteria on semi-selective media[8], immuno-fluoresence staining (IF); enzyme linked immuno-sorbent assay (ELISA)[9]; molecular analysis using the polymerase chain reaction (PCR)[10]; and pathogenicity testing. These methods are effective at detecting *R. solanacearum* in plant material, particularly when used in combination, as they are in the UK[11]. However, detection in more complex substrates such as soil or waste effluents may need to be improved to enable effective monitoring of survival and spread of the pathogen in the environment.

Measures in Scotland The UK has applied plant quarantine measures for many years to prevent the entry of non-indigenous pests and diseases. Until

Statistics behind sampling for *R. solanacearum*

The EC sample 200 tubers from 25 tonne seed lots for *R. solanacearum* testing. If the population to be sampled is very much larger than the size of the sample taken and it is assumed that diseased tubers are randomly distributed in the lot, then the following equation can be applied to the sampling with some accuracy:

$$P_{sampling} = 1 - (1-d)^n$$

where:

$P_{sampling}$ is the probability of collecting an infected tuber in a sample of n tubers from a stock in which the proportion of infected tubers is d. NB. This is the chance of getting an infected tuber in the sample and has nothing to do with subsequent detection of infection in the tuber by appropriate diagnostics (for which there will be another probability).

The chance of collecting and detecting disease in a sample will be as follows:

$$P_{detection} = P_{sampling} \times P_{diagnosis}$$

where $P_{detection}$ is the probability of detecting infection in a sample and $P_{diagnosis}$ is the probability of the diagnostic test detecting the infection in the infected tubers. This latter probability will vary and will depend on the technology employed to detect the infection and on the severity of infection. Obviously, the larger the size of the sample, the higher the proportion of tubers infected, and the more effective the diagnostic test, the greater will be the chance of detecting infection. The following table shows the effect of the first two of these factors.

Probability of getting an infected tuber in a sample related to size of sample and severity of infection

Sample size	Percentage infected tubers in seed lot			
	1	3	10	30
20	0.18	0.46	0.87	1*
200	0.87**	>0.99	1	1
2000	>0.99	1	1	1

* In sampling, probabilities are never exactly 1.0 (always slightly less) but in this and other cases they are so close to 1 as to be given as such in the table.

** Figure used by EC for sampling.

1993, all potato planting material entering Scotland had to complete post-entry quarantine testing at the Scottish Agricultural Science Agency (SASA), before it could be planted. This quarantine requirement protected Scotland from the entry of non-indigenous potato pathogens such as *R. solanacearum.*

The introduction of the European Single Market in 1993, to enable free trade of products within the EC, altered the controls on entry of potato material into Scotland. Under the new legislation, certain areas including Scotland, were recognised for the high health status of their seed potato production and designated as 'protected regions'. Seed potatoes from other EC countries are only eligible for entry into these 'protected regions' if they are of an equivalent health status, as defined by EC legislation, and designated as Community grade. Potato material from outside the EC still requires quarantine testing at the UK Potato Quarantine Unit at SASA before planting.

Seed potato production in Scotland is regulated by the UK Seed Potatoes Regulations 1991, under the control of SOAEFD. Seed production is maintained free from *R. solanacearum* by ensuring that all the starting material used to initiate seed production is derived from pathogen tested *in vitro* microplants. These microplants are held in the nuclear stock collection at SASA, and issued on request to licensed micropropagation laboratories for production of pre-basic stocks. All Scottish seed potatoes, other than those from Community grade stocks, are derived from these initial microplants which have all been comprehensively tested.

The increased trade in seed potatoes throughout the EC, together with outbreaks of EC quarantine diseases, has increased the need for disease monitoring. In Scotland, seed and ware potatoes have been surveyed annually for latent infections of *R. solanacearum* since 1993. In addition, systematic surveys of *S. dulcamara* in Scottish rivers, in potato growing regions, have been conducted since 1994. Plants are sampled and tested for infection by *R. solanacearum.* Finally, imported ware potatoes from outside the EC are inspected visually for brown rot at the port of entry to the UK. Additional EC measures introduced in 1997 include laboratory testing of a proportion of the ware potatoes imported from Egypt.

Is brown rot a problem for the future?

Brown rot is unlikely to result in significant crop losses in the UK under our current climatic conditions[12]. However, it could have a drastic impact on the seed industry through loss of confidence in the health of Scottish seed potatoes, and additional costs of testing and surveillance required by legislation. The introduction of brown rot into Scotland would, therefore, be a very serious and damaging development, and it is imperative that diligence is maintained in testing imports both now and in the future.

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Plant viruses

Peter F. Palukaitis

In the year of the bicentenary of the development of the first viral vaccine by Jenner, many are asking quo vadis, virosi? The question applies to plant virology at several levels, since as we reassess where the field of plant virology is going, we continue to see progress on the complex question of how viruses get around. The investigation of virus movement, whether at the level of cell-to-cell, leaf-to-leaf, or plant-to-plant, continues to lead to new and exciting observations, which in turn provide information essential for our understanding of how plants restrict virus infection; i.e., mechanisms of resistance. The axiom, most plants are resistant to most pathogens, applies equally to viruses as to other plant pathogens. Research at SCRI endeavours to understand these mechanisms of intra- and inter-plant movement and restriction, through the work done by three of the units within the Virology Department. Hence, virology at SCRI continues to thrive and to use various tools and systems to examine important mechanisms of interaction between viruses and plants, as well as between viruses and their vectors. Even though we are making excellent progress in these endeavours, we continue to reassess where we are going as a discipline and as a department. Thus, we will see further changes in the structure of our virology research programme and an increased focus on multidisciplinary research, which is already one of our strengths.

The virology programme at SCRI took some new directions in 1996, with the appointment of P. Palukaitis (ex Cornell University) as the new Head of Virology, and the amalgamation of the Deputy Director's (T.M.A. Wilson) Agrobiotechnology Unit with the Virology Department. The appointment of a former virologist (W. De Jong) as a Molecular Geneticist in the Crops Genetics Department, as well as the continued association of a virologist (A.T. Jones) with the Soft Fruit and Perennial Crops Department, highlight that not only viruses but those that study them are mobilised, disseminated and become established in new locations.

The Agrobiotechnology Unit is highly focused towards the expression of foreign proteins in plants, using the OVERCOAT™ Technology described by Chapman *et al.* in the SCRI Annual Report for 1995 (pp.135-137). This work has attracted considerable outside funding and multidisciplinary partnerships. This group has prepared a new biologically active cDNA clone of an attenuated strain (i.e. showing reduced symptoms) of potato virus X (PVX) for development as a vector. They have also purified an OVERCOAT™ PVX carrying a *Chlamydia* major membrane protein epitope, which is being assessed in vaccine trials.

Research in the Antibody Biotechnology and Diagnostics Unit also attracted industrial support as well as government funding, e.g. a substantial DTI LINK award for the application of novel antibody technology to the detection and monitoring of pollutants in water. The SOAEFD-supported research continues to explore the potentials of obtaining antibody-like proteins from a phage display library and refining and improving that technology (see pp.125-127 of the SCRI Annual Report for 1995).

In 1996, this group developed or showed the following:

(i) An ELISA could be devised that incorporated cucumber mosaic virus (CMV)-specific single-chain variable fragment (scFv) antibody fused to alkaline phosphatase. This enabled the production of the conjugate in bacteria, which should decrease the costs of the ELISA reagents.

(ii) scFv antibodies could be obtained specific to blackcurrant-associated nepovirus, as well as a plant enzyme, enoyl acyl carrier protein reductase. This further confirmed the utility of methods to detect reagents using phage display libraries that express a diverse range of antibody genes.

(iii) Three assay procedures (ELISA, dot-blot hybridisation, and RT-PCR) were tested for detection of potato mop top virus (PMTV) in tubers of five different potato cultivars. ELISA was the most robust and readily applicable method for use on large sample numbers.

(iv) An ELISA protocol could be developed to detect potato virus Y (PVY) in dormant potato tubers within 1 month of harvest. This showed about the same sensitivity as a currently used growing-on test, which requires approximately 3 months.

The programme of P. Palukaitis in the Molecular Virology and Plant Interactions Unit is concerned with the molecular biology of the replication and movement of CMV and the application of cell biology techniques developed at SCRI to the well-defined CMV genetic system. The first results of this interdisciplinary co-operation are described in a separate report in this chapter. Other progress made by members of this unit includes further characterisation of the molecular biology and plant interactions involving PVX and the tobraviruses, tobacco rattle virus (TRV) and pea early browning virus (PEBV).

An analysis of four proteins encoded by RNA2 of PEBV showed that the 9K protein could not be detected as a readthrough of the 5'-proximal coat protein. The 29K protein could be detected readily in both leaves and roots of infected plants; however, the 3'-proximal 23K protein could not be detected in infected plants. Deletion of the 23K protein did not affect viral RNA accumulation, but resulted in the development of chlorotic ringspots on the leaves of *Nicotiana* species, in contrast to the situation with the wildtype PEBV, which is symptomless on these plants. A series of hybrid viruses was generated between RNA2 of PEBV and TRV to determine what sequences of RNA2 regulate its replication by RNA1. The data showed that only the 5' terminal sequences of RNA2 need to be derived from the virus corresponding to the source of RNA1. This is consistent with an observation that an RNA2 containing TRV terminal sequences and PEBV RNA2 internal sequences was found in nature, in association with TRV RNA1.

The PVX coat protein, which is required for cell-to-cell movement, was shown to localise to plasmodesmata, but was not involved in plasmodesmatal gating. The PVX 8K protein, encoded by the triple gene block of proteins involved in movement, was shown not to be essential for cell-to-cell movement, although it did affect the rate of local spread; however, it was shown to be necessary for long-distance movement in plants. The use of PVX constructs expressing the jellyfish green fluorescent protein (GFP), continued to be a valuable aid in studying virus movement, either as free GFP or fusions of GFP with movement proteins of other viruses (see reports in the 1994 and 1995 Annual Reports, as well as in this chapter).

The Resistance Unit has also made use of the PVX vector system, expressing sequences encoding an scFv antibody. These sequences were cloned from hybridomas producing monoclonal antibodies to potato virus V (PVV) coat protein. This has enabled functional scFv to PVV to be expressed in *N. clevelandii* for testing its ability to inhibit PVV accumulation. Other research results on virus resistance include the following:

(i) In the potato cultivar Barbara, the Ry_{sto} gene from *Solanum stoloniferum* conferred extreme resistance to potato viruses A, Y and V.

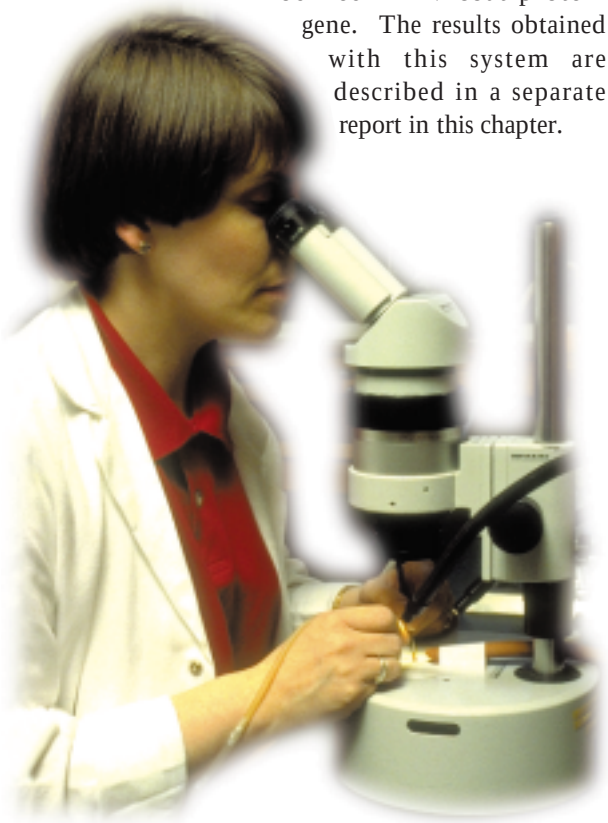
(ii) Transgenic *N. benthamiana*, expressing the coat protein gene of PMTV, conferred very strong resistance to PMTV, irrespective of whether the levels of transcript mRNA or coat protein accumulation were very high or very low. This novel form of resistance will be described in more detail in a separate report in this chapter.

(iii) Transgenic *N. benthamiana* and *N. clevelandii* plants, expressing a variety of nucleotide sequences derived from raspberry bushy dwarf virus, showed resistance to virus accumulation. The specifics of this resistance are described in detail in another chapter of this Annual Report.

(iv) Transgenic tobacco plants expressing a full-length cDNA clone of the potato leafroll virus (PLRV) genome, were generated. Infectious particles of PLRV developed in phloem tissue of such transgenic plants from seedling emergence. However, little virus seemed to accumulate in mesophyll tissue.

(v) Transgenic potato plants expressing the PLRV genome, were also generated for further studies on characterising natural and transgenic (pathogen-derived) mechanisms of resistance to PLRV.

Work on PLRV also continued in the Virus-Vector Interactions Unit, where it was shown that PLRV and some mutant strains could be propagated by agroinoculation of *N. clevelandii* using a cDNA clone of PLRV. This will enable mutants to be tested that can affect PLRV transmission by its aphid vector. Another approach to studying the interactions between PLRV (mutants) and its vectors involves the production of virus-like particles in insect cells, by expression of recombinant baculoviruses containing a modified PLRV coat protein gene. The results obtained with this system are described in a separate report in this chapter.



Other results obtained by research on virus-vector interactions include the following:

(i) The nucleotide sequence analysis of viruses (done in collaboration with virologists at ICRISAT and CIP) has led to the identification of two new luteovirus: one of these is associated with chickpea crops and may be related to chickpea stunt virus: the other virus has been named sweet potato leaf speckles virus and is more related to PLRV than to any other luteovirus, although it is not transmitted by all of the aphid-vector species of PLRV.

(ii) Two new vector species for PLRV were found. Several clones of *Myzus antirrhinii* transmitted PLRV more efficiently than the closely related, standard laboratory clone of *M. persicae*. On the other hand, *M. certus* transmitted PLRV only occasionally.

(iii) The melon or cotton aphid, *Aphis gossypii*, was detected on potatoes in western Scotland. This is a potentially new virus vector for potato crops in the UK, even though it was a poor vector of our isolate of PLRV, since it has been reported to transmit PLRV and PVY in warmer climates.

(iv) The nucleotide sequence of RNA2 of the TRV isolate PAY4 indicates that the virus does not have a 9K-like gene, even though it is transmitted by the same vector as PEBV. This casts some doubt on the role of the 9K protein in nematode transmission of PEBV, where a mutant of the 9K protein previously was demonstrated to affect transmission of PEBV.

(v) Serial passage of TRV under different selection conditions resulted in the loss of vector transmission. This was probably caused by deletions in TRV RNA2.

(vi) The nematodes *Xiphinema intermedium* and *X. tarjanense* (both members of the *X. americanum*-group) were shown to differ in their transmission of the nepoviruses tobacco ringspot (TRSV) and tomato ringspot (ToRSV) virus; *X. intermedium* was able to transmit both viruses and *X. tarjanense* was unable to transmit TRSV.

(vii) Transmission evaluation of 29 *X. americanum*-group populations revealed that 20 could transmit both TRSV and ToRSV, four could transmit TRSV alone, four could transmit ToRSV alone, and one could transmit neither. This indicates a low level of specificity between vector and virus.

Other highlights of the year included the following:

• Resistance-breaking isolates of TRV that caused spraing disease in potato cultivar Bintje were obtained from a site in Germany. One of these TRV isolates contained two distinct RNA1 molecules, which dif-

fered in their severity on *N. clevelandii*. Such RNA1 markers are rare.

• The pea enation mosaic virus (PEMV) satellite RNA could not substitute for the groundnut rosette virus (GRV) satellite RNA in its role in aphid transmission of the groundnut rosette assistor virus - GRV-satellite RNA complex, even though GRV could support the systemic accumulation of the PEMV satellite RNA. A detailed analysis of functional domains in the GRV satellite RNA is described in a separate report in this chapter.

• GRV open reading frames (ORFs) 3 and 4 were expressed as fusions with GFP from the PVX vector in infected *N. benthamiana*. The ORF4-GFP accumulated in plasmodesmata as well as in cytoplasmic inclusions. (ORF4 is similar in sequence to the CMV movement protein.) The ORF3-GFP accumulated only in cytoplasmic inclusions and in nucleoli.

• Two distinct geminivirus variants were found in samples of leaf curl-affected cotton from Pakistan. They showed similar epitope profiles, but differed in DNA-A sequence. Closely-related viruses were found in some other plant species, notably okra.

• In old, infected plants, cotton leaf curl virus produced small circular DNAs derived from DNA-A by various combinations of deletion, duplication, inversion and rearrangement.

• An epidemic of severe cassava mosaic disease in Uganda was shown to be associated with a geminivirus, designated the Uganda variant (UgV). The complete nucleotide sequence was determined for DNA-A of both a UgV isolate and of a Tanzanian isolate of east African cassava mosaic virus (EACMV), and partial nucleotide sequences were determined for several other isolates of each virus. DNA-A of UgV was shown to be extremely similar to that of EACMV, except for the central portion of the coat protein gene, which is like that of African cassava mosaic virus (ACMV). Serologically, UgV is identical to ACMV. Apparently, UgV arose by interspecific recombination between ACMV and EACMV.

• The C-terminal portion of the coat protein readthrough domain of PMTV was cloned and expressed in *E. coli*. An antibody, produced against the expressed protein, was used for immunogold labelling to demonstrate that the readthrough domain is present at one extremity of some virus particles.

• The third symposium of the International Working Group on Plant Viruses with Fungal Vectors was organised by L. Torrance and held in Dundee from 5-8 August. It was attended by 60 delegates from 15 countries.

Ultrastructural studies of the transmission of potato leafroll virus by aphids

F.E. Gildow[1], B. Reavy, J.A.T. Woodford, G.H. Duncan & M.A. Mayo

Potato leafroll virus (PLRV) causes the most widespread and important disease in potato crops world wide. It is classified in the genus *Luteovirus*, which also contains a number of other important pathogens of several of the major food crops of the world. One of the defining features of luteoviruses is that they are transmitted by aphids. Controlling populations of vector aphids is often used as a measure to limit the impact of PLRV infections, but such insecticidal control has environmental impact and is costly. Partly in pursuit of alternative approaches to the use of insecticides, both PLRV and its aphid vectors have been studied at SCRI for a number of years.

Properties of PLRV PLRV is transmitted in a persistent, circulative, non-propagative manner. Electron microscopy has established the complexity of the route by which particles of luteoviruses, such as PLRV, are acquired from infected plants and then transmitted to new plants. Figure 1 shows a longitudinal section of an aphid, alongside a diagrammatic section which shows the relationships among the

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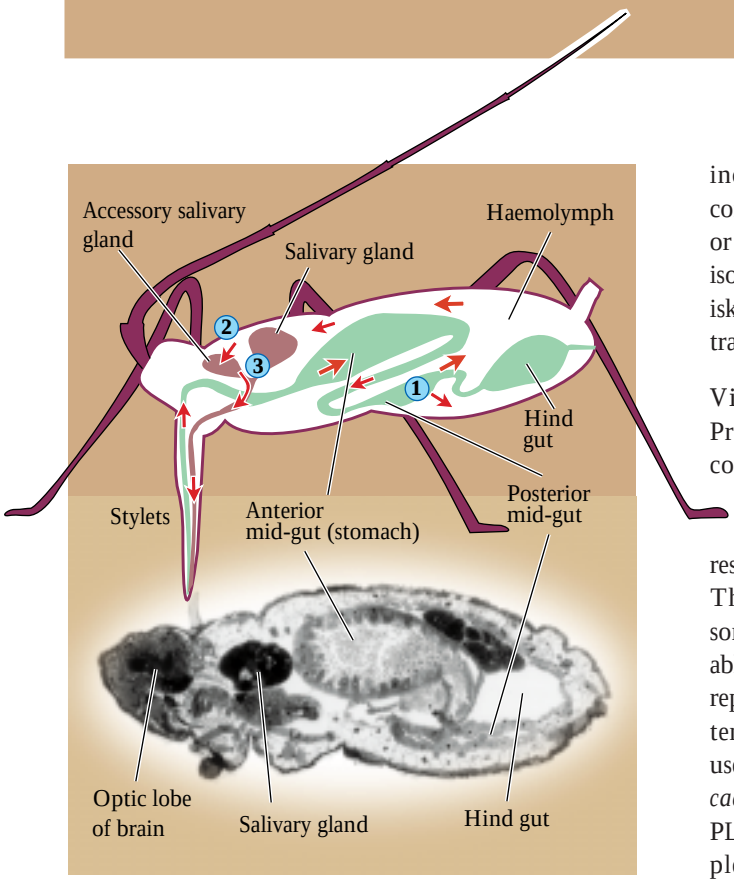


Figure 1 A longitudinal section of an aphid (lower) together with a diagrammatic section showing the relationships among the structures. The red arrows represent the passage of PLRV particles from a host plant, up the stylets, through the stomach, into the posterior mid-gut, then into the haemocoel and from there into the accessory salivary gland and thence with saliva into a new host plant. The numbered circles (blue) are the stages mentioned in the text.

structures and summarises the route of transmission. Particles are ingested with phloem contents by the feeding aphid and are then taken up from the gut into the haemocoel [Stage 1]. From there they enter the accessory salivary gland [Stage 2] from where they are then excreted in the saliva [Stage 3]. There is no evidence that PLRV multiplies in its vector aphid and thus the virus particles must remain intact during passage through the aphid body. It is therefore presumed that the transmission process is controlled by the structural proteins of the virus, in particular those parts which are on the surfaces of intact virus particles. Molecular studies have established the genetic map of PLRV and shown that the genome codes for six proteins (Fig. 2). The major protein present in virus particles is P3. Sometimes when the gene which codes for P3 is translated in the host cells, the ribosomes bypass the stop codon of the P3 gene and continue translation into the gene for P5. The resulting readthrough protein is a fusion of P3 and P5, and it is incorporated into PLRV particles. The results of comparisons among the amino acid sequences of P3 or P5 of poorly transmissible and readily transmitted isolates, suggested that a few amino acids in P5 (asterisked in Fig. 2) might be involved in efficient aphid transmission.

Virus-like particles from recombinant DNA

Previous work showed that when a modified PLRV coat protein gene was engineered into baculovirus DNA behind the polyhedrin promoter, expression of the recombinant baculovirus DNA in insect cells resulted in the synthesis of virus-like particles (VLP). The VLP comprised PLRV coat protein (P3) and some non-viral nucleic acid, and were indistinguishable in appearence from PLRV particles. In the work reported here, we have used these VLP as a model system for studying mutated virus particles. We have used two approaches. In the first, aphids (*Myzus persicae*) were allowed to feed on solutions of VLP or PLRV at about 100 µg/ml overnight and then sampled. In the second, aphids were injected intra-abdominally, directly into the haemolymph, with 0.02 µl inocula containing about 1 to 2 ng of PLRV or VLP. They were sampled after recovering on turnip leaves overnight. Typically, aphids were either transferred to test plants or, at intervals, fixed and embedded for electron microscopy. Most test transmissions of PLRV to *Physalis floridana* were successful. No infections resulted when aphids fed on, or injected with, VLP were tested.

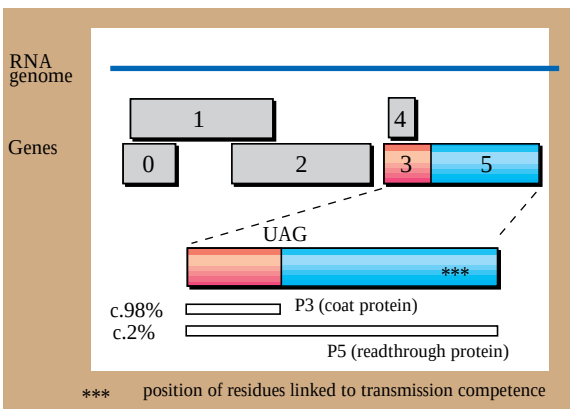


Figure 2 Diagram of the genome of PLRV. There are six open reading frames (0 to 5). Translation of ORF 3 produces the coat protein (red) and in about 2% of translations the UAG termination codon is suppressed and ORF 5 is translated as well to produce the readthrough protein. Asterisks indicate the approximate position of amino acids thought to be involved in aphid transmissibility.

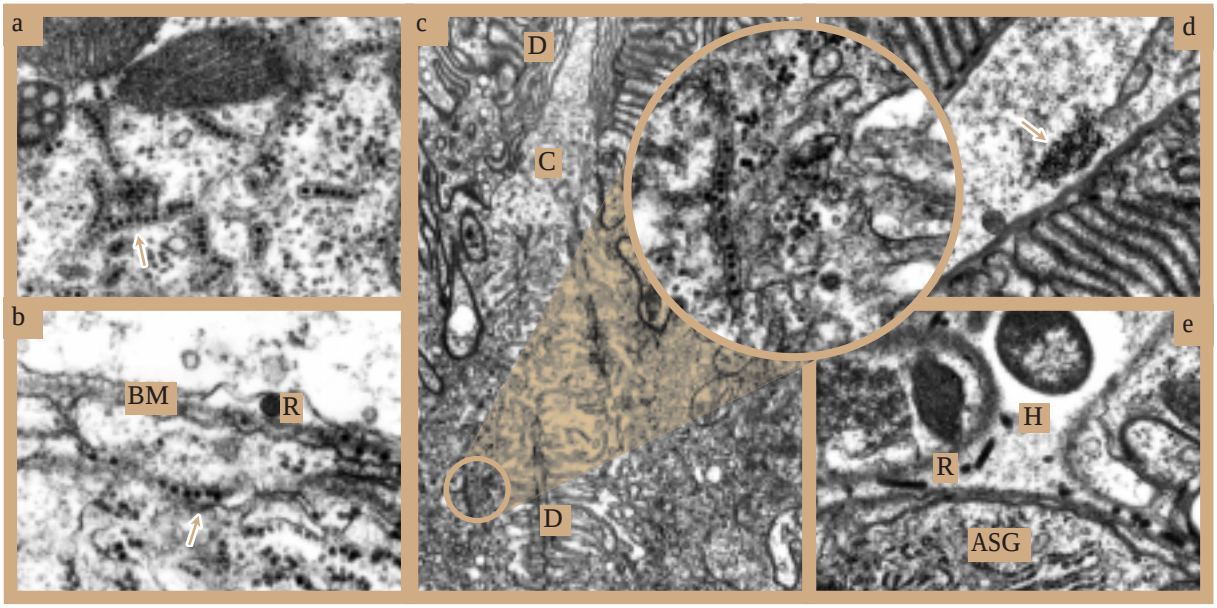


Figure 3 a) Part of a cell lining the posterior mid-gut. VLP (arrowed) are in membrane-bound structures that were formed by invagination at the gut surface.
b) Part of a cell of the accessory salivary gland. VLP (arrowed) are present in the basement membrane (BM) and in membrane-bound structures formed by invagination. A rhabdovirus particle (R) is in transverse section in the basement membrane.
c) Low magnification view of part of the accessory salivary gland. The saliva is secreted into collecting ducts (D) which drain into the gland canal (C). The circled area is enlarged as the inset and shows VLP in a membrane-bound structure close to some collecting ducts.
d) Part of the salivary gland canal (C) in an aphid injected with both VLP and antiserum to PLRV. The VLP (arrowed) are aggregated in the canal because of reaction with the antibodies. The canal drains towards the top right.
e) Part of the accessory salivary gland showing rhabdovirus particles (R) in the haemocoel (H) and along the edge of an accessory salivary gland cell (ASG).

Observations of virus or VLP in aphid tissues PLRV particles were observed at all the stages illustrated diagrammatically in Figure 1. Virus particles were seen being taken up by cells lining the posterior mid-gut and being released into the haemocoel (Stage 1), being bound to the basement membrane of the accessory salivary gland cells and taken up by these cells (Stage 2), and being released from the accessory salivary gland cells into the salivary gland canals prior to excretion in the saliva (Stage 3). Unexpectedly, when sections of aphids fed on, or injected with, VLP were examined, all the stages observed for PLRV particles were detected. Figure 3 illustrates these results. Figure 3a shows part of a cell lining the posterior mid-gut. VLP are present in membrane-bound structures which have formed by invagination of the cell membrane at the gut surface. Like PLRV particles, VLP were only taken up by cells in the posterior mid-gut. This contrasts with the uptake by cereal aphids of barley luteoviruses which takes place from the hind-gut. Figure 3b shows a cell of the accessory salivary gland which has VLP attached to the basement membrane, i.e. adjacent to the haemocoel, and also VLP in invaginated structures. No other cells were found that took up VLP in this manner. Figures 3c,d and e show part of the accessory salivary gland cell in which VLP have been ejected from the cell by exocytosis into the canals which feed into the salivary duct. Figure 3c shows a low magnification view of the gland; the inset shows part of the gland at high magnification in which VLP can be seen adjacent to the canals which collect the secreted saliva. Figure 3d shows particles that have collected in the salivary duct after exocytosis. The particles are aggregated because the aphid was injected with antibodies to PLRV at the same time as the VLP, and reaction with the antibodies has resulted in clumping of the particles. It was confirmed that the structures thought to be VLP were composed of PLRV coat protein by their specific labelling with gold-labelled antibodies.

Rhabdovirus infection of *Myzus persicae* It is known that aphids can be infected with certain viruses, and in our studies we have observed direct evidence for such

an infection. In sections of some aphids (e.g. Figure 3e), virus-like particles were observed in the haemolymph and between aphid cells. The particles were cylindrical with rounded ends, and measured about 260 nm long and 55 nm in diameter. The shape and size strongly suggest the virus was a member of the family *Rhabdoviridae*. Anecdotal evidence suggested that aphids infected with this rhabdovirus (or viruses) were less effective transmitters of PLRV than were rhabdovirus-free aphids. The observation prompts speculation that such viruses might represent a possible route for the control of virus vectors.

Summary The results help to narrow down the factors which influence the transmission of PLRV by aphids. It appears that it is only the major coat protein (P3) which determines that a virus particle, or VLP, passes through the barriers in the bodies of aphids and is injected into potentially fresh hosts with aphid saliva. This raises the question of what is the rôle of P5 in aphid transmission of PLRV. This is being tackled in current research work.

Acknowledgement

The work of Prof. Fred Gildow at SCRI was supported in part by the awards of a Fulbright Fellowship and a Mylnefield Research Services Fellowship

Potato mop-top virus: new insights into transgene-mediated resistance

H. Barker, B. Reavy, K.D. McGeachy & S.M.S. Dawson

The advantages of transgenic resistance to plant viruses using pathogen-derived sequences are well established and there are many examples where this approach has been successfully applied against potato viruses. The most common pathogen-derived transgene used for resistance is that encoding the coat protein, from which the term coat protein-mediated resistance (CP-MR) is derived. Potato mop-top virus (PMTV) is responsible for economic losses in potato crops grown in areas with cool climates and has been identified in Northern Europe (particularly Scotland, Sweden, Denmark and Finland), Canada, China, Japan and the Andean region of South America. PMTV is transmitted by motile zoospores of the plasmodiophoromycete fungus, *Spongospora subterranea*, which causes powdery scab on tubers. Infection with the virus can cause some yield loss but more importantly, qualitative damage, known as 'spraing', which can occur as brown arcs and circles in the flesh of tubers of susceptible cultivars. Some potato cultivars are particularly sensitive and when infected with PMTV, produce tubers with severe spraing symptoms. Effective and environmentally acceptable chemical control of the fungal vector is not commercially available, and there are no sources of resistance or tolerance to PMTV that have been deliberately used in breeding programmes.

In previous work[1,2], *Nicotiana benthamiana* was transformed with a translatable version of the CP gene from a Scottish isolate of PMTV (Fig. 1). Transgenic lines were found to have very strong resistance to infection by two Scottish isolates of PMTV and this resistance was effective following manual, graft, or fungal inoculation. If transgenic resistance is to be used in a potato breeding programme, several questions need to be considered. Firstly, what is the sequence variation in a spectrum of virus isolates that might be encountered by potato crops containing the transgene, and is the transgene effective against variant isolates? This is particularly important because CP-mediated resistance is not always effective against iso-

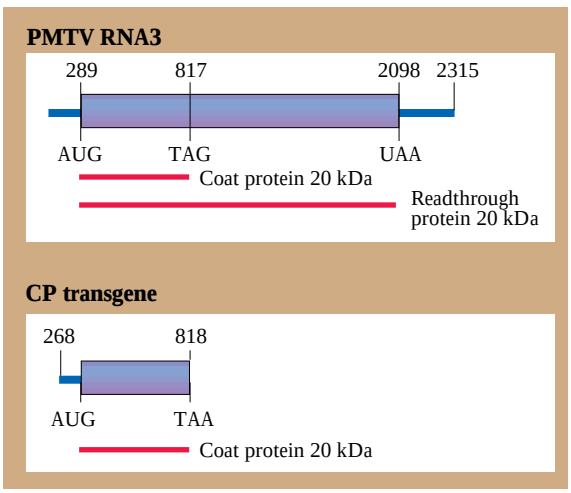


Figure 1 Genome organization of PMTV RNA 3 and the cloned CP transgene.

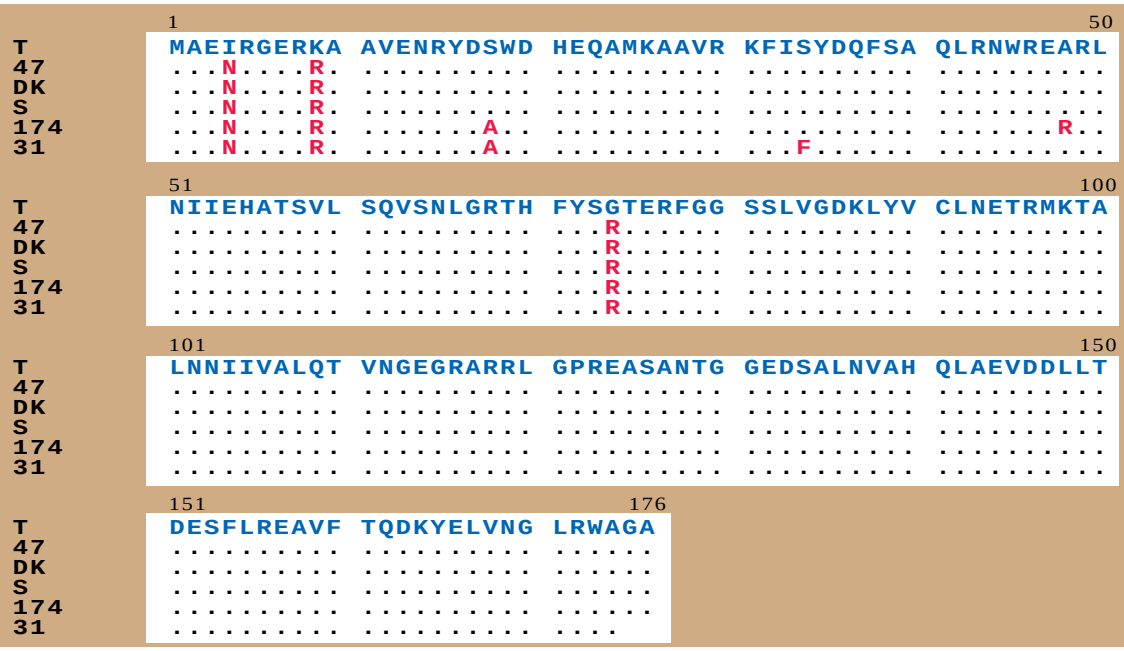


Figure 2 Comparison of the amino acid sequence of the CPs of the PMTV-S (Scottish isolate) and four Scandinavian isolates with PMTV-T from which the CP transgene was obtained.

lates whose CP gene sequences differ substantially from that of the transgene. Secondly, what characteristics of the transgene could be readily assessed in order to identify useful transgenic lines without the labour of exhaustive resistance testing? Thirdly, will the transgene remain effective through the crossing procedures encountered in a breeding programme? Our recent work has produced some answers to these questions.

Coat protein sequence variation in some European PMTV isolates The CP sequences from two Swedish, two Danish and two Scottish PMTV isolates were compared. The amino acid sequences of the four Scandinavian PMTV isolates were very similar to those of the Scottish isolates PMTV-T and PMTV-S (Fig. 2). Some isolates were identical, whilst others only differed by three amino acids. In addition, there were <2% non-coding changes in the nucleotide sequences of the other isolates when compared to PMTV-T, the isolate from which the transgene was obtained.

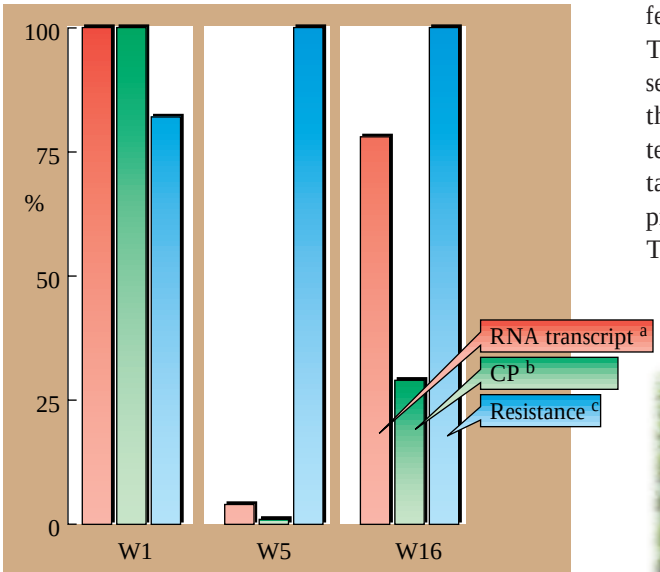
Effectiveness of transgenic resistance to Scandinavian isolates of PMTV Resistance was assessed by manually inoculating T_1 seedlings from *N. benthamiana* transgenic lines W1, W5 and W16 with the Scottish PMTV-S or one of four Scandinavian isolates. Approximately 17 days after inoculation, a sensitive infectivity assay was made on challenge-inoculated plants; the failure to recover infectious virus was used as an indication that a plant is resistant. Results indicate that apart from a few plants of some lines which became infected, infectious virus could not be detected in the inoculated transgenic plants (Table 1). In these tests, >93% of non-transgenic control plants became infected.

Features of the transgene expression in relation to resistance When tissue was sampled from transgenic seedlings and the levels of transgene mRNA transcript and endogenous CP accumulation were assessed, lines were found to differ substantially. Thus, levels of CP gene transcript and CP were high in some lines such as W1, intermediate in others such as W16 and low in

		Resistance[a] to manual inoculation with PMTV isolates				
Line	No.[b]	S	174	DK	31	47
W2	34	98%	100%	NT[c]	NT	NT
W5	13	NT	NT	100%	100%	100%
W8	34	97%	97%	NT	NT	NT
W15	16	NT	100%	NT	NT	NT
W16	14	NT	100%	NT	100%	100%

[a] Resistance calculated as the percentage of transgenic plants which remained virus-free after challenge inoculation.
[b] For each line, No. represents the number of plants inoculated with each isolate.
[c] NT = not tested.

Table 1 Resistance of five transgenic lines of *Nicotiana benthamiana* expressing the PMTV-T CP gene to manual inoculation with one Scottish and four Scandinavian PMTV isolates.



[a] RNA transcript accumulation levels estimated by hybridization of total leaf RNA with CP specific labelled cDNA probe in a northern blot and quantification of transcript bands. Data is expressed as percentage of the maximum amount of transcript detected which was in line W1.

[b] CP was assessed by quantitative ELISA of leaf extracts. Data is expressed as percentage of the maximum amount of CP detected which was in line W1.

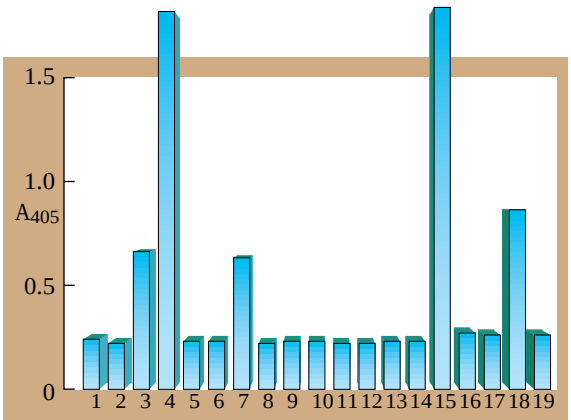
[c] Resistance is expressed as the percentage of transgenic plants that were not infected following challenge inoculation.

Figure 3 Expression of mRNA transcript, CP and resistance in three transformed lines of *N. benthamiana.*

others such as W5 (Fig. 3), although all three lines were highly resistant to PMTV. Indeed, of 10 independent lines of *N. benthamiana* transformed with the PMTV CP gene, all are highly resistant to infection (only a few transgenic plants of some lines become infected following inoculation) despite substantial differences in levels of CP gene transcript and CP.

Expression of the PMTV CP transgene in different generations For comparisons of CP accumulation in the different lines, samples were obtained by bulk sampling leaf tissue from many T_1 seedlings of a transgenic line. However, when individual plants of certain lines were assessed, it was evident that many plants contained only a very low level of coat protein, whilst a few contained high levels (e.g. line W8, Fig. 4). When a batch of such plants was challenge-inoculated with PMTV, most were resistant to infection and a few plants that did become infected were randomly distributed between the seedlings which accumulated different levels of CP. Several representative high- and low-expressing plants were allowed to self fertilise, and the T_2 seed progenies were collected. The level of CP accumulation in bulk samples of T_2 seedlings matched closely the level of accumulation in the original individual T_1 parent plants. In resistance tests, the T_2 seedling progenies were all equally resistant to infection (and as resistant as the original T_1 progeny), irrespective of whether their original parent T_1 plant expressed high or low levels of coat protein.

Discussion Our results show that several European isolates of PMTV are highly conserved in the amino acid sequence of the CP gene. These results and those reported elsewhere[3] suggest that the coat protein gene of PMTV isolates is highly conserved with isolates appearing to be more like minor variants. We have now shown that CP-MR, using a transgene from a Scottish isolate, is effective against four Scandinavian PMTV isolates. Given the high degree of coat protein sequence conservation between PMTV isolates, we



A leaf extract from each plant (abscissa) was tested by a quantitative ELISA. Thus, the A_{405} signal provides an estimate of the amount of CP accumulation in leaves. The A_{405} given by virus-free extracts was 0.23.

Figure 4 CP accumulation in individual transgenic plants from line W8.

expect this transgene to be effective against other isolates including those from the Andes.

The version of the PMTV CP transgene used in the experiments described here gives a high level of resistance, irrespective of the amounts of mRNA transcript and coat protein accumulated. Consequently, it seems that rigorous selection to identify those lines with the best resistance may not be necessary. Furthermore, it is encouraging that the PMTV CP transgene remains effective in two generations. Further tests in potato will be necessary to establish if transgenes are fully functional after crossing parents which contain homologous and heterologous transgenes.

More tests are in progress to establish whether the PMTV CP transgene is effective in potato, and to improve the functionality of the transgene for a potato breeding programme. The present evidence suggests that it may only be a few years before this type of biotechnology is used for the practical purpose of improving new potato cultivars.

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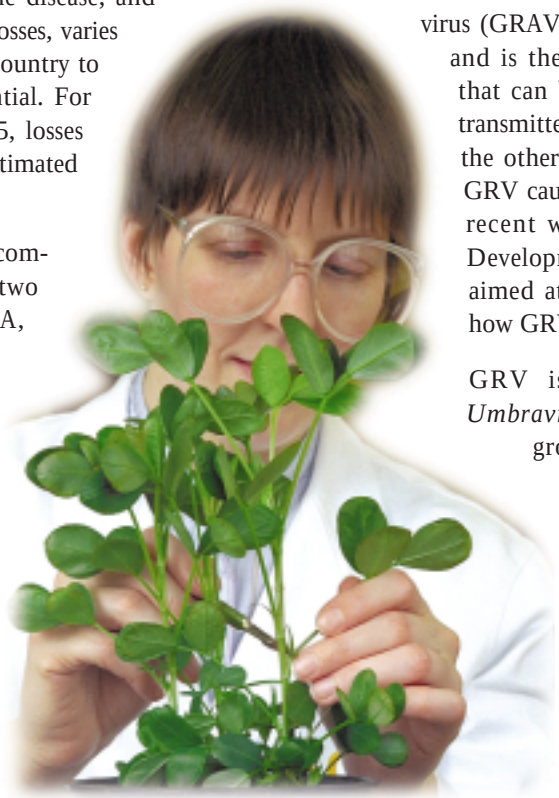
The causal agents of groundnut rosette disease: understanding the complex

D.J. Robinson & M. Taliansky

Rosette disease of groundnuts occurs only in Africa, south of the Sahara, but, in that area, it is the economically most important virus disease of groundnuts. Incidence of the disease, and therefore the extent of crop losses, varies from year to year and from country to country, but can be substantial. For example, in Zambia in 1995, losses due to rosette disease were estimated at $5 million[1].



The disease is caused by a complex of agents, comprising two viruses and a satellite RNA, which are transmitted by the aphid, *Aphis craccivora*. The interactions among these agents and their vector have been elucidated in earlier work at SCRI and are described in the *Annual Report for 1992*. In brief, symptoms of rosette disease are caused by the satellite RNA (sat-RNA), which relies on groundnut rosette virus (GRV) for its replication in infected plants. GRV and sat-RNA can be transmitted by aphids only from plants that are also infected with groundnut rosette assistor virus (GRAV). GRAV is a typical luteovirus, and is the only member of the complex that can both infect groundnuts and be transmitted by the vector independently of the other agents, but neither GRAV nor GRV cause symptoms on their own. Our recent work, funded by the Overseas Development Administration, has been aimed at understanding in more detail how GRV and the sat-RNA function.

GRV is a member of the genus *Umbravirus*. The plant viruses in this group have single-stranded RNA genomes but do not produce conventional virus particles. Because of this, they are rather difficult to work with and have been only imperfectly characterized. They are mechanically transmissible but each depends on a luteovirus (or luteo-like virus) for

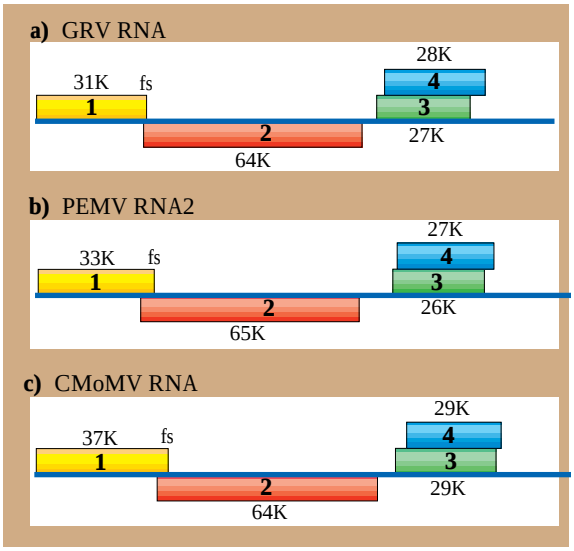


Figure 1 Diagram showing the arrangement of ORFs in (a) GRV RNA, (b) PEMV RNA-2 and (c) CMoMV RNA. In each case, the continuous horizontal line represents the RNA and the numbered coloured blocks the correspondingly numbered ORFs. The molecular weight of the predicted translation product is shown adjacent to each ORF. The ORFs coloured (1) and (2) are probably translated as a single polypeptide by frameshift at 'fs' and function as RNA-dependent RNA polymerases. The ORFs coloured (4) encode probable cell-to-cell movement proteins.

transmission by aphids, as GRV does on GRAV. The nucleotide sequence of the entire genome of GRV was determined; it comprises 4019 nucleotides and contains four large open reading frames (ORFs) (Fig. 1). ORFs 1 and 2 overlap, but ORF 2 does not contain an AUG initiation codon near its 5' end. However, immediately before the stop codon in ORF 1, there is a seven nucleotide sequence associated with frameshifting in several plant and animal viruses. It seems likely therefore that ORFs 1 and 2 are expressed as a single protein by ribosomes that change reading frame in the overlap region. Motifs present in the predicted amino acid sequence of this protein suggest that it is an RNA-dependent RNA polymerase, involved in replication of GRV RNA. The other two ORFs are almost completely overlapping in different reading frames, and are probably expressed from subgenomic RNA. The predicted protein product of ORF 4 is similar in sequence to proteins involved in cell-to-cell movement of several other plant viruses, and that is probably the function of this protein too. There are no clues as to the function of the ORF 3 protein, but it is probably not a coat protein because GRV does not form conventional nucleoprotein particles.

The only other umbravirus whose genome has been sequenced is one isolated from carrots in Australia and known as carrot mottle mimic virus (CMoMV). The arrangement of ORFs in CMoMV RNA is very similar to that in GRV RNA (Fig. 1), and the putative gene products are similar in size and sequence. Another viral RNA that resembles these two is RNA-2 of pea enation mosaic virus (PEMV; Fig. 1). PEMV is the sole member of the genus *Enamovirus* and has a bipartite genome. RNA-1 has similarities with the genomes of luteoviruses and it is now clear that RNA-2 is umbravirus-like. The biological properties of PEMV also resemble those of a luteovirus - umbravirus complex in several respects, and it seems likely that PEMV has evolved from such a complex, with increased mutual dependence of the two parts. Some isolates of PEMV contain a sat-RNA, which has been characterized by workers at Michigan State University[2]. Although the PEMV sat-RNA is somewhat smaller than that of GRV, there are sequence similarities between them. Therefore, in collaboration with the Michigan group, we examined their interchangeability. GRV was able to support the replication of the PEMV sat-RNA in several host species, including groundnut, which is not a host for PEMV itself. However, unlike the GRV sat-RNA, the PEMV sat-RNA did not induce any symptoms in groundnut plants. Conversely, PEMV supported replication of the GRV sat-RNA, in hosts including pea, which is not a host for GRV. Different variants of the GRV sat-RNA cause different symptoms in infected plants, and one in particular, called YB3b, caused brilliant yellow blotch symptoms in *Nicotiana benthamiana* whether the helper virus was GRV (Fig. 2) or PEMV. These results further strengthen the hypothesis of an

Figure 2 *N. benthamiana* plants showing (left) brilliant yellow blotch symptoms induced by infection with GRV and sat-RNA YB3b and (right) mild symptoms induced by infection with GRV and sat-RNA MC3a.

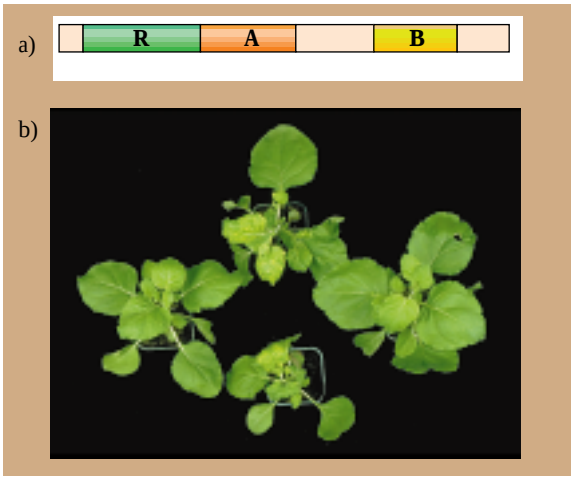


Figure 3 a) Diagram showing the positions in sat-RNA YB3b of the domains, designated A and B, involved in production of yellow blotch symptoms in *N. benthamiana* and of the domain, designated R, essential for sat-RNA replication.
b) *N. benthamiana* plants infected with GRV together with (top) sat-RNA YB3b, (left) sat-RNA YB3b with domain A deleted, (right) sat-RNA YB3b with domain B deleted or (bottom) mixture of sat-RNAs with either domain A or domain B deleted.

evolutionary relationship between PEMV and the luteovirus-umbravirus complexes, such as GRAV-GRV, and also suggest that the ability of the sat-RNAs to affect symptom production is an intrinsic property, largely independent of which helper virus they are associated with.

GRV sat-RNA YB3b consists of 903 nucleotides, and contains four small ORFs. Site-directed mutagenesis was used to replace the initiation codon of each of the ORFs with another triplet, in such a way as not to make any other change to the coding capacity. All four mutants replicated to the same extent as sat-RNA YB3b when inoculated together with GRV, and all produced yellow blotch symptoms in *N. benthamiana*. Thus, the ability to be replicated by the helper virus as a sat-RNA and the ability to induce symptoms do not require any of the potential translation products of the sat-RNA but are properties of the RNA sequence itself. Experiments with deletion mutants identified three functional domains in sat-RNA YB3b (Fig. 3a). One (designated R) comprises nucleotides 47 to 281 and is essential for sat-RNA replication. Sat-RNAs from which this region had been deleted could not be replicated and were functionally dead. Two other domains, nucleotides 280 to 470 (designated domain A) and nucleotides 629 to 849 (designated B), are both involved in production of the yellow blotch symptoms. Deletion of either of these domains prevented induction of yellow blotch symptoms (Fig. 3b), although sat-RNA replication was not affected. However, when *N. benthamiana* plants were inoculated with a mixture of sat-RNAs, one with domain A deleted and one with domain B deleted, together with GRV, yellow blotch symptoms were produced (Fig. 3b). Thus, production of these symptoms requires both domains A and B, but they can act *in trans*, i.e. they can be provided on different sat-RNA molecules. Preliminary experiments suggest that the same two domains are involved in the production of rosette symptoms in groundnut plants, and that here too they

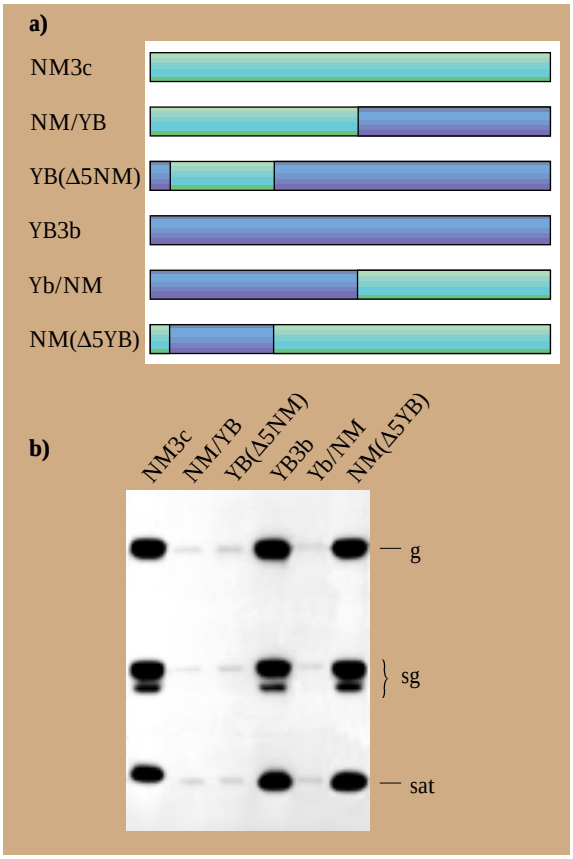


Figure 4 a) Diagram illustrating the construction of chimeric sat-RNAs between YB3b and NM3c. Sequences derived from sat-RNA NM3c are one colour and those from sat-RNA YB3b are another.
b) Northern blot of RNA extracted from inoculated leaves of *N. benthamiana* plants infected with GRV together with each of the sat-RNAs shown above. The top panel was probed with a GRV-specific probe and the bottom panel with a sat-RNA specific probe. 'g' indicates the position of GRV genomic RNA, 'sg' that of GRV sub-genomic RNAs and 'sat' that of sat-RNA. Note that the replication of all the RNAs is diminished when domain R of the sat-RNA is derived from NM3c.

Figure 5 *N. benthamiana* plants infected with GRV + sat-RNA YB3b (top left); uninfected (bottom left); inoculated with GRV + sat-RNA NM3c and challenged with GRV + sat-RNA YB3b (top right); inoculated with GRV + sat-RNA MC3a and challenged with GRV + sat-RNA YB3b (bottom right).

can act *in trans*. Experiments with chimeric sat-RNAs, in which domains A and B were obtained from different sat-RNA variants, showed that it is domain A that is unique to YB3b. Yellow blotch symptoms were produced only if domain A was provided by sat-RNA YB3b, whereas domain B could be obtained from other sat-RNA variants which do not themselves induce yellow blotch symptoms. Yellow blotch symptoms are induced only in *N. benthamiana* and *N. occidentalis* but not in other systemic hosts, such as *N. clevelandii*. It seems, therefore, that sat-RNA YB3b carries a specific determinant in domain A, together with a second determinant in domain B, which is common to several sat-RNA variants, and that one of these determinants is host specific. These two determinants are able to interact with some host factor or factors, leading to changes in the metabolism of the host plant and thence to symptom development. This process of symptom induction by interaction between two untranslated RNA elements, which can complement each other, is unlike anything that has been described before.

Another sat-RNA variant, called NM3c, which produces only very mild symptoms, drastically diminishes the replication of the helper GRV in infected *N. benthamiana* plants. In consequence, replication of the sat-RNA is also very low. Experiments with deletion mutants and chimeric sat-RNAs showed that this ability is a property of domain R of sat-RNA NM3c (Fig. 4). Furthermore, inoculation of *N. benthamiana* plants with GRV + sat-RNA NM3c protects them against the production of yellow blotch symptoms by inocula containing sat-RNA YB3b (Fig. 5), provided the two inoculations are no more than two days apart. Sat-RNA NM3c is able to down-regulate the replication not only of the helper GRV inoculated with it, but also of the helper virus in a challenge inoculum applied up to two days before or after it. Protection against yellow blotch symptom production is also afforded by a sat-RNA called MC3a, which neither itself induces symptoms in *N. benthamiana* nor affects GRV genome replication (Fig. 5). However protection by MC3a is less complete and occurs by a quite different mechanism from protection by NM3c, because replication of the GRV helper virus is not affected. It seems that, in this case, the MC3a and YB3b sat-RNAs compete for replication by the helper virus. Total sat-RNA concentration in plants that have received both sat-RNAs are the same as in plants that have received only one but, provided the MC3a-containing inoculum is applied before or not more than two days after the YB3b-containing one, half or less of this total sat-RNA is YB3b. The diminished level of YB-derived sat-RNA molecules in the plants apparently prevents the production of yellow blotch symptoms.

In groundnut plants, infection with either sat-RNA YB3b or MC3a together with GRV produces rosette symptoms, so that the kind of protection afforded by sat-RNA MC3a may not be of any practical value. However, sat-RNA NM3c produces few, if any, symptoms in groundnut, and a more attractive idea is to use sat-RNA NM3c, or perhaps just its R domain, introduced into groundnut plants by genetic engineering, to protect them against rosette disease. Other possible novel forms of resistance involve using a modified version of the RNA polymerase gene from GRV or the coat protein gene from GRAV. Practical application of these ideas has to await the development of a reliable protocol for the transformation of groundnuts, but meanwhile they are being tested in *N. benthamiana*.

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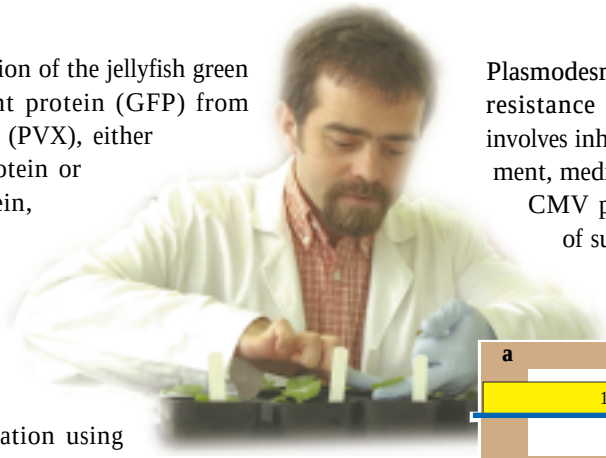
The green fluorescent protein and cucumber mosaic virus movement: lighting the way

P. Palukaitis, P. Boevink, T. Canto, K.J. Oparka & S. Santa Cruz



The expression of the jellyfish green fluorescent protein (GFP) from potato virus X (PVX), either as a fusion protein or as a free protein, and its application as a dynamic marker of virus movement and protein localisation using confocal laser scanning microscopy, was described in the 1995 Annual Report (pp.78-82). Here, we describe an extension of that technology to an analysis of the movement of cucumber mosaic virus (CMV) and the role of various CMV gene products in virus movement, which have provided new insight into the mechanism of movement of an agriculturally important virus with a wide host range.

Localisation of PVX:3a-GFP vs. PVX:GFP-3a The expression strategy of the CMV 3a movement protein (MP) as a fusion with GFP from the PVX vector (Fig. 1b & c), resulted in the GFP being fused to either the N-terminus or the C-terminus of the CMV 3a MP. Inoculation of *Nicotiana clevelandii* plants by such modified PVX vectors resulted in the normal course of infection for PVX. However, the subcellular distribution of the fluorescence was quite different for the two fusion proteins. The GFP-3a fusion protein showed a random distribution within the cell (Fig. 2a), often forming small aggregates, with some GFP being associated with plasmodesmata, while the 3a-GFP fusion protein was localised exclusively to plasmodesmata (Figs. 2b & c). Since the C-terminus of the CMV MP can be deleted, whereas the N-terminus can not be deleted without affecting movement[1], our data demonstrate that plasmodesmatal localisation of the CMV MP is dependent upon either some function or proper folding associated with the N-terminus of the CMV 3a protein.

Plasmodesmatal localisation and replicase-mediated resistance Replicase-mediated resistance to CMV involves inhibition of virus replication and virus movement, mediated by a transgenically-expressed defective CMV polymerase gene[2]. Microinjection studies of such transgenic tobacco plants have revealed that the resistance mechanism also inhibits the gating of plasmodesmata by the

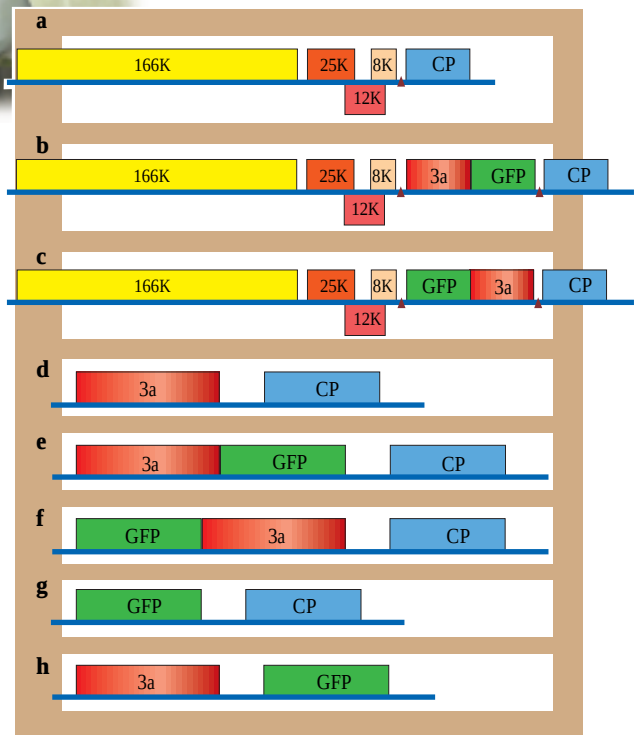


Figure 1 Schematic representation of potato virus X (a), cucumber mosaic virus RNA 3 (d) and modified viral genomes (b,c,e-h). a) Wild-type PVX RNA; b) Expression of the CMV movement protein fused at its C-terminus to GFP, under transcriptional control of a duplicated PVX subgenomic promoter (triangles); c) Expression of the CMV movement protein fused at its N-terminus to GFP; d) Wild-type CMV RNA 3; e) CMV RNA 3 with GFP fused to the C-terminus of the 3a movement protein; f) CMV RNA 3 with GFP fused to the N-terminus of the 3a movement protein; g) CMV RNA 3 with GFP replacing the 3a movement protein; h) CMV RNA 3 with GFP replacing the coat protein.

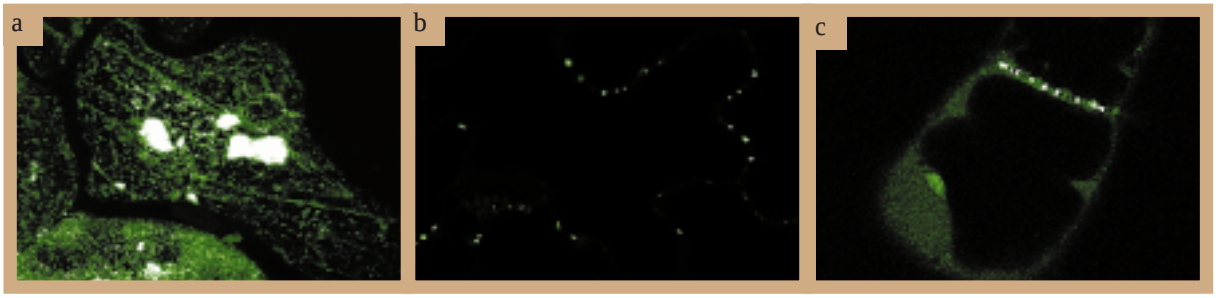


Figure 2 Subcellular distribution of 3a-GFP and GFP-3a fusion proteins expressed from PVX. The GFP-3a fusion protein (a) shows a random distribution in infected *N. clevelandii* epidermal cells. The 3a-GFP fusion protein (b&c) shows targeting to plasmodesmata, both in *N. clevelandii* epidermal cells (b), as well as in cells of *N. clevelandii* trichomes (c).

CMV MP, and it has been suggested that the transgenic defective polymerase might inhibit the MP from becoming associated with plasmodesmata[3]. We have directly tested this hypothesis, and shown that in such transgenic tobacco plants, which have no resistance to infection by PVX, the CMV 3a-GFP fusion protein still localised to plasmodesmata (as in Figs. 2b & c). Thus, while plasmodesmatal gating still may be affected in such transgenic plants, localisation of the MP to the plasmodesmata is not. Interestingly, plasmodesmatal localisation of 3a-GFP expressed from PVX also was not inhibited in transgenic tobacco expressing the CMV 3a gene, indicating that the 3a-GFP is able to compete with the wild type (wt) 3a protein for plasmodesmatal binding sites.

Localisation and function of CMV:3a-GFP vs. CMV:GFP-3a Fusions between the CMV MP and the GFP were also constructed in and expressed from CMV RNA 3 (Figs. 1e & f), which naturally contains the CMV 3a open reading frame as well as that of the coat protein (CP) (Fig. 1d). Infection of tobacco or *N. benthamiana* with CMV RNAs 1 plus 2 and the genetically modified RNAs 3, resulted in the expression of the fusion proteins in epidermal cells.

The subcellular distribution of the fusion proteins was the same as when these corresponding genes were expressed from the PVX vector (Fig. 2), except that infection by CMV expressing the fusion proteins was limited to single cells (see below). That is, neither fusion protein involving the CMV MP was able to translocate the movement of the virus. Curiously, transgenic tobacco plants expressing the CMV MP, which could complement the movement of MP-defective CMV[1], also were unable to potentiate the movement of the virus-genome expressing the GFP fusions. This might be due to an inability of the fusion proteins to translocate viral RNA, but still bind to RNA (and in the case of 3a-GFP, to associate with plasmodesmata), and prevent binding and translocation by the wt MP, or it might be due to some problem involving the encapsidation of modified RNAs 3.

Requirement for MP and CP for CMV movement In separate constructs, the genes for the CMV MP or CP, both expressed from CMV RNA 3, were replaced with a gene encoding the GFP (Figs. 1g & h). RNA 3 of these constructs was co-inoculated to tobacco plants with CMV RNAs 1 plus 2, and either limited or no movement was observed (Figs. 3a & b). By

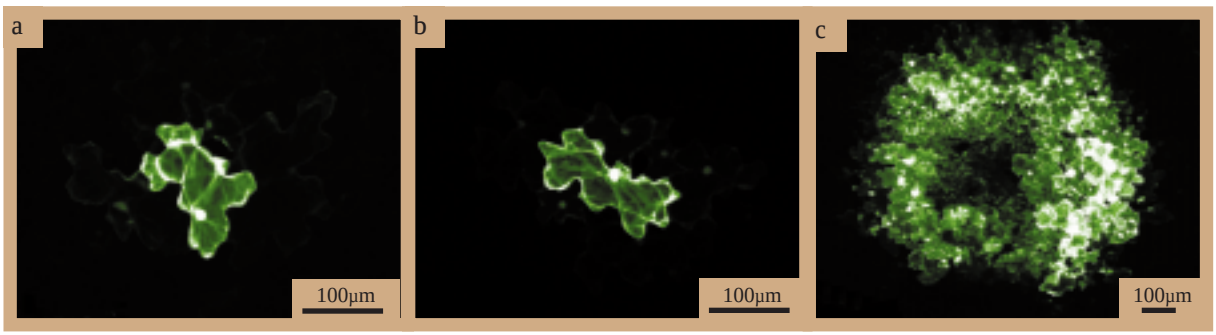


Figure 3 Expression of free GFP from CMV RNA 3 as a marker of genetic requirements for cell-to-cell movement of CMV. Tobacco plants were inoculated with transcripts of CMV RNAs 1+2 and transcripts of various RNA 3 constructs shown in Figure 1g & h. a) RNA 3 from Figure 1G, with the movement protein replaced by GFP; b) RNA 3 from Figure 1h, with the coat protein replaced by GFP; c) RNA 3 from Figure 1g & Figure 1h mixed together.

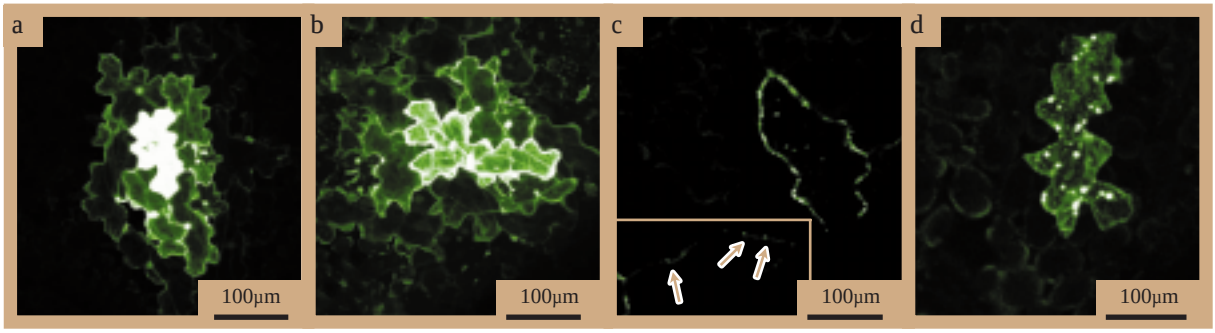


Figure 4 Expression of GFP in cells adjacent to a CMV inoculated cell forming a 'halo' of lighter fluorescence. Tobacco plants were inoculated with CMV RNAs 1+2 and transcripts of RNA 3 with either the CP gene replaced by the GFP gene (a), the 3a gene replaced by the GFP gene (b), 3a-GFP fusion (c) or GFP-3a fusion (d). Note the absence of halo from (d), and the magnified area (inset in c), showing GFP in the plasmodesmata of cells adjacent to the inoculated cell.

contrast, when the inoculum contained a mixture of both RNA 3 constructs expressing free GFP, there was complementation and virus movement (Fig. 3c). Thus, both the 3a MP and the CP are required for efficient cell-to-cell movement of CMV. However, inoculation involving two complementing RNA 3 molecules, did not result in systemic infection. The rate of cell-to-cell movement was slower than observed previously with wt virus, or in complementation experiments not involving GFP expression[1,2]. This may have allowed some host response to occur that precluded further movement of the virus.

Limited cell-to-cell movement and plasmodesmatal trafficking Although most infections involving the constructs in Figures 1e-h were limited to single cells, in most cases, a halo of much lighter fluorescing cells could be seen adjacent to or surrounding the brightly fluorescent cell (Fig. 4). This was observed for most of the CMV RNA 3 constructs (Figs. 4a-c) except the one expressing the GFP-3a fusion (Fig. 4d; see Fig. 1f). In the case of infections involving the 3a-GFP fusion, the fluorescence in the adjacent cells was also localised in the plasmodesmata (insert in Fig. 4c). This also occurred in leaf epidermal cells infected with RNA 3 expressing free GFP in place of the 3a protein, which implies that free GFP is trafficked by some host or other viral-encoded protein (while the GFP-3a fusion is not). Alternatively some limited translocation of the viral RNAs occurs, including the RNA 3 expressing the free GFP (but not RNA 3 expressing the GFP-3a fusion). In either case, the data suggest that there may be alternative trafficking mechanisms that can translocate some RNAs or proteins from cell-to-cell, albeit not as efficiently as the 3a MP. These conclusions are consistent with data obtained from other laboratories indicating that other CMV gene products play some role in virus movement[2,4,5].

Virus movement and the induction of a hypersensitive response *Chenopodium* species are often used as assay hosts for the presence of a virus. These plants can be infected by a broad range of plant viruses, and produce a hypersensitive response (HR) that often limits the infection to the inoculated leaf. The HR is usually manifested as chlorotic or necrotic lesions that appear at the sites of infection. Mutations of plant virus genes involved in virus movement, that cause an inhibition of movement, usually result in the absence of induced macroscopic lesions. To determine whether they also affect the HR from occurring at the single cell level, the various CMV constructs expressing GFP were inoculated to *C. amaranticolor* and the sites of infection were analyzed using epifluorescence microscopy. Thus, when sites of infection by CMV constructs, which only gave single-cell infections, detected by GFP fluorescence under UV light (Figs. 5a & c), were analyzed under visible light, there were no indications of any microscopic HR involving only the sites of infection (Figs. 5b & d). On the other hand, when the inoculum contained a mixture of GFP-expressing constructs, previously shown to complement each other (Figs. 1g & h and Fig. 3), fluorescence of multiple cells was observed (Fig. 5e) and a visible lesion developed. The visible lesion was still microscopic and included and surrounded the green fluorescent cells (Fig. 5f). Since infections involving the above constructs were slower to spread (Fig. 3), it is likely that the HR in *Chenopodium* has led to smaller lesions being produced because of more limited spread of the infection by the time the HR occurs (2-3 days after infection). It is also apparent that the formation of an HR leading to a visible lesion requires more than the infection of a single cell, but not a large number of cells. Future research will determine whether single-cell infections in general do not induce

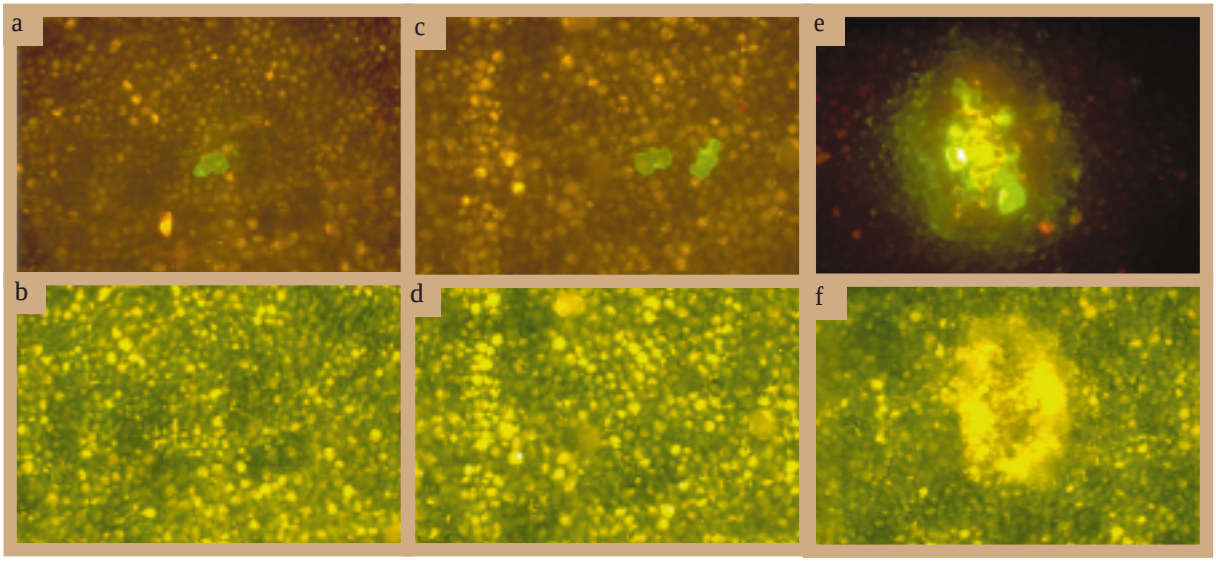


Figure 5 Analysis of lesion formation in *Chenopodium amaranticolor* inoculated with GFP-expressing CMV RNA 3 constructs. C. amaranticolor leaves were inoculated with CMV RNAs 1+2 and RNA 3 derived from constructs described in Figure 1g & h. The sites of infection were detected by green-fluorescence of the GFP under UV light (a,c & e) and the cells were then viewed under visible light (b,d & f). a,b) RNA 3 expressing the construct in Figure 1g with GFP replacing the CMV movement protein. c,d) RNA expressing the construct in Figure 1h with GFP replacing the CMV coat protein; e,f) A mixture of RNAs 3 expressing the constructs in Figures 1g & h, with GFP replacing either the movement protein or coat protein respectively; Note the visible lesion that forms in (f), where multiple cells were infected (e) vs. the lack of a visible lesion that forms in (b) and (d), when only single-cell infections occur (a & c).

HR in plants, or whether this observation is limited to *Chenopodium* species.

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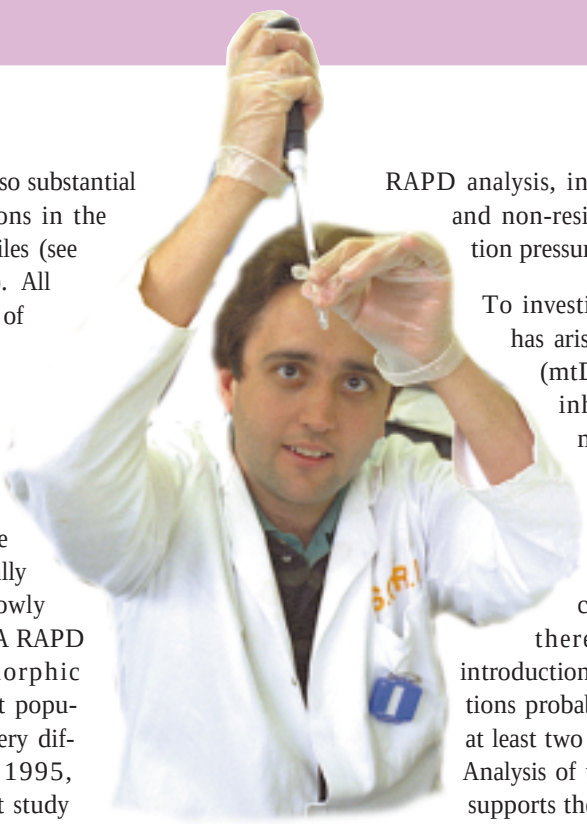
Nematodes

David L. Trudgill

Nematodes are ubiquitous. They cause serious diseases in man and animals, they are the major component of the soil fauna, and they are important pathogens of plants. Recent collaborative studies have shown that some of those that attack plants have extremely sophisticated interactions with their hosts and modify the expression of plant genes at their feeding sites. These changes in gene expression result in the induction of novel plant cells, which provide the developing nematode with abundant food. We work on two such groups of nematodes which have different strategies. The potato cyst nematodes have a very narrow host range, and reproduce sexually; the root knot nematodes have very wide host ranges and reproduce parthenogenetically. Both groups have been widely spread around the World and they have become serious pests. Consequently, much of our work has to be interactive with nematologists in Europe and other parts of the World and this requires external funding, an area where my colleagues have been exceptionally effective as we are currently involved in four different EU projects. Our contribution focuses on analysing the patterns of spread, particularly of different gene pools, and the implications for control using resistant cultivars. Other research is concentrated on understanding how nematodes induce the changes we observe in plants and on mechanisms of resistance. This report concentrates on these latter aspects.

Potato cyst nematodes Two species of potato cyst nematode (PCN; *Globodera pallida* and *G. rostochiensis*) were introduced into Europe from South America at some unidentifiable time. *G. rostochiensis* used to be the most widespread species and was present in most fields in much of western Europe which had a history of frequent potato production. It is extremely persistent between potato crops so that, in short rotations, populations rapidly increased and caused damage. The introduction in late 1960's of cultivars of potato resistant to *G. rostochiensis*, but not to *G. pallida*, has led to a progressive decrease of the former, so that it has almost been eliminated from many fields. However, *G. pallida* has progressively increased, so that it is now becoming a major threat, and it is on this species that our research is concentrated.

The *G. pallida* that was introduced into the UK appears to be much more variable (heterogeneous) than the *G. rostochiensis*, which all appears to derive from a single introduction. This heterogeneity within populations of *G. pallida* is maintained by sexual reproduction and is so great that none of the various sources of resistance tried are completely effective; some juveniles are always able to develop into adult



females with eggs. There are also substantial differences between populations in the proportion of 'virulent' juveniles (see Ann. Rep. for 1995, 151-154.). All of this adds to the difficulties of using resistant cultivars to manage populations of *G. pallida*.

Molecular techniques were used to analyse this variation and how it might affect the use and effectiveness of the partially resistant cultivars that are slowly starting to become available. A RAPD (random amplified polymorphic DNA) analysis confirmed that populations of *G. pallida* can be very different (see Ann. Rep. for 1995, 151-154.). However, a recent study has revealed an almost equal amount of within population variability. The genetic variation in a single population of *G. pallida* was fragmented by inbreeding a series of single cyst lines over six generations. The inbred populations were found to be almost as variable in their virulence to two sources of resistance, and in their RAPD patterns, as we had observed in the previous study where we had compared a range of different populations from around the UK. Selection of populations of *G. pallida* on clones of potato with resistance from different sources, and inbreeding on two different 'susceptible' cultivars, also led to populations that could be distinguished by RAPD analysis, indicating that both resistant and non-resistant cultivars exert a selection pressure.

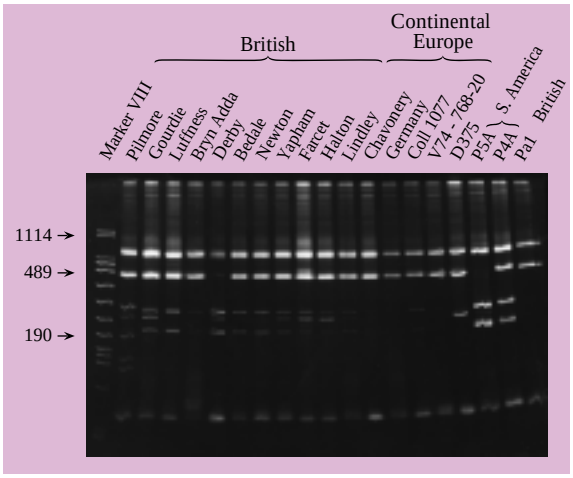


Figure 1 Digestion of PCR product from ITS region with restriction enzyme Rsal. The patterns with British populations are mixtures of the more simple patterns seen with South American and D375 populations.

To investigate how this heterogeneity has arisen, the mitochondrial DNA (mtDNA) of *G. pallida*, which is inherited mainly through the maternal line, has been examined to determine how many discrete introductions there have been into Europe. This study is now almost complete and indicates that there have been at least three introductions and that most UK populations probably arose from the mixing of at least two distinct mitochondrial types. Analysis of the ribosomal DNA (rDNA) supports the view that typical UK populations of *G. pallida* have arisen through hybridisation of populations with different rDNA (Fig. 1).

A minority of populations of *G. pallida*, including the extremely virulent Luffness population and the avirulent pathotype Pa1, are distinct and have less complex mtDNA profiles. Analysis of four populations from S. America showed that only one had similar mtDNA sequences to the majority of European populations. The others had distinct and different mtDNA profiles, indicating yet further variation.

Root knot nematodes The root knot nematodes (RKN; *Meloidogyne* spp.) provide an interesting contrast to PCN. The most damaging species reproduce by mitotic parthenogenesis, which is essentially a cloning process, and have Worldwide distributions. Comparative studies of populations of *M. incognita* from different continents have shown that they have very similar RAPD profiles and no differences could be detected in the ITS region of their rDNA. Even so, although in a host range study >90% of the plants tested were hosts for the different populations of *M. incognita*, a minority of plants were hosts for one or more populations but not for others, implying variation in the nematode which we had not detected in our molecular studies. Subsequent studies to characterise these populations concentrated on the intergenic spacer (IGS) region of the rDNA gene, a region which includes sequence repeats which are not transcribed. This has revealed differences between populations (Fig. 2) and should be extremely useful in future studies of the relationships between popula-

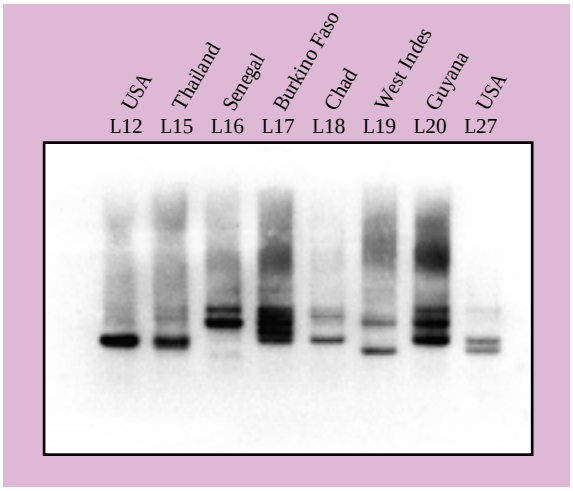


Figure 2 Southern blot of 18-28S - ribosomal PCR products from lines of *Meloidogyne incognita* probed with the 5S sequence contained with IGS from *M. javanica.*

tions in relation to their origins and host range differences. This region of DNA is also proving useful in collaborative studies, funded by the EU, on *M. chitwoodi* and *M. fallax*, two species which reproduce by facultative meiotic parthenogenesis, and which pose a threat to the European potato industry.

Integrated control of nematodes Modelling nematode population dynamics and their effects on crop yields, for which we have just been offered additional funding from the Potato Marketing Board, may appear unexciting. However, the interactions between the nematode, the crop and various environmental and management factors make it the most complex crop disease problem facing many growers and modelling is fundamental to providing the understanding needed to develop effective integrated control strategies. It emphasises the importance of achieving a substantial decline in populations by having a sufficient rotational gap between potato crops and shows that this is particularly true for the white species of PCN (*G. pallida*), whose increase is reaching epidemic proportions. The experience of farmers, laboratories processing soil samples, and Agricultural Advisors all indicate that *G. pallida* is now the most serious disease problem affecting ware potato producers. Population modelling shows that, in a typical rotation, the change from the yellow (*G. rostochiensis*) to the white species of PCN can be brought about in 20 to 30 years by the growing of cultivars resistant to the former. Modelling also shows that resistant cultivars, of which there is a lack for the white species, are crucial to the integrated management of both species. It also shows that nematicides are much more effective at controlling population increase when applied to low rather than high populations and makes a strong argument for controlling *G. pallida* before it reaches damaging population levels. Such a conclusion raises important questions about targeting non-fumigant nematicides to only the most heavily infested parts of fields, and the ethics and economics of treating light infections to prevent them becoming large and damaging.

Trap cropping of PCN Trap cropping by growing cv. Cara from small seed and then harvesting after 6 weeks has been tried and found to give variable decreases in *G. pallida.* Early harvesting is essential because Cara is not resistant to *G. pallida.* We have been seeking an alternative to potato as a trap crop, concentrating on Solanaceous spp. which have small seed which are easy to produce and sow, and which are totally resistant (immune) to both species of PCN. Hatching tests with a range of root 'diffusates' have identified several such plants which hatch PCN eggs, one of which is almost as effective as that from potato.

Nematode secretions Understanding nematode secretions is the key to understanding the complex changes nematodes induce in both susceptible and resistant plants. Both PCN and RKN induce (by different mechanisms) the plant cells at their feeding sites to become enlarged, multi nucleate and metabolically hyperactive. During feeding, nutrients are extracted through a 'feeding tube' which is thought to act as a molecular sieve, enabling the nematode to remove the cytosol without damaging the cell on which it is feeding. This feeding tube is formed from oesophageal gland cell secretions (saliva) and it is thought that other secretions from the same glands are involved in inducing changes in gene expression and cell development at the feeding site. In resistant plants, nematodes which cannot induce, or fail to maintain a healthy feeding site either die or become males (which need much less food than females).

In a collaborative EU project, it has been demonstrated that cyst and root knot nematodes modify plant gene expression at their feeding sites, with some genes being increased and expression of others suppressed. Promoters, tagged with GUS, have been isolated for use to differentially express various anti-nematode genes, including lectins, specifically at nematode feeding sites, as a means of producing transgenically resistant plants.

Systems for collecting nematode secretions have been developed to facilitate immunological and biochemi-

Figure 3 A hydroponic system for growing infected tomato to produce the millions of nematodes needed to collect their secretions.

cal analyses of its components (Fig. 3). An expression library has been screened using antibodies raised against collected secretions and several genes are currently being sequenced. The secretions are also being directly characterised and, in conjunction with the Moredun Institute, those from PCN shown to have high levels of SOD, an enzyme involved in protecting organisms from free radicals.

Engineering natural resistance Transgenic resistance based on 'foreign' genes is fraught with problems and an alternative is to use natural resistance genes. These specifically recognise and interact with a chemical derived from the pathogen and initiate a resistant response. Up to now resistance genes have been incorporated into new cultivars by traditional plant breeding whereby a susceptible plant is crossed with a resistant one. We are seeking to isolate a resistance gene (the '*Hero*' gene) from tomato, effective against both species of PCN. To do this, we are using a system developed at the Sainsbury Laboratory, whereby a transposon from maize has been incorporated into tomato. This transposon has the ability to change position in the chromosome when the appropriate enzyme is supplied and, if it moves into the Hero gene, then resistant plants will become susceptible. The transposon is 'tagged' so the gene whose effect has been disrupted can be subsequently isolated. We are making good progress, in collaboration with colleagues in Germany, towards isolating the *Hero* gene, which will also allow us to characterise the gene and provide a basis for exploring its mode of action and that of the chemical elicitor within the secretions of avirulent nematodes.

Nematode-induced promoters

N. Barthels[1], F.M.van der Lee[2], J. Klap[2], O.J.M. Goddijn[2], S.A. Ohl[2], P. Puzio[3], F.M.W. Grundler[3], M. Karimi[1], L. Robertson, W.M. Robertson, K. Lindsey[4], M. Van Montagu[1], G. Gheysen[1] & P.C. Sijmons[2,5]

The most damaging groups of plant parasitic nematodes include cyst (*Globodera*, *Heterodera*), root knot (*Meloidogyne*) and virus vector (*Xiphinema*, *Longidorus*) species. The cyst and root knot nematodes are endoparasites that become sedentary as the feeding site is established, whereas *Xiphinema* and *Longidorus* species are ectoparasites that invade the root only with their long stylets and can move from root to root to initiate their feeding sites. All these have complicated interactions with their hosts. Cyst and *Longidorus* spp. induce the plant cells at their feeding sites to greatly expand and, in the case of cyst nematodes, these coalesce to form a multi-nucleate syncytium with enlarged, polyploid nuclei (Fig. 1). Root knot and *Xiphinema* spp. induce similar changes, but the cells enlarge and become multi-nucleate by nuclear division without cytokinesis. The effect of these changes is to greatly increase the available food

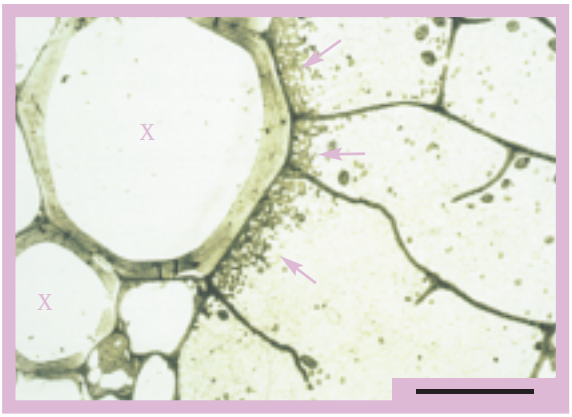


Figure 1 Electron micrograph of a transverse section through a feeding site induced by *Heterodera schachtii* showing the syncytium with cell wall ingrowths and the highly invaginated cell walls (arrowed) adjacent to the xylem vessels (X). Bar represents 10µm.

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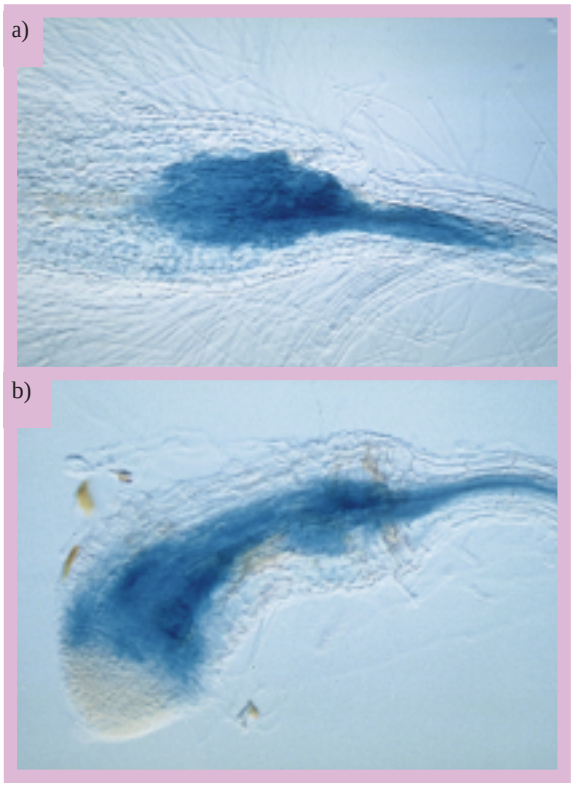

Figure 2 *Arabidopsis thaliana* roots showing *gus* expression associated with a nematode-induced promoter within galls induced by a) *Meloidogyne incognita* b) *Xiphinema diversicaudatum*.

to the developing nematode, and it has been long apparent that such modifications must involve changes in gene expression within the cells that form the feeding site.

To understand these changes, both the secretions injected by the nematode (see p. 183) and their effects on plant gene expression are being analysed. The latter studies have been part of an EU-funded Concerted Action Programme project, in which SCRI took responsibility for the studies with *Xiphinema*. A similar approach has been used with all three groups of nematodes, and involves studying the changes in gene expression at nematode feeding sites in *Arabidopsis* plants into which the ß-glucuronidase reporter gene (*gus*) has been randomly inserted. The *gus* insert lacks the promoter required for its expression, but if the point of insertion is close to a plant promoter, then the *gus* gene may be specifically expressed with the associated plant gene, resulting in a blue coloration in the tissues where this has occurred (Fig. 2). Changes in gene expression induced by nematode feeding are identified by changes in coloration at feeding sites, and both increased and decreased expression have been observed.

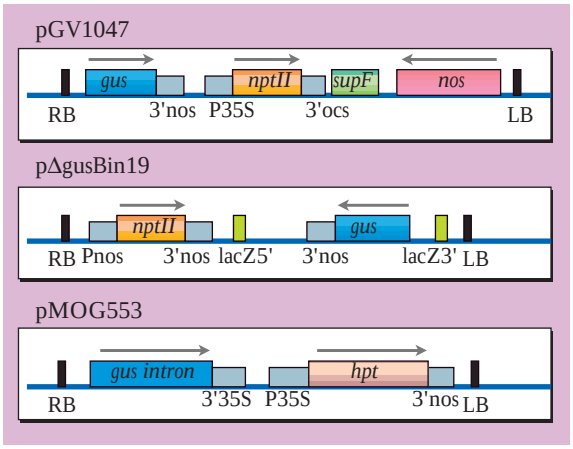

Figure 3 Diagramatic representation of T-DNA constructs pGV1047, pΔgus Bin19 and pMOG553 used to identify *Arabidopsis thaliana* lines with GUS labelled nematode-induced promoters.

Three classes of *Arabidopsis thaliana* promoter tagged lines were generated using the binary T-DNA vectors pGV1047, pΔgusBin19 and pMOG553 (Fig. 3). The potential use of these lines is extensive, and this technique has previously been used to identify molecular markers in plant development[1] and in the isolation of environmental and hormonal stress - responsive regulatory sequences or genes[2]. It is clear that these lines could have a wide range of applications and substantial numbers of them are now available from the Arabidopsis Seed Stock Centre, Nottingham, U.K. In the initial screening with *Heterodera schachtii*, approximately 1500 lines were tested both in sterile culture and in soil to exclude the possibility of artefacts. The bioassays showed that 103 of the lines had varying degrees of GUS activity within the syncytia. Mechanical injury did not

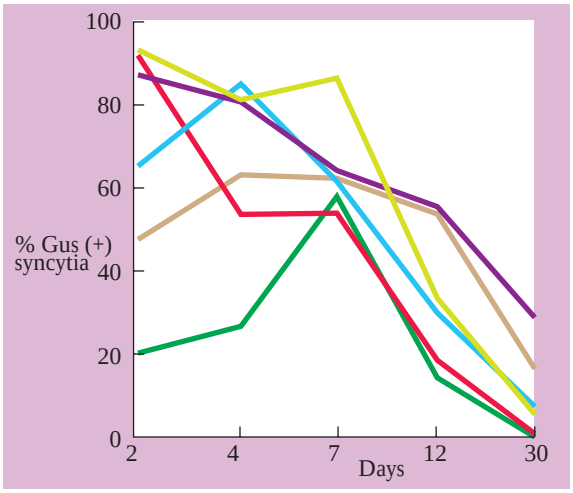

Figure 4 Graph showing a comparison of the percentage of syncytia in six selected *A. thaliana* lines stained for GUS activity at 2,4,7,12 and 30 days post infection with nematodes.

induce changes in any of these lines and therefore the promoters were not thought to be wound-inducible. More detailed analysis of six lines showed that four had strong *gus* expression in the feeding site between 2-7 days after infection but in two of the lines expression lasted longer, with more than 50% of the syncytia showing GUS activity at 12 days post infection (Fig. 4).

Despite the differences in the life cycles of the cyst and root-knot nematodes compared with *Xiphinema*, all these induced *gus* expression at their feeding sites (Fig. 2). Interestingly, in one of the six selected lines, neither root-knot nor *Xiphinema* nematodes could induce galling.

The DNA of several tagged promoters has been isolated from *A. thaliana* and more details will be published elsewhere[3]. These promoters have been sequenced and re-inserted into *A. thaliana* by *Agrobacterium*-mediated transformation. Further work has demonstrated that these versions of the nematode-inducible promoters had similar expression patterns to the promoters present in the original lines. Consequently, experiments are in progress to link these promoters to anti-nematode genes, such as lectins and protease inhibitors. These will be used to study the effect on the development of the target nematode species and compare the effects with those observed using a constitutively-expressed promoter, which gives continuous and wide-spread expression of the products throughout the plant.

These studies have a direct practical value as a means of developing transgenic plants which can inhibit nematode development where no other type of resistance is available. Fundamental studies such as these, which characterise the molecular interactions between the nematode parasites and host plants, may yield further insights which can be exploited for controlling these important agricultural pests.

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Acknowledgement:

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Characterisation of the secretions of the potato cyst nematode and their role in the host-parasite relationship

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The potato cyst nematodes (PCN, *Globodera rostochiensis* and *G. pallida*) are damaging pests of potato crops. In the UK, yield losses and the costs of controlling PCN with nematicides are thought to be £20-30 million each year. In the absence of effective natural resistance against many pathotypes of PCN, effort is currently being focused on gaining an understanding of the basic biology of plant parasitic nematodes in the hope that such information will uncover new targets for novel control methods.

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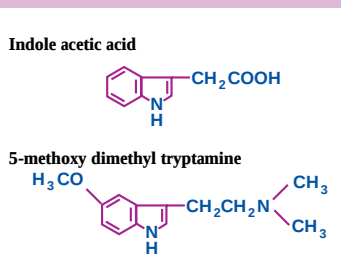


Figure 1a Structures of DMT and indole acetic acid showing similarities between these molecules

Nematodes have been known to produce secretions in response to a range of chemicals for some time. One of the best at stimulating the production of secretions is the neurotransmitter analog 5-methoxydimethyl tryptamine (DMT). Comparison of the structure (Fig. 1a) of this compound with that of the plant hormone, indole acetic acid (IAA) showed these molecules have a similar structure. Subsequent experiments showed that IAA, and a range of other plant hormones could produce the same effect as DMT in inducing the production of secretions from nematodes.

Recent work has shown that PCN may contain a protein with similarities to hormone receptors from plants; an antibody raised against the hormone binding domain of these plant proteins recognises a protein in nematodes when tested on western blots (Fig. 1b). Current work is aimed at biochemical purification of this putative receptor from PCN and isolation of the gene coding for this molecule from an expression library.

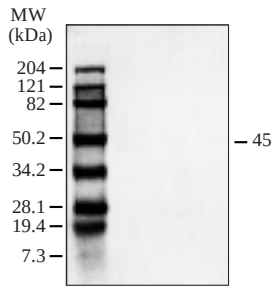


Figure 1b Immunoblot of whole body extracts of *G. pallida* probed with antiserum raised against the conserved hormone binding domain of plant auxin receptors. A band of molecular mass 45 kDa is labelled.

Figure 1 Nematodes respond to plant hormones.

When a potato crop is grown, the second stage juveniles of PCN are stimulated to hatch from cysts in the soil left behind from the previous potato crop. The juveniles locate the potato root and penetrate it just behind the growing root tip before migrating intracellularly through the root to a site where they can begin to feed. At this point each second stage juvenile induces the formation of a syncytium, a large multinucleate cell formed by the breakdown of plant cell walls and subsequent fusion of neighbouring cells, in the root tissue. To form the syncytia, nematodes have to induce changes in the expression of cell cycle genes and a range of other plant genes, leading to a more active metabolism and altered osmotic condition in the syncytium. Understanding how nematodes induce such fundamental changes in gene expression of their hosts remains a major goal in plant nematology.

The secretions of PCN are in more intimate contact with the plant than any other nematode molecules and are therefore thought to play important roles in the interaction between parasite and host. Secretions present on the cuticle surface may serve to mask the nematode from its host and to anchor the nematode at its feeding site. Two large sense organs, the amphids, situated on the head of the nematode, also produce secretions which may form a feeding plug, through which the nematode inserts its stylet to feed on the syncytium. However, it is the secretions of the dorsal and subventral oesophageal gland cells, which are injected into plant tissues through the nematode stylet, which have been implicated in a range of processes associated with invasion and feeding. These include enzymatic effects on plant tissues during invasion, induction of the syncytium and production of the feeding tube through which the nematode withdraws nutrients. Our work has focused on the isolation and characterisation of a range of secretory components of PCN in order to determine how these molecules are used by the nematode to parasitise plants.

Invasive stage juveniles of PCN are extremely small and difficult to culture in large numbers, making study of their secretions extremely challenging. Work

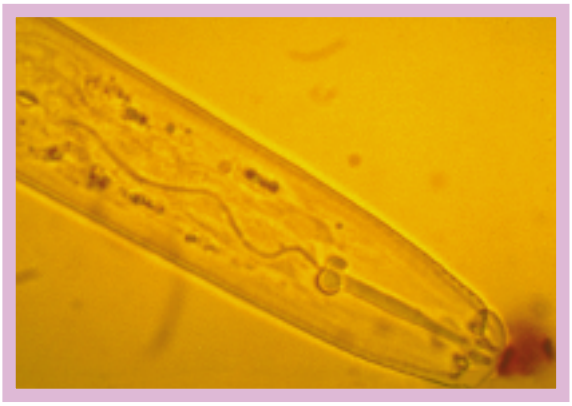


Figure 2 Second stage juvenile of *G. pallida* producing secretions at the tip of its stylet.

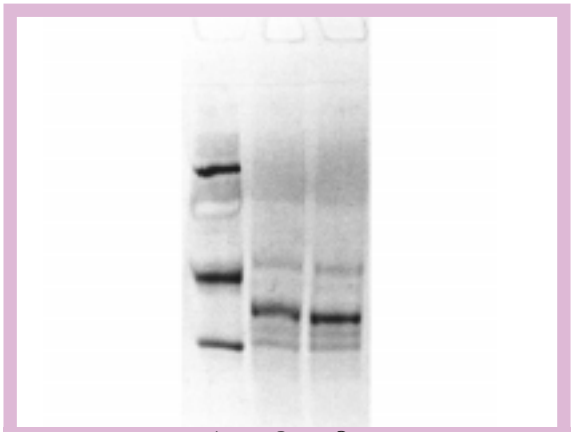


Figure 3 SDS PAGE analysis of collected secretions from second stage juveniles of *G. pallida*. Lane 1 - Molecular weight markers; Lanes 2&3 collected secretions.

in this area was stimulated by our observation that exposure of the invasive stage juveniles to plant hormones (Fig. 1) stimulates them to produce secretions (Fig. 2). This allowed sufficient secretions to be collected to undertake biochemical analysis and to raise antibodies against secretory components.

Electrophoretic separation of collected secretions using SDS - PAGE revealed that at least eight distinct proteins are present ranging from approximately 15 to 70 KDa in molecular mass (Fig. 3). Fractionation of secretions on the basis of their molecular mass confirmed this figure and also permits individual components to be purified for further analysis allowing probes against each component to be generated. Biochemical assays for a range of enzyme activities have already been carried out on collected secretions from PCN. Activities of a range of enzymes have been detected, including substantial superoxide dismutase (SOD) activity. This enzyme may have a role in protecting the nematode from the plant resistant response, neutralising oxygen free radicals produced in the oxidative burst. The production of SOD as a defensive measure is widespread throughout the nematodes; a number of animal parasites have also been shown to secrete this enzyme in response to oxidative stress. Future work in this area will examine whether expression levels of SOD gene transcripts in PCN are correlated with oxygen free radical levels in plants.

Antibodies have also been raised against secretions from PCN to provide probes to examine the spatial and temporal localisation of secretory components.

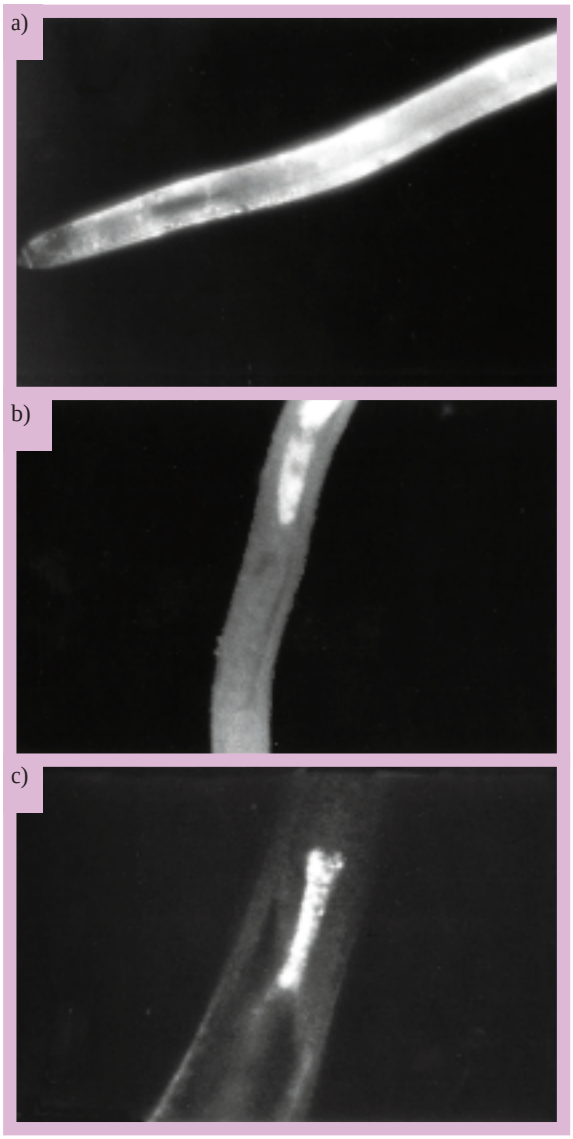


Figure 4 Binding of antibodies to second stage juveniles of *G. pallida* shown using indirect immunofluorescent staining. a) Antibody binding to the surface; b) Antibody binding to the subventral gland cell; c) Higher magnification showing binding of antibody to secretory granules within the subventral gland cell.

These antibodies show a range of binding patterns on nematodes. One antibody recognises surface secretions of PCN invasive stage juveniles (Fig. 4a). Another recognises the subventral gland cells of the nematodes (Figs 4b & c). This demonstrates that secretions collected from PCN emanate from a range of body sources. Some of the antibodies which have been raised recognise protein, rather than carbohydrate epitopes. These have been used to screen expression libraries to isolate and subsequently characterise the genes coding for the secreted proteins. To date we

MQRLLLCLTGASFIVLLFGASLPPIDISSIPEQYRELIPKEVIDFYN
TLTAEDKQALKEVAERHEEFQTEEQAMEALKAKSEKLHSKAVELRNL
VKEKIDKLVPGAKTFVTETIEKLKAMRPKSGEKPNLEELRKGANDTI
EKFKALSVEAKESLKANFPKITGVIQSEKFQALAKSLLKTEGAAPAA

Figure 5 Amino acid sequence of PCN secreted molecule GPSEC-2. A predicted signal sequence is shown in red and a potential glycoslylation site is underlined. Four long stretches of alpha helix are shown in blue.

have fully characterised two genes, *gp-sec-2* and *gp-sec-3* and several others are currently being studied. The first, *gp-sec-2* was isolated from the PCN expression library using an antibody which recognises secretions located on the surface of PCN (Fig. 4a). The full length cDNA of 743 nt contains a single long open reading frame of 564 nt which encodes a protein of 188 amino acids with a predicted molecular mass of 20.9 Kda (Fig. 5). Experiments suggest that *gp-sec-2* is expressed throughout the life cycle of PCN (Fig. 6a). As expected for a secreted protein, a stretch of predominantly hydrophobic amino acids is present at the N-terminus which may function as a signal sequence for extracellular transport. A single N-linked glycosylation site is present at amino acid residues 138-141. Structural predictions for the GPSEC-2 protein suggest that four long stretches of strongly amphipathic alpha helix are present, separated by short loop regions. Such structural features are commonly found in proteins which bind and transport hydrophobic ligands; the hydrophilic regions of the helices face the outside of the molecule to allow contact with the aqueous environment whilst the hydrophobic regions form a pocket at the interior of the protein in which hydrophobic ligands can reside.

Comparison of the GPSEC-2 sequence with others in various databases revealed similarity to secreted proteins from two animal parasitic nematodes, *Onchocerca volvulus* and *Brugia malayi* and to two proteins from the free living nematode *Caenorhabditis elegans*. Comparing these sequences showed that extremely high similarity was present across the entire lengths of the sequences. Comparison of the predicted structure of GPSEC-2 with those of the homologues from the other species suggests that the extensively helical structure is also highly conserved.

The wide phylogenetic distribution of this molecule coupled with the strong similarity at the sequence and structural level, suggest it plays an important role in nematode physiology and its presence in a range of nematodes with a wide range of lifestyles suggests, at first sight, a function in the general metabolism of nematodes. Alternatively it is possible that this molecule has become adapted in parasitic nematodes in order to fulfil a specific role in the parasitic process (see below). Expression of cloned GPSEC-2 will allow functional studies on the protein which will help

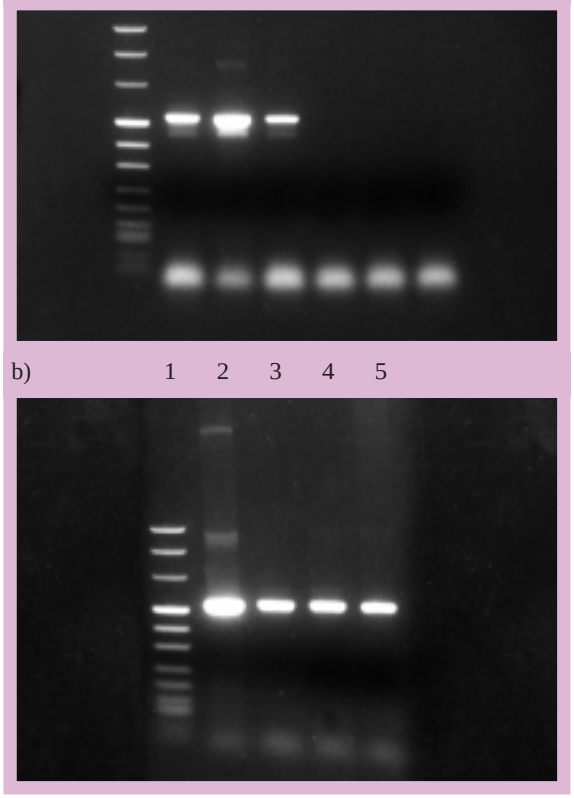


Figure 6 RT-PCR analysis to examine expression of genes coding for secreted molecules throughout life cycle of PCN. a) RT-PCR reactions showing *gp-sec-2* is expressed in all life cycle stages tested. Lane 1 - molecular weight marker; Lane 2 J2; Lane 3 J4/virgin females; Lane 4 Adult females. Lanes 5,6&7 - negantive controls for each stage. A band of 520bp is present, as expected if *gp-sec-2* mRNA is present in the sample. b) RT-PCR reactions showing *gp-sec-3* is expressed in all life cycle stages tested. Lane 1 - molecular weight marker. Lane 2 - positive control showing product produced if *gp-sec-3* is present; Lane 3 - J2; Lane 4 J4/virgin females; Lane 5 adult females.

Figure 7 Amino acid sequence of PCN secreted molecule GPSEC-3. A potential N-linked glycosylation site is underlined.

uncover its role in the host-parasite interaction. It may also be possible to compare the role of GPSEC-2 in plant parasites and *C. elegans* by transforming *C. elegans* mutants lacking the homologous gene with *gp-sec-2*. Rescue of the mutant phenotype would imply a similar role for the gene product in PCN and *C. elegans.* Alternatively, wild-type *C. elegans* may be transformed with antisense transcripts of *gp-sec-2* and effects on phenotype observed. Transformation of *C. elegans* with PCN genes may also provide a means of producing large quantities of protein, glycosylated as it would be in PCN, for functional analysis.

We have also extensively characterised another PCN gene, *gp-sec-3*, which encodes a secreted molecule produced in the subventral gland cells of *G. pallida*. The nature of the protein it codes for is far less clear than that of GPSEC-2. *gp-sec-3* gives rise to a full length mRNA sequence of approximately 900 nt which contains a single long open reading frame of 507 nt. As for *gp-sec-2*, experiments suggest that *gp-sec-3* is expressed throughout the PCN life cycle (Fig. 6b). The protein encoded by this gene, GPSEC-3, has a predicted molecular mass of 18,900 KDa and contains one N-linked glycosylation site at residues 29-32. (Fig. 7). In contrast to GPSEC-2, comparisons of GPSEC-3 to other sequences in the database reveal very little significant similarity to proteins from other nematodes. One stretch of GPSEC-3 shows good similarity to a small part of a much larger protein from *C. elegans* but the rest of GPSEC-3 bears no resemblance to the rest of this *C. elegans* protein. Furthermore, no similarity to any animal parasite genes is observed. Given the extent to which the *C. elegans* sequencing project has proceeded and the number of ESTs sequenced from animal parasitic nematodes such as *O. volvulus*, it seems likely that GPSEC-3 represents an entirely novel nematode protein. While potentially interesting, this makes deducing a function for GPSEC-3 extremely difficult. Currently, we are working towards expressing this protein in yeast so that functional studies can be undertaken. In the longer term, it will be necessary to develop a transformation system for PCN in order to fully understand the function of molecules such as this, which apparently have no homologues in *C. elegans.*

That some genes coding for secreted molecules have clearly defined homologues in *C. elegans* and in animal parasitic nematodes whilst others do not, raises the question as to how parasitism arises in a group as diverse as the nematodes. It is possible that parasitic nematodes use largely the same genes as their free living counterparts with genes or expression of these genes, modified slightly to allow for parasitism. The use of SOD as a defence enzyme in a wide range of nematodes is an example of this strategy. Functional analysis of GPSEC-2 may show that it also falls into this category. Alternatively, entirely novel genes, not present in other nematode groups, may be used by parasitic nematodes to attack hosts. It is possible, given the absence of *C. elegans* or animal parasite homologues of GPSEC-3, that this gene falls into this category. Study of PCN genes with roles at the host-parasite interface will allow this question to be investigated.

A mitochondrial DNA perspective on diversity in the potato cyst nematode, *Globodera pallida*

M.R. Armstrong, V.C. Blok, B.E. Harrower, M.S. Phillips & D.L. Trudgill

The relationship between potato, and potato cyst nematode (*Globodera rostochiensis* and *G. pallida*), is a complex host-parasite interaction, where it is possible to distinguish multiple races, or pathotypes, within the nematode species. Pathotypes are distinguished by differences in virulence that depend on the interaction between resistance genes in the host and avirulence genes in the nematode. Several pathotypes of *G. pallida* are thought to have been introduced into Europe from South America during the last century. Distinguishing these pathotypes has become increasingly important, as the widespread use of potato cultivars resistant to *G. rostochiensis* has resulted in *G. pallida* becoming increasingly prevalent. European populations of *G. pallida* are assigned to two main pathotypes. Populations belonging to pathotype Pa1 are unable to replicate on potato cultivars into which the H2 gene from *Solanum multidissectum* has been introgressed, whilst populations designated as Pa2/3 are able to replicate.

Population	Pathotype	% reproduction on partially resistant cv. Morag	Source
Bedale	Pa2/3	19.8	England
LOC	Pa2/3	12.6	England
E	Pa2/3	12.5	England
Pilmore	Pa2/3	12.5	Scotland
Friskney	Pa2/3	17.0	England
Wainfleet	Pa2/3	25.2	England
B/Pa1	Pa1	29.3	Scotland
Gourdie	Pa2/3	10.5	Scotland
2-22	Pa2/3	6.3	The Netherlands
D375	Pa2/3	7.4	The Netherlands
Sleen	Pa2/3	44.1	The Netherlands
Chutanbajo	-	7.3	Ecuador
Luffness	Pa2/3	87.6	Scotland
Falkland	Pa2/3	-	Falkland Islands
Chavornay	Pa2/3	26.9	Switzerland
BBA1	Pa2/3	12.6	Germany
Rookmaker	Pa2/3	20.5	The Netherlands
P4A	P4A	14.5	Peru
P5A	P5A	47.1	Peru
El Salto	-	13.4	Ecuador

Table 1 Populations of *Globodera pallida*, their pathotype classification, percentage reproduction, and country of origin.

Most British and European *G. pallida* populations are classified as pathotype Pa2/3 for which there is no major gene resistance. These populations exhibit a continuous range of virulence, from very low to high levels of reproduction, on the partially resistant potato cultivars that are available. The present inability to sub-divide this broad Pa2/3 pathotype phenotypically, limits its practical significance. Our molecular studies attempt to establish if genetic sub-divisions exist. Two hypotheses are being tested; either that virulence differences can be associated with separate, genetically distinct, introductions from South America, or that during the spread of *G. pallida* throughout Europe, the range of virulence was generated by a process of fragmentation which resulted in a range of sub-populations with different virulence characteristics.

To explore these two hypotheses, a study of the genetic relationships of *G. pallida* populations was made through the analysis of their mitochondrial DNA (mtDNA). The mitochondrial genome has two important features that make it especially attractive for this purpose. Firstly, the mechanism of mitochondrial inheritance usually prohibits recombination. Consequently, the mitochondrial genotype of an individual is unambiguously related to its progenitors. Secondly, with the exception of some specific circumstances, mtDNA is selectively neutral, hence selection pressures which have acted subsequent to the introduction of *G. pallida* into Europe should not obscure any relationships between the founder populations.

Twenty one populations of *G. pallida*, differing in virulence, were selected for analysis, ten British, seven from continental Europe, and four from South America, along with two populations of the sibling species *G. rostochiensis* (Table 1). To assay variation in the mtDNA, the total genomic DNA of each population was digested, Southern blotted and hybridised with short fragments of mtDNA which were either isolated from a mtDNA 'library' or generated by PCR (the polymerase chain reaction which copies sequences of DNA). For example, Figure 1 shows the pattern of

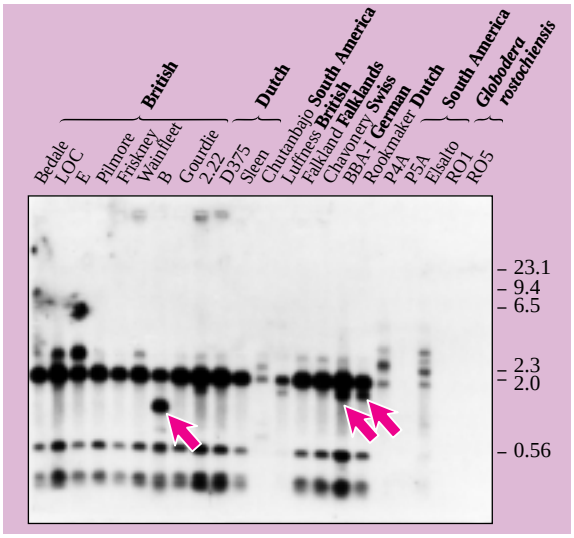


Figure 1 Southern blot of genomic DNA digested with DraI and hybridised with a 1.2Kb mtDNA probe (p226) homologous to the NU2 gene of *Drosophila sechellia.* Arrows indicate restriction fragments indicative of the presence of multiple mitotypes within some populations.

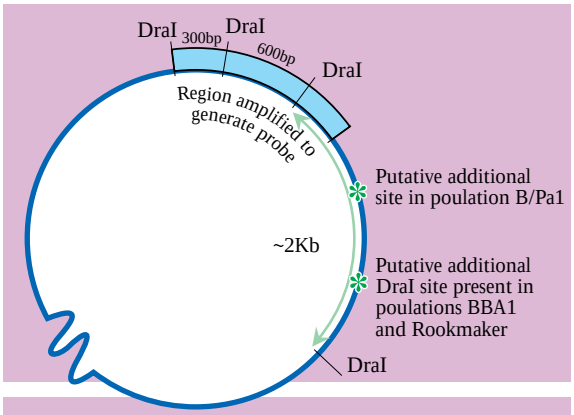


Figure 2 Putative restriction map of clone p226, and external region of the *G.pallida* mtDNA, showing positions of confirmed internal DraI sites, and proposed external DraI sites. The additional DraI sites (marked with an asterix) present in the mtDNA of populations Pa1, Rookmaker, and BBA1 would prevent the generation of the observed ~2Kb restriction fragment, if they were present on the same mtDNA as the most distant DraI site. Consequently, it is proposed that they must be present on separate mitotypes.

hybridisation obtained when a radioactively labelled clone, derived from a British *G.pallida* population, and homologous to the NADH ubiquinone oxidoreductase chain 2 gene (NU2), was used to probe genomic DNA which had been digested with the endonuclease DraI. Some variation was observed amongst European populations, with more still evident amongst those from South America. Not all the additional restriction fragments observed in the distinct populations can be accounted for simply by the presence of additional DraI sites within the region homologous to the probe (Fig. 2). It is proposed therefore, that mitochondrial variants (mitotypes) within the populations are responsible for this variation. Consequently, a population can exhibit combinations of mtDNA characteristics. For example, populations B/Pa1, Rookmaker and BBA1 share restriction fragments with the majority of British populations, but also have unique restriction fragments. Other restriction enzyme/probe combinations have revealed similar complex patterns, for these and other populations, further suggesting the existence of multiple, cross-hybridising, mtDNA mitotypes in *G.pallida* populations.

In addition to observations such as these, evidence also emerged suggesting the presence of highly diverged mitochondrial variants in *G.pallida* populations. Clones pe31 and pe91, obtained from the mtDNA library made from *G. pallida*, are both homologous to the same region of the cytochrome oxidase sub-unit 1 gene (COI) but are 23% dissimilar in nucleotide sequence (Fig. 3). Both mitochondrial

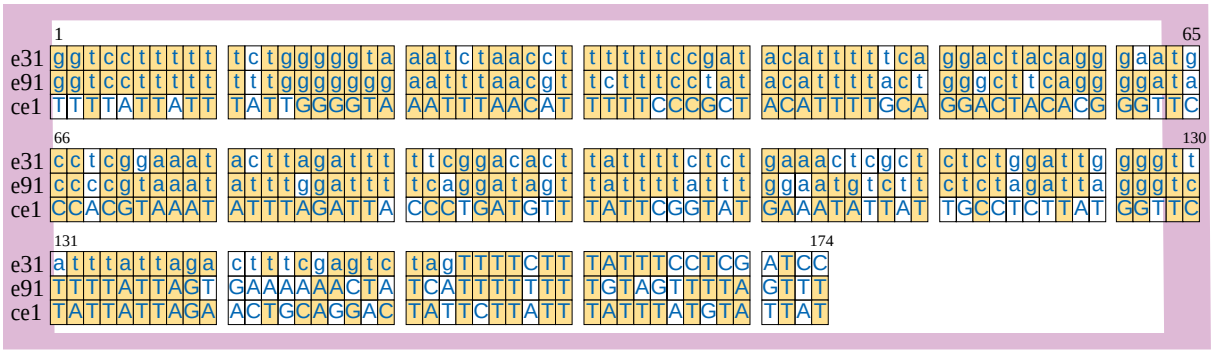


Figure 3 Sequence alignment of the nucleic acid sequences of clones pe31 and pe91 with the COI sequence from *Caenorhabditis elegans.*

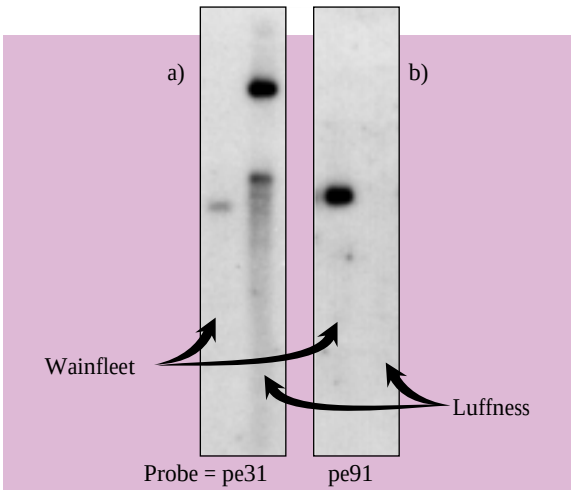


Figure 4 Southern blot of genomic DNA from populations Wainfleet and Luffness, digested with HindIII and hybridised with; panel (a) clone pe31, panel (b) clone pe91.

forms are apparently present in the majority of European populations with the exception of Luffness which fails to hybridise to pe91 (Fig. 4).

Figure 5 summarises the results for a number of mtDNA probe/restriction enzyme combinations. The ten British *G. pallida* populations are virtually indistinguishable, with the exceptions of the phenotypically distinct B/Pa1 population, and Luffness, a highly virulent Pa2/3 (Table 1). This suggests that Luffness, like Pa1, is likely to be a separate introduction, rather than representing a fragmented sub-population generated from a central British introduction. Continental European populations generally are mitochondrially similar to the majority of British populations, with the exception of Rookmaker (a Dutch population) and BBA1 (a German population). These populations also have additional mitotypes that occasionally share the characteristics of Luffness mtDNA. The populations from South America were distinct, both from each other and from the European populations (with the exception of El Salto). Frequently they were apparently so diverged that probes failed to hybridise, potentially suggesting speciation or sub-speciation within *G. pallida*. It is clear that a considerable amount of genetic diversity exists within South America and that European *G. pallida* isolates are a sub-sample of this diversity. The DNA from the two *G. rostochiensis* populations failed to hybridise to any of the probes.

The analysis of the mtDNA of *G. pallida* populations has suggested relationships not apparent from previous analyses, potentially due to the unique characteristics of mitochondrial inheritance, and at the same time added crucial supporting evidence to differences already observed. Not relying exclusively on PCR-based methodologies enabled the mixtures within populations to be investigated. Earlier RAPD studies (Ann. Rep 1995) and current investigations of ribosomal DNA, indicated that the South American population P5A is distinct. This is confirmed by the mtDNA data, raising questions about the taxonomic relationship of P5A to the other *G. pallida* populations.

The mtDNA study reported here, previous RAPD investigations, and RFLP and isozyme studies have all indicated that both the Luffness and B/Pa1 populations are different to the majority of British populations. However until now, it has not been clear whether these are separate introductions, or unique genepools resulting from either a very limited, or atypical sample from an already established population. The mtDNA data clearly indicates that Luffness and

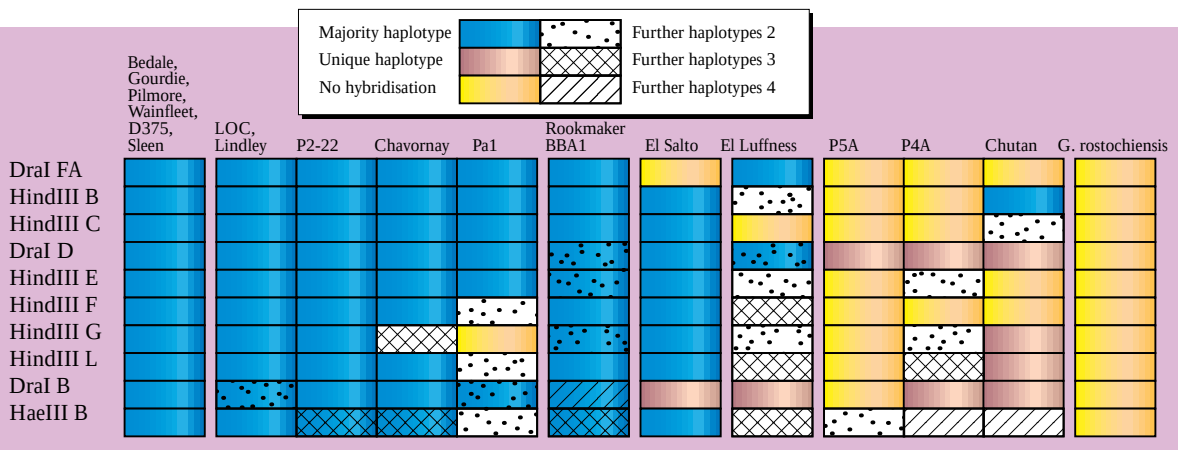


Figure 5 Summary of the RFLP data for a number of restriction enzyme/mtDNA probe combinations.

Pa1 are two separate introductions distinct from the remainder of the British populations. The majority of the continental European *G. pallida* populations also appear to be derived from the same introduction as the British, but this study indicates that additional introductions, as represented by Rookmaker and BBA1, have also been made on the continent. The significance of the presence of two highly diverged mitotypes within the majority of British and European populations is currently under investigation.

A major concern for potato production in north-western Europe: the plant-parasitic nematodes *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla*

V.C. Blok, J. Wishart & M.S. Phillips

The Columbia root-knot nematode, *Meloidogyne chitwoodi*, is a new potential threat to European agriculture. *M. chitwoodi* is a major pest of potato in the USA. It produces external galling and internal spots on tubers (Fig. 1) and it now has been found to damage potato and other crops in some EU countries. Damaged potato tubers are unmarketable, either for direct consumption or processing. Seed potatoes can also be infested and as they are a major means of spreading the nematodes, will fail to pass quarantine requirements. *M. chitwoodi*, and another more recently described species, *M. fallax*, are now found in north-western Europe and are thought to have been introduced from the USA. Both species are difficult to control by rotation as they have a wide host range and are able to reproduce on, and sometimes damage most major crops, including lucerne, the cereals, wheat and maize, beet, carrot and salsify, as well as potato. The problems caused are exacerbated by the pressure to decrease the use of nematicides because of environmental concerns. A further complication is that these two species have been confused with *M. hapla* which is indigenous in Europe and also attacks potato.

A rapid method of identifying the different species is crucial if effective use of resistant cultivars and crop rotation are going to be used to control the different

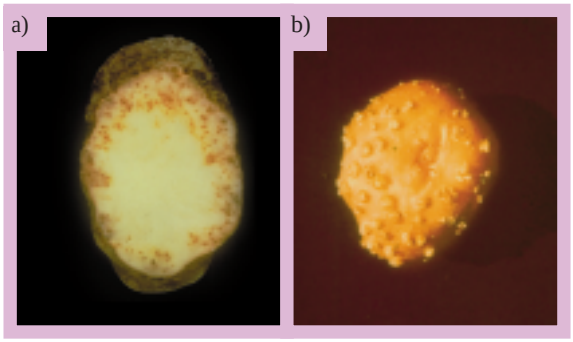


Figure 1 Cut tuber showing a) infection with *M. chitwoodi*, and b) whole tuber showing galling with *M. fallax*.

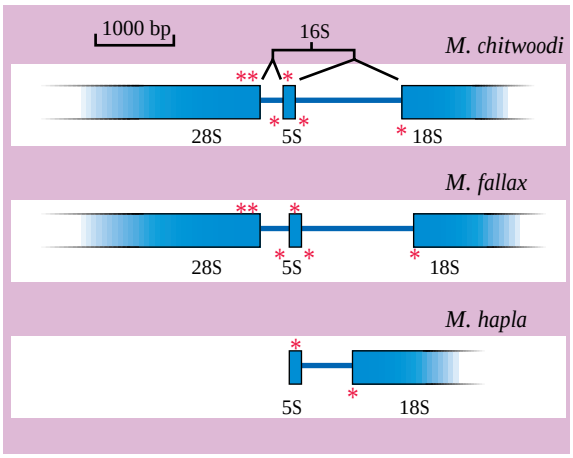


Figure 2 Schematic diagrams showing organisation of the IGS regions of *M. chitwoodi*, *M. fallax* and *M. hapla*. Primers sites for amplifying the region between the 5S and 18S genes are indicated by (*) and the region between the 5S and 28S genes with (**).

Species	Code	Place of origin	Race
M. chitwoodi	IPO-Ca	Rips, NL 1	1
	IPO-Ci	Maasbree, NL	1
	IPO-Cl	Smilde, NL	1
	IPO-Ck	Spijkenisse, NL	
	IPO-Cx	Kessel, NL	1
	IPO-Cy	Herckenbosch, NL	1
	IPO-Cbh (CAMC2)	California, USA	
	IPO-Cbf (ORMC8)	Oregon, USA	2
	IPO-Cbd (WAMC16)	Washington, USA	
M. fallax	IPO-Fa	Baexem, NL	
	IPO-Fd	Roggel, NL	
	IPO-Fe	Rilland, NL	
M. hapla	IPO-Hi	Smilde, NL	
M. incognita	Line 19	French West Indies	
M. arenaria	Line 28	French West Indies	
M. mayaguensis	Line 13	Puerto Rico	

Table 1 Isolates of *Meloidogyne*, their codes, place of origin and race if known.

species. Sensitive and quick diagnostic tools are also needed to detect infested potato tubers and control the spread of infected plant material. Studies to find such tools have been funded by an EU grant to several European laboratories including SCRI. Within the first year of this project, we have been able to identify an effective, accurate, PCR-based method for species identification. This technique is not life-stage specific and is sufficiently sensitive to be used with individual nematodes, presenting an attractive alternative to traditional methods such as morphological examination or host range testing. The method is based upon dif-

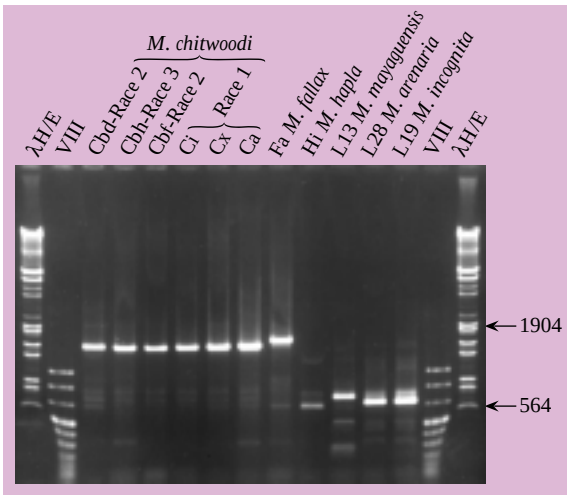


Figure 3 PCR products from the IGS region between the 5S and 18S genes from various *Meloidogyne* spp.

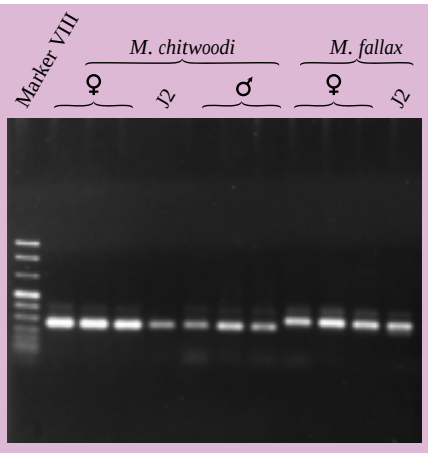


Figure 4 PCR products from the IGS region between the 5S and 18S genes from different life stages of *M. chitwoodi* and *M. fallax*.

ferences in the intergenic spacer (IGS) region of the ribosomal DNA, and distinguishes *M. chitwoodi*, *M. fallax* and *M. hapla*.

Schematic diagrams of the IGS region for the three species are shown in Figure 2. Amplification of the region between the 5S and 18S genes from different isolates (Table 1), yields different sized products, characteristic of each of these three species and they are also distinguished from the other main *Meloidogyne* species (Fig. 3). The IGS region between the 28S and 5S genes also yields different sized products for *M. chitwoodi* and *M. fallax* and this region can be readily amplified with extracts from single juveniles or individual female or male nematodes (Fig. 4).

The ability to distinguish species is but the first stage in identification. Biological differences between isolates within *M. chitwoodi* complicate management problems. Three races of *M. chitwoodi* have been identified, the first two of which are differentiated by their abilities to reproduce on lucerne and carrot. This distinction has implications for rotational cropping regimes. The third race is distinguished by its ability to reproduce on the wild potato, (*Solanum bul-*

	Host Reaction			
	Tomato	Lucerne	Carrot	*Solanum bulbocastanum*
Race 1	+	-	+	-
Race 2	+	+	-	-
Race 3	+	+	-	+

Table 2 Differential hosts to distinguish races of *M. chitwoodi*.

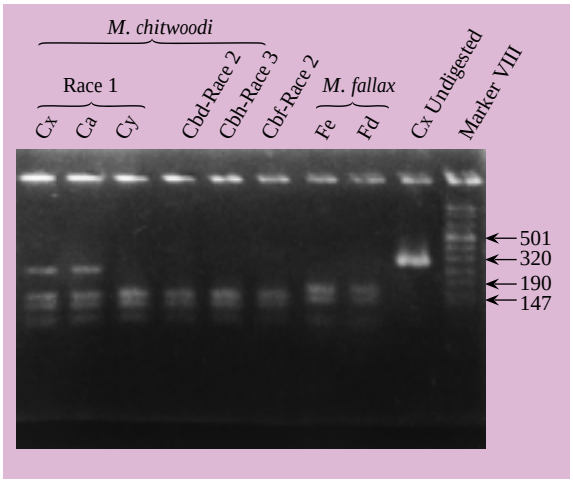


Figure 5 Digestion products from PCR products from the IGS region between the 5S and 28S genes using the restriction enzyme Hinf I from different isolates of *M. chitwoodi* and *M. fallax.*

bocastanum) (Table 2), and is important for determining the effective deployment of resistant genotypes. Consequently, further molecular studies have been initiated to develope markers to differentiate these races. No variation in the size of the 5S to 18S IGS region has been observed, however, isolates have been differentiated following digestion of the amplification product from the 28S to 5S region with the restriction enzyme Hinf I (Fig. 5). The isolates of *M. chitwoodi* form two groups. The first contains only race 1 isolates, whereas the second contains isolates from all three races. The implications of these groupings in relation to the biological characterizations of these isolates are unclear, and further molecular studies, together with biological assessments of these isolates on various plants including accessions from the Commonwealth Potato Collection are underway.

Biomathematics and Statistics Scotland

Rob Kempton

Biomathematics and Statistics Scotland (BioSS) is devoted to the application of statistics and mathematics to the biological sciences. It contributes research, consultancy and training to agricultural and biological research institutes in Scotland, and collaborates in research programmes with leading UK and European universities and laboratories. In January 1996, BioSS received a regular 4-year Review from an international group of assessors. The Review was extremely positive and praised BioSS for the substantial contribution it was making to biological research in Scotland. With its core of methodological expertise, it formed an unique bridge between academia and a broad range of applied research in the public sector and industry.

The BioSS research programme is presented under five broad themes outlined below. These span the R&D programmes funded by SOAEFD and allow extensive interaction with scientists in client organisations. Following the recommendations of the Review Group, research has been focused on a reduced number of topics within each theme. Spatio-temporal modelling is a feature of several themes, and a bridging programme is now established to coordinate work in this rapidly developing area.

Theme 1: Statistical design and analysis. This theme is designed to improve the efficiency, cost-effectiveness and impact of research across the biological science base. Topics include the investigation and control of interference in observer perception and recording, and the development of methods for controlling spatial heterogeneity in field experiments.

Theme 2: Mathematical biology. The study of mathematical models can improve understanding of real-world systems and enhance the information from experimental observations. Space-time models studied under this theme include models for spread of plant epidemics, which have been applied to the study of epidemiology of fungal pathogens at SCRI.

Theme 3: Image analysis. Biological data are often in the form of images whose information can be analysed most efficiently by computer. This theme contributes to research into computer vision systems, giving particular emphasis to developing solutions for problems in photography, microscopy and scanning systems.

Theme 4: Environmental modelling. This theme focuses on applications in environmental research, an important area for statistical modelling. It includes a

coordinated project, undertaken with SCRI, MLURI, SAC and the University of St Andrews, to investigate appropriate models for vegetation dynamics, working at scales ranging from individual plants to vegetation communities. Here, as with other types of environmental data, observations may be made infrequently and correlated in time and space, posing particular problems for statistical modelling.

Theme 5: Genetic analysis. This theme is central to work at SCRI. The rapid spread of molecular technology, means that BioSS is working with many different types of data, including DNA sequences, RAPDs, RFLPs, AFLPs and micro satellites, and a wide range of taxonomic groups, from plants to viruses. Particular expertise has been developed in linkage methods, selection strategies, the analysis of population structure and phylogenetic analysis.

Collaboration with SCRI scientists across these five themes resulted in 12 co-authored papers appearing in refereed journals, or in press, in 1996.

Performance indicators, league tables and the assessment of crop cultivars

R.A. Kempton & M. Talbot

The last decade has seen a strong drive to increase the accountability and efficiency of public sector activities, such as education, health and the social services, and the vigorous promotion of quantitative indicators of institutional performance to achieve this aim. Initially introduced by management to improve internal monitoring, performance indicators are now increasingly used for institute comparison, as a basis for funding decisions, and to provide the public with the information to exercise consumer choice. Comparisons are often published in the form of league tables. Examples of published rankings include: schools, based on results at public examinations; hospitals, based on risk-adjusted mortality rates; and prisons, based on numbers of disciplinary cases, though in this case the freedom for users to exercise choice would seem extremely limited.

If performance indicators are to be used to shape the development of the public sector, it is essential that administrators and the public know how they should be interpreted. In particular, there needs to be greater awareness of the limitations of a single indicator for assessing performance, the sources of uncertainty associated with a ranking, and the natural variation that might be expected from year to year. To illustrate the issues, we draw on our experience of using performance indicators in a well established area, the assessment of crop cultivars and publication of a Recommended List.

Choice of performance indicator A performance indicator is a quantitative measure of an object or system which is purported to represent some aspect of its 'quality' of function. Quality is difficult to define and usually impossible to encapsulate in a single measure.

Unfortunately, it is all too easy for a straightforward, easily measurable, performance indicator to come to be viewed as *defining* overall quality, rather than merely representing one of its components. For administrators and users, the superficial attraction of adopting a single performance measure is that it immediately provides a simple ranking of a set of objects in a 'league table'. For example, league tables based on GCSE examination scores have rapidly become established among parents and education authorities as the standard way of comparing schools, despite widespread recognition that many different qualities in a school contribute to a young person's development and prospect of future happiness and success.

How is overall performance assessed for crop cultivars? Here, important components of quality may include yield potential under ideal conditions, resistance to different diseases, maturity and, for cereal crops, malting or bread-making quality. One approach is to combine the different quality aspects in a single measure, called by breeders a selection index. This requires choosing relative weightings for performance attributes. The choice is often subjective and the combined index difficult to interpret. Animal breeders attempt to base their weights on the relative economic importance of the attributes (Table 1), but this approach has found little favour among plant breeders who prefer to adopt a more pragmatic approach. For staple food crops, consistent yielding ability remains the most important attribute for producers. However, in early generations of selection, yield assessment is unreliable, so population performance is improved by discarding those genotypes which fall below acceptable levels for disease resistance and other important quality characteristics. In later stages, selection is based primarily on yield, but other attributes are still taken into account.

This breeding approach provides farmers with a list of finished cultivars with diverse quality attributes. An example is shown in the Recommended List of spring barley cultivars for 1996, produced by the Scottish Agricultural College (Table 2). In presenting the trial results, cultivars are ranked on yield under disease-free

	Lamb growth	Mature size	Litter size	Maternal ability
Sire	100	-	-	-
Lowland ewe	25	-	64	11
Hill ewe	60	15	1	24

Table 1 Relative contribution of four traits to a selection index for sheep (Meat and Livestock Commission 1986). Different trait weightings are used for lowland and hill ewes. In lowland meat production systems, profitability is highly correlated with litter size. However, higher litter size results in smaller individual birth weights, and higher rates of lamb mortality. Consequently, in very harsh hill conditions, the optimum litter size is close to 1 and lamb growth and maternal ability are key traits.

Good
Tends to be good
Intermediate
Tends to be poor
Poor

Year first listed	Spring barley (100=6.78t/ha, 54.0cwt/acre)		Grain yield as % of treated controls: Fungicide treated	Grain yield as % of treated controls: Untreated	Resistance to ear loss + grain shedding (1-9 poor -good)	Malting quality	Screenings <2.5mm (1-9 poor -good)	Specific weight (1-9 poor -good)	1000 grain weight (1-9 poor -good)	Maturity (days later (+) or earlier (-) than average)	Straw strength (1-9 weak - strong)	Brackling risk (1-9 high - low)	Straw length(cm taller(+) or shorter(-) than average)	Disease resistance (1=susceptible, 9 = resistant): Mildew	Rhyncho-sporium	BYDV	Diversification group
1994	G	Delibes	105	97	8	Medium	7	4	7	0	8	7	-6	6	7	6	7
1991	O	Nomad	101	87	7	Poor	7	6	7	0	6	4	+1	6	6	5	8
1994	G	Cooper	100	93	8	Good	4	6	5	+1	9	8	-8	7	5	7	7
1992	G	Chariot	99	94	7	Good	7	8	6	0	8	7	+4	9	3	6	1
1988	G	Prisma	98	75	5	Good	7	4	7	-1	7	4	+4	3	7	4	0
1986	O	Camargue	97	79	6	Medium	5	7	7	+1	7	5	0	3	8	5	4
1992	G	Derkado	96	92	8	Good	7	7	7	0	8	7	-1	9	3	3	1
1988	G	Tyne	95	86	8	Poor	4	6	4	-2	5	5	-1	7	5	7	4
1995	PG	Optic	108	96	6	Good	7	7	7	+3	7	7	+2	7	5	8	9
1995	PG	Rivieria	102	96	7	Medium	7	7	7	-1	7	7	-10	9	4	4	1

Table 2 SAC Recommended List of spring barley cultivars for 1996 based on trials on farms in northern Britain over the previous 5 years. Cultivars Optic and Riviera are Provisionally Recommended as their results are based on only 4 years in trial.

Cultivar	Locations ES2	ES3	NS2	NS3	NE3	NE10	Unadjusted mean control (% control)	Adjusted mean (% control)
Delibes	7.81	8.93		7.18		7.00	7.73 (113)	7.43 (109)
Nomad	7.53	8.98		6.31		6.25	7.27 (107)	6.96 (102)
Cooper	7.22	8.89		6.61		5.85	7.14 (105)	6.84 (100)
Chariot	7.31	8.22	7.44	6.44	4.87	6.02	6.72 (99)	6.72 (99)
Prisma	7.51	8.65		6.26		6.36	7.20 (106)	6.89 (101)
Camargue	7.29	8.69	7.43	6.09	4.99	6.49	6.83 (100)	6.83 (100)
Derkado	6.67	8.14		5.98		5.37	6.54 (96)	6.23 (91)
Tyne	7.32	8.21		6.38		5.82	6.93 (102)	6.63 (97)
Optic	8.10		7.95		5.43		7.16 (105)	7.39 (108)
Riviera	7.87		8.29		5.54		7.23 (106)	7.46 (109)

Table 3 Yields (t/ha) of spring barley cultivars grown at six locations in 1993 with means adjusted for missing locations using REML. Cultivars Chariot and Camargue are present in all trials and are consequently unaffected by adjustment. The unadjusted means for Provisionally Recommended cultivars, Optic and Riviera, are downwards biased, as they appear in more low-yielding environments, while unadjusted means for other cultivars are upwards biased.

conditions, with exceptional performance (good or bad) for other attributes highlighted in colour. In many cases, these other attributes will be more influential than yield in determining farmers' choice. For example, the SCRI cultivar Tyne, which now yields 5 - 10% below the best cultivars, is still the earliest maturing one on the Recommended List, and retains a niche market, particularly in northern Scotland. Quoting an overall index of economic merit for a cultivar may be of interest to producers, but would not provide farmers with the information to select cultivars for their own purposes and environmental conditions. Presenting performance indicators for a range of quality attributes, gives users the opportunity to decide on their own attribute weightings (importance values) to suit their individual circumstances.

Adjusting for background environment The most controversial aspect of using performance indicators in the public sector has been the construction of league tables for institutions based on crude output measures. Criticism has been particularly intense in respect of school league tables, where ranking has been based on GCSE scores with no adjustment for differential pupil intakes among the schools. Statisticians have argued that comparisons among institutions should always be based on the added value that they provide.

In the context of plant breeding, the added value of a new cultivar is assessed against the current commercial standard, or mean of several standards, when grown under the same environmental conditions. The performance (e.g. yield) of the cultivar can be expressed as

$$Y = G + E$$

where E is the performance of the standard in a particular environment and G is the added (genotypic) value from growing the new cultivar. Hence, when the environments differ, the recorded yields of a set of cultivars need first to be adjusted by the appropriate environmental effects, to allow comparison based on added values. In cultivar testing, this adjustment may take place both within individual trials and across trials. We illustrate the importance of adjustment for the latter case using the results for a short series of yield trials (Table 3), where most cultivars are grown in only a proportion of trials. Direct comparison of cultivar means ignores differences in environmental effects between trials. Various methods have been used for adjustment, though some are inefficient and can produce inconsistent results. Residual maximum likelihood (REML), a general estimation method developed by BioSS and its predecessors over the last 25 years, provides efficient cultivar comparisons for incomplete series of trials. When the method is applied to the yields of Table 3, some cultivar means are adjusted by as much as 5% and the ranking of cultivars is substantially changed.

Non-linear responses So far we have assumed implicitly that the added value of a cultivar over the standard is constant for all environmental inputs, i.e. that G and E effects are additive (Fig. 1a). The added values for cultivar performance, and related rankings, are then applicable to all environments. However, assessment is invariably complicated through cultivar by environment interactions, often expressed as differential sensitivities of cultivars to an environmental stress (Fig. 1b). When interactions are of the cross-over type and affect cultivar rankings, recommendations to

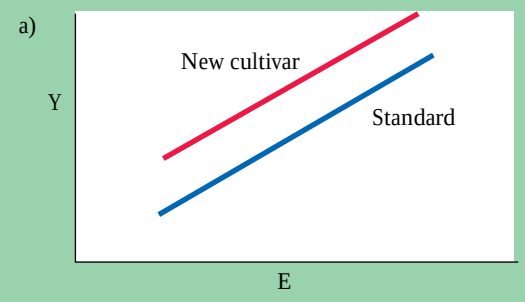


Figure 1 a) The performance Y of a new cultivar may show a constant added value over the current standard cultivar for all environments E, or b) there may be a cross-over interaction such that the new cultivar outperforms the standard in only some environments.

farmers need to take account of their particular environmental conditions (Fig. 2). The distinctive performance of cultivars in northern Britain justifies a separate Scottish Recommended List for most crops. NIAB also produces separate regional recommendations for England and Wales. In the UK, the weather makes a major contribution to cultivar by environment interactions, but its unpredictability can obscure regional patterns and add to the uncertainty in predicting cultivar performance.

Presenting uncertainty If performance indicators are to be used in decision making, it is essential that users are fully aware of their inherent variability, even after adjustment for known confounding factors. There is particular ignorance about the sensitivity of rankings to variation in the underlying measures. Variation may arise from a number of sources: for example, the overall variation in morbidity after a particular hospital operation may include contributions from the patient, surgeon, subsequent medical treatment, hospital and time of year. When using performance indicators as predictors, it is important that all relevant sources of variation are included in deriving an overall error estimate. Evidently, the clear presentation of variability when publishing performance indicators is both important and complex. We illustrate this for

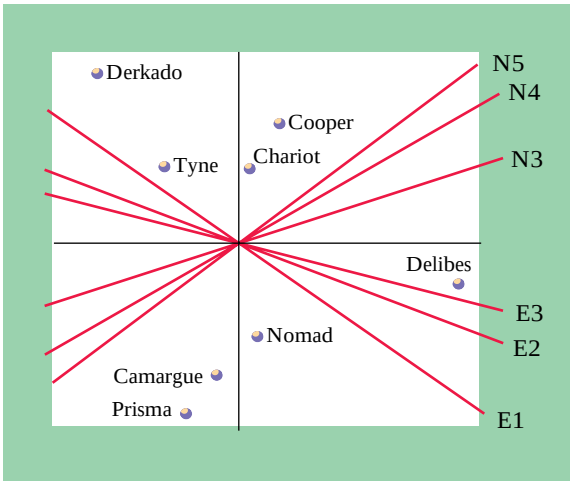


Figure 2 The effect of cultivar by environment interaction on the ranking of cultivars at different locations can be illuminated in a biplot display. This is illustrated for the yields of the eight Fully Recommended barley cultivars of Table 2 at six locations (labelled E1, E2, E3, N3, N4, N5) in 1996. The horizontal axis ranks cultivars on their overall mean. The vertical axis gives the first principal component of the cultivar by location interaction. The relative yields of cultivars at a particular location are approximated by projecting the cultivars onto the respective location axis. The plot above suggests that Delibes outyields all cultivars at all locations in 1996. The other cultivars fall into two groups: Nomad, Camargue and Prisma are ranked above Cooper, Chariot, Tyne and Derkado at locations in south-east Scotland (labelled E1, E2, E3), but are outranked by this second group at the more northern locations (N3, N4, N5).

comparing the yields of crop cultivars.

The unequal replication of cultivars in a series of Recommended List trials gives rise to different errors for the estimated cultivar means. These standard errors relate to the average performance across the region sampled by the trial locations. The cultivar means can also be used to predict performance at a random location (or field) in the region, but their error estimates should then be increased to take account of cultivar by location interaction. This is displayed graphically in Figure 3 which plots cultivar means in rank order, with their 95% confidence intervals at the regional and field scale. The degree of overlap of intervals for two cultivars is directly related to the uncertainty in differentiating between their performance, and this uncertainty is followed in their ranking.

Most information on variability of crop yield relates to locations. Within UK, however, the variability in cultivar differences from year to year is usually larger than that across locations. Thus confidence intervals

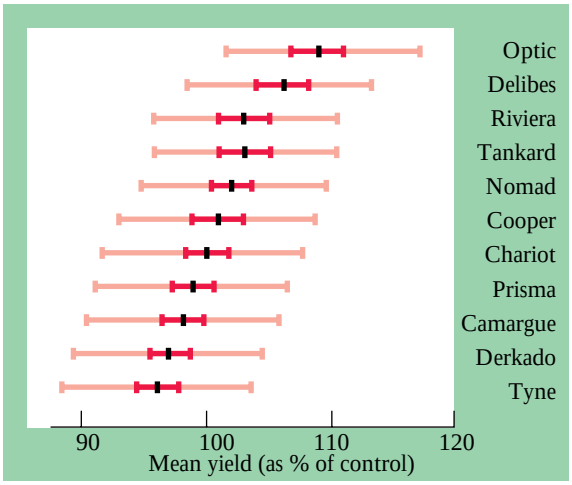


Figure 3 Relative mean yields for Recommended List cultivars (Table 2) plotted in rank order with their 95% confidence intervals for prediction on a regional ( ) and field ( ) basis.

based on yields for a single year will underestimate the uncertainty in comparing the performance of the cultivars in future years.

Impact of performance indicators Quantitative indicators are essential for the objective assessment of performance, but their introduction into the public sector has been controversial. Their crude application in, for example, setting performance targets, ranking institutions, and formula funding, has led to much professional cynicism and accusations that, by targeting effort, individuals and institutions can raise their performance against specific indicators without increasing the overall quality of outcome. Indeed, the resulting distortion in behaviour may even lead to a deterioration in quality. Thus in education, identification of the proportion of A-C passes at GCSE as a key performance indicator for rating schools has led to the unwarranted concentration of resources on potential D-grade pupils. In the USA, surgeons are reported to have increased risk factors relating to their patients before operation so as to improve their own risk-adjusted mortality rates. It has even been suggested that some surgeons may refuse to operate on patients with poor prognosis who are likely to spoil their own league placing!

The impact of the Recommended List on farmers' choice of cultivars is worthy of a fuller study. However, the emphasis on providing farmers with a wide range of performance indicators on which to base their decisions, rather than a simplistic league table, means that the Recommended List can bring a trustworthy currency to an intensively competitive market place. Our concern with the uncritical promotion of performance indicators to encourage competition and efficiency in the public sector is that, in many cases, the currency is flawed or too readily forged.

Research services

Analytical facilities

W.W. Christie

Laboratory Accreditation

The first phase of the introduction of an approved Quality Assurance (QA) System within SCRI was completed in December, 1996, with the award of certification to ISO 9002 by SGS Yarsely International Certification Service Ltd, for the Stable Isotopes Laboratory and the Lipid Analysis Unit of MRS Ltd. Expansion of the system to other areas, including phytochemical and lipid research, mass spectrometry and the media preparation facility, is underway. This process will also include an upgrade of certification to the ISO 9001 standard to encompass the design and development elements of research. The consultancy project initiated to assist in the introduction of our QA system was concluded successfully in December on the award of certification. An important element of this project was the training of internal Quality Auditors, which was conducted on site in March. A data archival facility is being set up, which will allow for archival of both hard copy and electronic files. Electronic data will be archived on writeable compact discs in order to meet the accreditation requirements for data permanence. Further details concerning Laboratory Accreditation, including a description of the Quality System and its function, are given in a review article in this report, 'Laboratory Accreditation'.

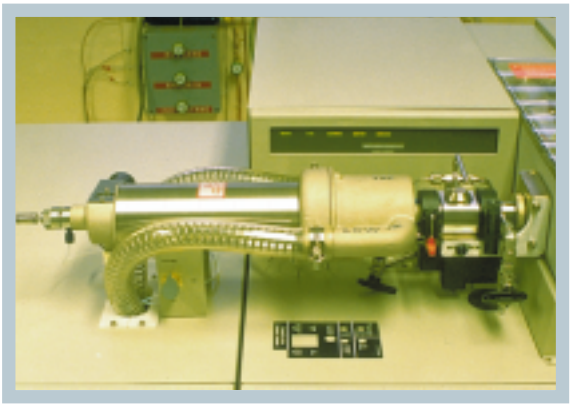
Stable Isotope Facility

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 - ^{13}C, ^{15}N, ^{18}O and ^{34}S in a wide range of solid, liquid and gas samples. All the instrumentation is based on continuous-flow isotope-ratio-mass spectrometers that are fully automated and operated through computer data systems. Automation allows a high through-put of samples, essential for many biological experiments where large data sets are required. For solid samples, the Europa Scientific Tracermass and 20-20 mass spectrometers are interfaced to Roboprep CN and ANCA-*NT* SL combustion sample converters. A Roboprep G+ gas purification unit is used for gas analysis. Plant samples of one to five milligrams are used, containing 25 to 100µg of the element of interest. Where possible, analytical protocols are devised to minimise sample preparation and fully exploit the automation.

SCRI also has expertise and resources for sample preparation from a wide range of 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.

Services to Molecular Biologists

DNA synthesis The DNA synthesis facility is based on an Applied Biosystems model 394 DNA/RNA synthesiser. We also have a single column model 391 synthesiser which provides cover during servicing and repairs. The 394 instrument allows the simultaneous synthesis of four oligonucleotides and is equipped with automated amidite dissolution, on line trityl monitoring and automatic cleavage facilities. Side-chain deprotection and recovery of the DNA remain manual operations. Both instruments synthesise DNA by a step-wise solid phase method which allows over 100 nucleotides to be coupled in a single nucleic acid molecule. Oligonucleotides containing between 20 and 30 residues are normally required and cycle times are such that eight oligonucleotides can be made in a day.



The particle beam interface for the mass spectrometer

Scientific Liaison & Information Services

W.H. Macfarlane Smith

The importance of good communication, both within and outwith research organisations, has never been of greater consequence. Recent events such as the BSE scare have caused many of the public to regard research with, at best, disdain, and, at worst, outright suspicion. While such perceptions are unfounded, the need to explain the relevance of science in a clear and easily understood way, free from jargon, is paramount. Within research organisations, the rapid pace of scientific progress imposes further demands in ensuring that staff keep up-to-date with new developments. The pressures for SCRI to operate in a commercial manner and to sell its many achievements and new products also make many demands. All of this is set against a background where Health and Safety legislation has never been more directly relevant to research and imposes its own constraints. The Scientific Liaison and Information Services Department endeavours to meet these diverse and increasing needs in a variety of ways through its several units.

Information Technology Services (ITS) has an ever-increasing number of customers to satisfy in terms of advice on, and assistance with, the purchase of both hardware and software, as well as the routine provi-

SLIS staff with a group of farmers and consultants from Norfolk.

sion and maintenance of services through the UNIX and Novell systems. The demand for installation of, and support for, a wide array of software packages is also increasing rapidly. The conversion of older buildings to provide modern laboratories and offices, has necessitated the further extension of the Local Area Network (LAN), which has also required the upgrading and replacement of some of its older components. The substantial increase in the use of electronic mail and the need to access electronic information sources, is reflected in the following statistics. The number of registered Novell processes rose by one third, from 340 to 441, central disk space usage on Novell doubled from 2GB to 4GB, software usage also doubled, and electronic mail is now averaging around 1500 messages per day. Without doubt, these demands will continue to increase in the next few years and will have to be matched by further extensions to, and upgrading of, the LAN, and the need for greater computing power, disk space and memory. It will also be necessary to explore means of lightening the information overload on scientists and other colleagues and to develop faster, but more targeted, methods of disseminating information. With the above background of increasing demand in mind, it is with special regret that we record the retirement of the Unit Head, Ron Clark, and the departure of Rob Kidger to undertake voluntary services overseas. Both individuals made a major contribution to the development of ITS as we know it today.

The Library continues to perform a vital rôle as a source of information for research staff. Here again, increasing demands and expectations are evident, with over 300 new books requested and 166 actually purchased or donated. The increase in loans from the bookstock also reflects the demand. BIDS ISI usage continues to increase and there has been a steady requirement for the EDINA BIOSIS abstracting system which was introduced this year. The latter has abstracts for all the journal articles which it cites and allows readers to be more selective over articles requested on inter-library loans. The number of Journals available in electronic format is growing, though most publishers continue in parallel with the production of a hard copy version. A few are uniquely electronic, with access to them available by hypertext link from the Library's Intranet Web pages. Steady progress on the Internet is being made, despite limited resources, and the majority of Departments now have their own entry. A start has been made on the production of Intranet pages for a range of matters relevant only to the internal activities of SCRI.

The Visual Aids unit also continues to see a rising demand for its services. While much of this is due to an increase in scientific presentations of various kinds, a significant part is due to the Institute's heightened commercial activities. The latter require support for the launch of new products, through the production of brochures and displays which are used at grower days, crop- and trade-events, and press briefings. These demands are reflected in a 5% increase in poster production, a 14% increase in the production of publicity photography, a 45% increase in desk top publishing activities, and a 50% increase in the number of display boards constructed. The year also saw SCRI's quadrennial Open Days. These were well supported by both the scientific community and the general public. The interest of the press in our science was manifest by the large number of newspaper and magazine articles which followed.

Health and Safety continues to satisfy all legal requirements, with ongoing programmes for training staff in the safe operation of equipment e.g. fork-lift trucks and chainsaws, the extension and annual review of risk assessments, the medical monitoring of staff involved in pesticide application, etc., and the implementation of the new standards specified in safety signs.

SLIS continues to receive a wide range of visitors, with commercial companies, politicians, scientists and students all eager to learn about SCRI's science. The number of such visits again increased over the previous year and an even wider range of countries, from South Africa to Mongolia, was represented. The number of articles placed with newspaper, magazines and journals about the work of the Institute increased by 55% over the 1995 figure. A number of televisions crews visited SCRI and various members of staff appeared on radio and television to talk about their work.

The representation of the Institute at public events is important, and staff from both SLIS and various Science Departments have attended a range of events, including the PMB Potato Planting Demonstration, ScotGrow 96, The Royal Highland Show and the Edinburgh Science Festival. It is clear from the interest at such events, and numerous follow-up enquiries, that SCRI's science has a great relevance and importance to a wide range of commercial and other interests.

Estate, Glasshouse & Field Research Department

G. Wood

The Field Research Unit lost two members of staff during the year when Michael Soutar transferred to Engineering and Maintenance to become a caretaker, and Ron Dalrymple resigned to take up another appointment. Ron was a senior member of the unit, with vast experience and well respected by colleague and client alike. In addition, the Department lost its Administrator when Lorna Doig retired due to ill health. This post is increasingly vital to fulfil the modern pro-active, financial and quality-assured management and operational systems in place throughout the Institute. We were fortunate to get Wendy Patterson to take on this rôle when she transferred from the FBPP Department on a half-time basis.

The Department's internal quality control reporting system (see the Annual Report for 1995) completed its first full year. Each report is filled in by the client for whom field or glasshouse research assistance has been provided. From over 1200 returns, 98% of clients were entirely satisfied with the work undertaken; in only 2% of cases were corrective action responses required. These latter serve to improve feedback, improve quality and raise standards even higher.

In order to make best use of resources, we have continued to pay particular attention to the upgrading of existing glasshouse facilities. Utilisable space to hold increasing numbers of plants is one limiting factor. We have made great progress in this regard by further installations of modern fixed and mobile benching. The latter allows up to 90% of the floor area to be used, and in many glasshouse cubicles this has improved the area of usable bench space by more than 50%. A Tomtech HC80 computer auto-controller of internal thermal/shade screens and supplementary lighting was installed in a complete wing of four Cambridge glasshouse cubicles, as a first step towards exploiting the technologies which are now available. As well as improving the environment for plant growth, this facility also automatically saves energy since lights are switched off at high ambient light levels and night time shading reduces radiant heat loss. In its first full year of operation, the new system has provided an energy saving of more than 40%, an equivalent saving of more than £30 per light. Extrapolating this over the total number of lights used throughout the glasshouse complex, potential cost savings would reach £20k to £30k per annum. At current prices, such an auto-lighting control system, if introduced throughout, could pay for itself within 5 years.

As well as the SSCR soft fruit and potato walks, the Open Days in June necessitated significant inputs from staff, machinery, equipment and facilities in the EGFR Department. Approximately 5000 plants were sold to the public attending the Open Day on Saturday 8 June.

The land resource for field trials was reduced by 5 ha when the lease for a parcel of land was terminated by the Ninewells Hospital Trust. Also during the year, Historic Scotland imposed scheduling orders on four sites within the remaining land area of the Institute. Photographic evidence from crop marks, and some limited physical detection studies in the field, have clearly identified residues of ancient settlements in three quite widely separated areas. The scheduling imposes limitations on cultivations and cropping, as well as on any plans to develop such sites for buildings.

The recording and provision of data to the Meteorological Office, from the Climatological Station at SCRI, is now made by Colin McCreadie and Gillian Pugh in this Department. Gordon Dunlop (CEP) initiated a training programme to familiarise them with the equipment and procedures, and we have retained access (where appropriate) to

Agricultural fleeces (Don & Low Nonwovens Ltd) being put to the test under adverse conditions.

Donald MacKerron (CEP) on a consultancy and advisory basis. The meteorological data is also made available to various research and commercial clients.

Staff in the Glasshouse Research Unit cooperated with W H Macfarlane Smith (SLIS) to undertake a research trial for International Seed Producers on germination, emergence and relative growth curves of various brassica seed lots.

The Field Research Unit undertook a number of special trials during the year. Variety trials assessed the suitability for our conditions of 14 and 15 cultivars of spring and winter barley respectively. Cultivars Chariot, Optic and Tankard were selected as the most suitable spring barleys. Whilst the 6-row cultivars Manitou and Muscat yielded well in excess of 10 t/ha, cultivars Melanie and Regina were selected as the most suitable winter barleys. One of a series of soil management and crop nutrition studies investigated fertilizer nitrogen levels that do not adversely effect yield and quality in malting spring barley. A single cultivar was combine-drilled with full- and half-rate compound fertilizer. At harvest, hectolitre weights were identical, grain nitrogen levels were 1.48 and 1.39%, respectively, and screenings below the 2.2 mm sieve were both low at around 1%. Yield was less in the half-rate fertilizer area but the biggest difference was in the amount of straw, the full-rate fertilizer producing twice the quantity. Significantly, soil N levels before sowing and after harvest were identical, at 30 kg/ha, in both areas.

Soil N levels are important environmentally and economically, and need to be kept to a minimum, or utilised effectively, by a growing crop, such as potato, which is a fairly large user of N. A large scale trial was set up to mirror work done on small plots by D K L MacKerron and his colleagues in CEP. The results of the trial with potato cultivar Maris Piper confirmed those workers' findings, which showed that it was possible to give a specific recommendation for a crop, given available N from the soil, and the modelled requirements of the crop. Having taken a soil N reading before planting, maximum yield was obtained by an application of granular fertilizer at planting which gave a combined total of 150 kg N/ha. In a non-irrigated crop, this resulted in a 20% saving in fertilizer compared with standard recommendations.

An efficacy trial against foliar blight symptoms in potato, compared the fungicides Dithane, Fubol and Shirlan against a water-only control. Shirlan gave the best control under high disease pressure. On a scale from 0% - no infection, to 100% - total leaf destruction (stems infected and turned brown), when the water-only control plots were at the 98% level, Shirlan treated plots were averaging only a 30% score.

Assistance was given to colleagues in other Departments for field trials researching natural plant products for control of PCN (Nematology and British Technology Group); the use of cowpea trypsin inhibitor to confer field resistance to vine weevil in strawberries (Soft Fruit and Perennial Crops, Axis Genetics Ltd, and the Department of the Environment); and pesticide efficacy and residue analysis trials in blackcurrants (Soft Fruit and Perennial Crops, SmithKline Beecham and Inveresk Research International).

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.

In previous years, capital works programs provided the means for the Institute to refurbish and modernise its older facilities. Once again, in 1996, no such funding was available. In an effort to ensure that improvements continued to be made, the Buildings, Equipment and Maintenance Committee, was created in 1995 under the chairmanship of Dr J M Duncan. This utilises a small amount of funding, set aside from core grant, for refurbishments and upgrades to the areas of science where such investment would provide most benefit to the Institute as a whole. Works carried out in 1996, *via* funding from this Committee, included an extension to the tissue culture facilities within the Virology area, and refurbishments to laboratories within both the Fungal & Bacterial Plant Pathology and Nematology Departments.

To continue the improvements to glasshouse facilities, which have been carried out through capital works in the past, a small team from within the Engineering and Maintenance Department was formed to refurbish individual glasshouse compartments. This prevents such areas from falling into disrepair, or in some cases below licencing authority levels.

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. In-house maintenance cover has been extended to counter-balance such measures and to minimise any reduction in the service provided to staff.

The Department also has responsibility for maintaining and extending the cabling associated with the Institute's Local Area Network, and key staff have been trained to install and test cables to Category 5 standards. Over the past year a number of areas in outlying buildings have been incorporated into the Network, all to a Category 5 level.

In addition to this, there is an on-going replacement program to upgrade the original thin Ethernet cabling which still serves the majority of Institute buildings. Retaining the ability to carry out such work by our own staff has two main benefits to the Institute in that it reduces costs, and results in a broader in-house knowledge of the Network infrastructure.

Scottish Society for Crop Research

D.L. Hood

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. It provides a link between SCRI and farmers, processors and other interested bodies by organising meetings for the exchange of information between members and staff of the Institute. It sponsors occasional publications and provides financial assistance to staff for travel and other activities. It is open to membership by any interested person or corporate body on application to the Secretary and it is controlled by a Chairman and Committee of Management. Several crop-orientated sub-committees maintain contact with members on specialised topics relevant to their interests. Membership of the Society was 317 on 31 December 1996.

The AGM of the Society was held on 2 April when Professor J.R. Hillman, Director of SCRI spoke on 'Foresight in Agriculture'.

The Committee of Management met on two occasions (2 May and 7 November).

Members cast a critical eye over the latest potato releases.

Travel Grants were awarded to:-

Dr John Bradshaw, to attend the 13th Triennial Conference of the European Association for Potato Research, 14-19 July 1996, Wageningen, The Netherlands.

Dr Brian Williamson, to attend the 11th International Botrytis Symposium, 23-27 June 1996, Wageningen, The Netherlands.

Dr Amar Kumar, to attend the 4th Potato Molecular Biology Symposium, 17-21 July 1995, Wageningen, The Netherlands.

Copies of the reports are available from the Secretary.

A Potato Walk was held on Thursday 8 August when the emphasis was on potato breeding and genetics. Topics covered included blackleg resistance, *Solanum phureja* potatoes for salads and 'gourmet' potato chips, and non-food uses such as starch.

A Soft Fruit Walk was held on Thursday 25 July when an update was given on integrated control of raspberry root-rot. A field trial of genetically modified strawberry cultivars was described, as was the *Ribes* breeding programme.

Crop Sub-Committees were active for Soft Fruit, Potatoes, and Cereals, holding several meetings throughout the year, bringing forward topics for discussion and research.

The Society continues to fund Spring and Winter Barley Trials with 3 years' results forming the basis for further research.

Officers of the Society

Trustees:

G.B.R. Gray, OBE, Smeaton, East Linton, East Lothian EH40 3DT

A. Patullo, MC, 6 Castle Way, St Madoes, Glencarse, Perthshire PH2 7NH

A.G.M. Forbes, Omachie, Kingennie, nr Dundee DD5 3RE

I.E. Ivory, Ruthven House, Meigle, Blairgowrie, Perthshire PH12 8RF

Chairman: T.P.M. Thomson, Brae Mount, Sidlaw Road, Blairgowrie PH10 7DB

Vice-Chairman: Dr D.A.S. Cranstoun, Corehouse, Lanark ML11 9TN

Members of Committee of Management

J. Arbuckle, East Flisk, Newburgh, Fife KY14 6HP

A.C. Bain, Flocklones, Invergowrie, Dundee DD2 5LE

D. Craib, Stynie, Fochabers, Morayshire IV32 7LE

J.M. Drysdale, Kilrie House, Kirkcaldy, Fife KY2 5UW

R.G. Galloway, East Balmirmer, Arbroath DD11 2PN

W.P. Laird, Cairnie Lodge, Cupar, Fife KY15 4QD

A. Logan, Tarvit Home Farm, Cupar, Fife KY15 5SU

G. Rennie, Stenton, St Monans, Fife KY10 2DF

Secretary and Treasurer: D.L. Hood, 25 North Balmossie Street, Monifieth, Angus DD5 4QL

Registered Office: c/o Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA

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.

Occasional Publications

Raspberry cultivar trial 1980-1985. Cormack, M.R. & Gordon, S.L. Occasional Publication No. 9. 1990. Scottish Crop Research Institute, Dundee, 22pp. £10

Raspberry cultivar trial 1975-1979. Cormack, M.R.& Brown, J.McD. Occasional Publication No. 8. 1981. Scottish Crop Research Institute, Dundee, 25pp. Out of print.

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.

Raspberry cultivar trial 1971-1975. Cormack, M.R. Occasional Publication No. 2. 1976. Scottish Horticultural Research Institute, Dundee, 29pp. Out of print.

Strawberry cultivar trial 1971-1974. Cormack, M.R. Occasional Publication No. 1. 1974. Scottish Horticultural Research Institute, Dundee, 10pp. Out of print.

Mylnefield Research Services Ltd

N. W. Kerby

Mylnefield Research Services (MRS) Ltd was incorporated in 1989 to exploit, commercially, the resources and expertise of SCRI. Enhancing competitiveness has been fundamental to the development of the business activities of MRS Ltd/SCRI and successful innovation is an essential part of this. SCRI fosters a pioneering culture which is exemplified by the number of patents applied for, those granted (see SCRI Annual Report 1995), the number of potential plant cultivars submitted into National Listing schemes and those gaining Plant Variety Rights.

MRS Ltd 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.

Company Aim

The primary objective of MRS Ltd is to exploit, commercially, the scientific expertise and resources of SCRI while protecting its charitable status and intellectual property.

MRS Ltd acts as the gateway to a variety of skills unique within the UK biological, agricultural and horticultural research service, ranging from fundamental studies on genetics, molecular biology and physiology, through agronomy and pathology to glasshouse and field trials. MRS Ltd is in a position to offer a wide variety of services which typically utilise the unique range of modern facilities and resources resident at SCRI.

As in previous years, 1996 was another year of growth and MRS Ltd played a key role in financially supporting scientific research at the Institute. MRS Ltd gratefully acknowledges and is reliant on the valuable and essential contributions made by SCRI staff.

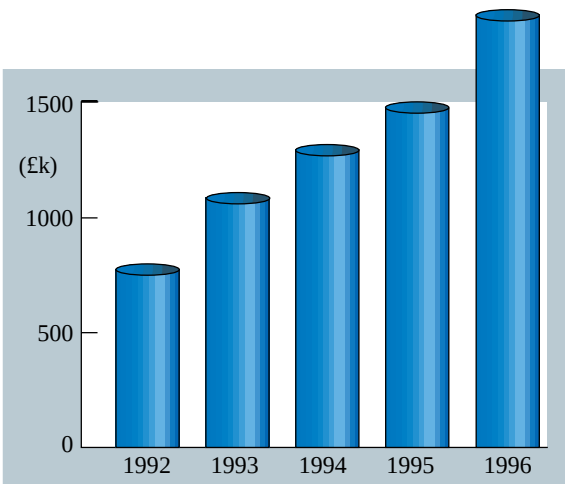


Figure 1 MRS Ltd income. The first five years.

Financial

The first five financial years Our fifth financial year ended on 31 March 1996. Turnover was in excess of £1.88M, representing a 27% increase on the previous year. This increase is a considerable achievement and outperforms the 1995 14% increase in turnover of which we were justifiably proud. Since our first financial year (1992), income which reflects business activity, has increased by 143% (Fig. 1). From the time of its incorporation, MRS Ltd has been self-sufficient in providing its own accommodation and facilities. This has been achieved without start-up funding, Government subsidy or venture capital. Gross profit in 1996 was 32% of turnover, broadly in line with previous years. As in previous years, the majority of income is Contract Research income (88.5%) (Fig. 2). As a percentage, this is expected to decline as Royalty and Analytical Service income increases. The financial contribution to SCRI, in addition to intercompany purchases for services provided by the Institute, included a Gift Aid of £100,000 (£72,000 in 1995), a management fee of £105,000, and a provision of £107,000 for the unique Nuclear Magnetic Resonance (NMR) imaging facility.

External Contracts During 1996, competitively awarded external contracts in excess of £2.11M were signed, thereby demonstrating the high level of activity and achievement of SCRI scientific staff. Sponsors

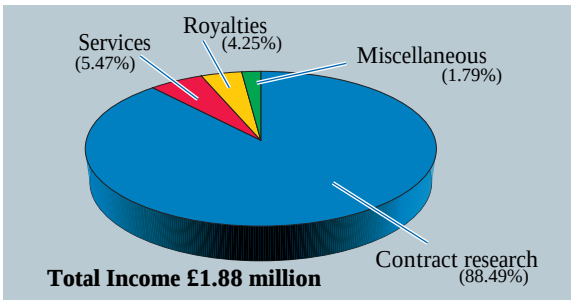


Figure 2 Analysis of MRS Ltd income 1996.

included UK Government Departments (excluding SOAEFD), European Union, Levy Boards and the private sector. Notably, significant contracts with Kentish Garden Ltd, Axis Genetics plc, the Potato Marketing Board and the Ministry of Agriculture, Fisheries and Food were awarded.

Strawberry breeding and soft-fruit biotechnology have attracted considerable support during the year. Kentish Garden Ltd have contracted MRS Ltd to breed and select superior strawberries for the fresh market. This programme fully complements the Pernod Ricard Strawberry Breeding Programme targeted to superior processing cultivars.

Intellectual Property IP and 'Know-how' are fundamental to successful technology transfer. They provide a major contribution to the future potential income of MRS Ltd, through licence agreements and collaborations with other organisations. In addition, IP brings increased publicity to MRS Ltd and SCRI, and greater recognition of the extensive range of expertise available at the Institute. SCRI has embraced the need for wealth creation, and MRS Ltd is increasingly aware of significant numbers of staff actively seeking protection for their innovative research.

This has been demonstrated in the past 5 years through the number of patents, trade marks and Plant Variety Rights applied for and subsequently granted. For instance, during 1995, six patent applications and one Patent Cooperation Treaty (PCT) application were made. A brief description of these applications can be found in the SCRI Annual Report of 1995. In 1996, one of our British Patent Applications for treating organic tissue, invented by J Pontes, R J McNicol and N W Kerby, was filed as a PCT application. Another of our PCT applications entered the National Phases in 12 European States, USA, Canada, Australia and New Zealand. The application relates to expression control polynucleotides, derived from spliceosomal protein gene promoters, and the inventors were J W S Brown, G C Clark and G G Simpson. A PCT was filed and assigned to Nickerson Biocem. The patent application relates to the use of a unique maltase gene for use in starch modification and sugar starch conversions in transgenic plants; the patent inventors were H V Davies and M A Taylor.

Of the five trade marks which were registered in 1995; DISCOVERY, SCRI and HEXAGON (SCRI logo) were all granted in 1996. We are proceeding with the remaining two trade marks, MYLNEFIELD and MRS and have registered another mark, OVER-COAT, in the UK, EU and USA.

Licence Agreements granted in 1996 During 1995, MRS Ltd had an opportunity to take control of the propagation and commercialisation of our strawberry cultivar Symphony. This enables us to work more closely with industry and increase the flow of information between the different sectors: consumers, retailers, growers, field-propagators, micro-propagators, plant breeders and research scientists of MRS Ltd and SCRI, whilst maximising Royalty income.

With MRS Ltd acting as Head Licensee, a UK Consortium was established in 1996 to propagate, market and sell Symphony. The members of the Consortium are Darby Bros Farms Ltd, J Hargreaves & Sons, C R Melton & Sons and R W Walpole (Strawberry Plants) Ltd. The following overseas propagators also signed a Licence Agreement for Symphony in 1996: Haberli AG (Switzerland), Rapo Verkoop BV (The Netherlands), Konings Royal Plant (The Netherlands) and Goossens Flevoplant BV (The Netherlands). For sales of Symphony in the UK domestic market, Licence Agreements have been signed with Ken Muir and Unwin Seeds Ltd.

MRS Ltd was pleased to assign responsibility for Dalgety Agriculture's publicly-funded potato germplasm enhancement programmes to Greenvale Produce Ltd, following the management buy-out of Dalgety Produce by Greenvale Produce Ltd. MRS Ltd is looking forward to working with Greenvale Produce Ltd together with Nickerson Seeds Ltd. Dalgety Agriculture will still have an interest in cereals, and the interests of Nickerson Seeds Ltd in potatoes, brassicas and cereals will remain the same.

An Agreement was signed with Gordon & Innes for a collaborative Potato Breeding Programme.

GORDON & INNES LTD
Growing for the future

A significant number of cultivars completed National List trials in 1996, namely the brassicas, Caledonian, Massif and Interval, and the potatoes, Claret, Spey, Kirrie, Anya and Othello. Brief descriptions of each cultivar are given on pages 53-55.

Caladonian, Massif and Interval are marketed by Sharpes International Seeds Ltd.

SHARPES INTERNATIONAL

The potato cultivars Claret, Spey, Kirrie and Othello are marketed by Greenvale Produce Ltd and Nickerson Seeds.

Anya is exclusively owned and marketed by Whitworth's Food Group.

In addition, the blackcurrant Ben Connan was granted a United States Plant Patent.

The Lipid Analysis Unit The first year of the Lipid Analysis Unit proved to be extremely productive. A successful, internationally attended, conference entitled 'Fatty Acids and Lipids - Chemistry and Analysis' was held at SCRI in September 1996.

Due to the highly favourable feedback from the delegates of this course, it is the intention of MRS Ltd to hold similar events in future years.

MRS Ltd is grateful to Professor F D Gunstone, an MRS Ltd Consultant, and Drs W W Christie, G Dobson and C Scrimgeour of SCRI for their advice, expertise and continuing commitment to the success of the Unit.

Mylnefield Research Fellowship Dr Leila Blackman of the University of Sydney, Australia was awarded the second Mylnefield Research Fellowship in August 1996. Dr Blackman joined Dr Karl Oparka for a period of 6 months to study the assembly and movement of virus particles in plants.

Employees We welcomed the following MRS Ltd scientific staff who were appointed during 1996 on various external contracts: Angela Ingram, S Nikki Jennings, Sarah Miller, Jacqueline Murphy, Joanne Russell and Fiona Slack.

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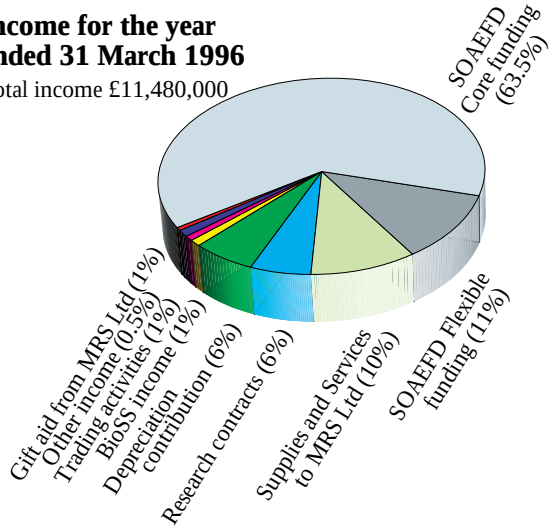
Summary of the Accounts

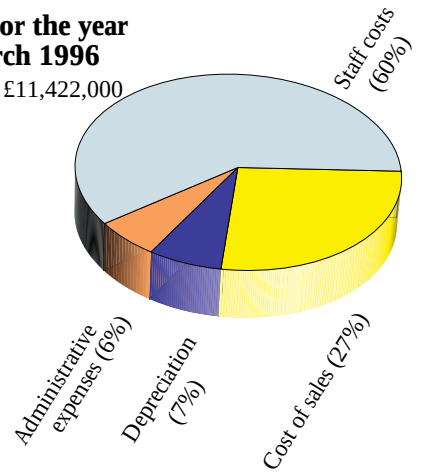
Income for the year ended 31 March 1996

Total income £11,480,000



Expenditure for the year ended 31 March 1996

Total expenditure £11,422,000

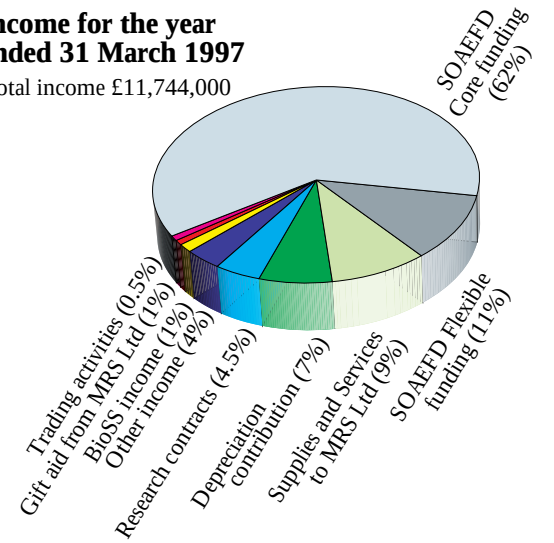


Balance sheet at 31 March 1996 **Total value £12,712,000**

Assets		Liabilities	
Fixed assets	92 %	Capital reserve	88 %
Stocks	1 %	Income & expenditure account	2 %
Debtors	7 %	Current liabilities	10 %

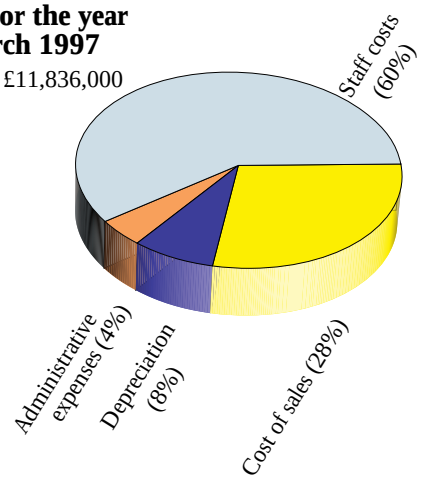
Income for the year ended 31 March 1997

Total income £11,744,000



Expenditure for the year ended 31 March 1997

Total expenditure £11,836,000



Balance sheet at 31 March 1997 **Total value £12,485,000**

Assets		Liabilities	
Fixed assets	93 %	Capital reserve	91 %
Stocks	1 %	Income & expenditure account	1 %
Debtors	6 %	Current liabilities	8 %

The Governing Body

Back row, l to r: J.M. Drysdale, P.C. Young, T.P.M. Thomson, J.E. Godfrey, J.B. Forrest
Front row, l to r: Heather M. Dick, A.N. MacCallum, D.L. Lee, K. Hopkins
(Absent: A.C. Bain, R. Cogdell, J.M. Sime, A.R. Slabas, P. Whitworth)

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 Chairman 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 in 1997.

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 in 1997.

Professor Heather M. Dick, M.D., F.R.C.P.Glas., F.R.C.Path., C.Biol., F.I.Biol., F.R.S.E., studied at Queen's College, Dundee (St Andrews University). She was Professor of Medical Microbiology, University of Dundee, from 1984 to 1996, and Visiting Professor (Immunology) at the University of

Strathclyde from 1981. She was Lecturer in the Department of Bacteriology at the University of Glasgow from 1964-71, and Consultant in Clinical Immunology at Glasgow Royal Infirmary from 1971-84. She joined the Governing Body of SCRI in 1992.

J. M. Drysdale is a specialist cereal grower and contract farmer, who farms near Kirkcaldy, Fife. He is Chairman of the Tayforth Marketing Group and a member of the Board of Directors for United Oilseeds, Devizes, Wiltshire. He was appointed to the Governing Body in 1997.

J.B. Forrest, F.R.Ag.S., farms at Whitmire, Duns, specialising in malting barley, milling wheat and oats, and pedigree cattle and sheep. He was a Nuffield Scholar in 1980. He was Vice-Chairman of the National Seed Development Organisation (NSDO) from 1982 until its privatization, and Vice President of the Scottish National Farmers Union (NFU) in 1981. He has been Chairman of British Cereal Exports since 1994, and is a local Director of the NFU Mutual Insurance, and Farms Advisor to the University of Newcastle. He was elected to the Council of the China Britain Trade Group in 1997. He joined the Governing Body of SCRI in 1983.

J.E. Godfrey, B.Sc., A.R.Ag.S, gained his degree in agriculture from the University of Reading, and is Director of family farming companies in Lincolnshire and Yorkshire. A former Chairman of the Potato Marketing Board, he is member or adviser to numerous agricultural committees, including Bishop Burton Agricultural College; 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 joined the Governing Body of SCRI in 1992.

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 in 1997.

Professor D.L. Lee, B.Sc., Ph.D., C.Biol., F.I.Biol., F.Z.S., F.R.S.A., studied Zoology at King's College (Newcastle), University of Durham, and was awarded his Ph.D. by the University of Cambridge, where he was a Fellow of Christ's College for 13 years, for research on parasitic nematodes. He was awarded the Scientific Medal by the Zoological Society of London in 1971 for research on nematodes. He is currently Professor Emeritus of Agricultural Zoology at the University of Leeds. He is an Independent Member of the UK Advisory Committtee on Pesticides; Vice-Chairman of the Grants & Education Sub-Committee; a member of the Council of the Yorkshire Agricultural Society; and is a Director of University of Leeds Farms Ltd, and Mylnefield Research Services Ltd. He was a member of the Governing Bodies of SHRI and SCRI from 1974-82, and was reappointed in 1986. He is a former Governor of the National Vegetable Research Station (now HRI Wellesbourne).

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 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 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.

T.P.M. Thomson, M.A., trained as a physicist and mathematician. He is Director of Thomas Thomson (Blairgowrie) Ltd, soft fruit growers of 40 ha of raspberries, strawberries and other soft fruit for fresh retail marketing and processing. He is a Director of Scottish Soft Fruit Growers Ltd and Kentish Garden Ltd, and

an ex-Director of NSA Plants Ltd (now renamed Meiosis Ltd). He was formerly Chairman of the SSCR and the Scottish Nuclear Stock Association Ltd, and Vice-Chairman of the Soft Fruit Panel of the Horticultural Development Council. He joined the Governing Body of SCRI in 1992.

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 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 horti-cultural 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 in 1997.

Staff list as at 31 December 1996

Director	Professor J.R. Hillman, B.Sc., Ph.D., D.Sc., F.L.S., C.Biol., F.I.Biol., F.R.S.E.[1,2,3]	Band 1
Deputy Director	Professor T.M.A. Wilson, B.Sc., Ph.D., C.Biol., M.I. Biol.[2]	Band 2
Secretary & Financial Manager	R.J. Killick, B.Sc., M.B.A., M.A., Ph.D., C.Biol., M.I.Biol.	Band 4
Assistant to Director	T.J.W. Alphey, B.Sc., Ph.D., C.Biol., M.I.Biol.	Band 4

Cell & Molecular Genetics Department (CMG)

Head : W. Powell, B.Sc., M.Sc., Ph.D., D.Sc.[4,5,10]	Band 3
J.W.S. Brown, B.Sc., Ph.D.[6]	Band 4
R. Ellis, B.Sc., Ph.D.[6]	Band 4
B.P. Forster, B.Sc., Ph.D.[6]	Band 4
G.C. Machray, B.Sc., Ph.D.	Band 4 (Prom. Apr)
W.T.B. Thomas, B.Sc., Ph.D.	Band 4
R. Waugh, B.Sc., Ph.D.[6]	Band 4
C.G. Simpson, B.Sc., Ph.D.	Band 5 (Prom. Apr)
J.S. Swanston, B.Sc., Ph.D., C.Biol., M.I.Biol.	Band 5
A. Young	Band 6
E. Baird, H.N.C., B.Sc.	Band 7
Gillian Clark, H.N.C., B.Sc.	Band 7
Jackie Lyon	Band 7
G.R. Young, H.N.C.	Band 7
Nicky Bonar, H.N.C.	Band 8
A. Booth, H.N.C.	Band 8
Diane Davidson	Band 8 (P/T)
R. Keith	Band 8
Jennifer Watters, H.N.D.	Band 8 (P/T)
A. Wilson	Band 8
Patricia E. Lawrence	Band 9
Alice Bertie	Band 10
J.D. Fuller	Band 10
Joyce I. Young	Band 10

Cellular & Environmental Physiology Department (CEP)

Head : H.V. Davies, B.Sc., Ph.D.[5]	Band 3
K.J. Oparka, B.Sc., Ph.D.[5]	Band 3 (IMP)
B. Boag, B.Sc., Ph.D.[6]	Band 4
J.W. Crawford, B.Sc., Ph.D.[7]	Band 4
J.M.S. Forrest, B.Sc., Ph.D.	Band 4
B.S. Griffiths, B.Sc., Ph.D.	Band 4
Linda L. Handley, B.A., B.Ed., M.Sc., Ph.D.[8]	Band 4
D.K.L. MacKerron, B.Sc., Ph.D.	Band 4
B. Marshall, B.Sc., A.R.C.S., Ph.D.[7]	Band 4
I.M. Morrison, B.Sc., Ph.D.[6]	Band 4
K. Ritz, B.Sc., Ph.D.[7]	Band 4
D. Robinson, B.Sc., Ph.D.[6]	Band 4
G.R. Squire, B.A., Ph.D.	Band 4
A.G. Bengough, B.Sc., Ph.D.	Band 5
G.J. McDougall, B.Sc., Ph.D.	Band 5
D. Stewart, B.Sc., Ph.D.	Band 5 (Prom. Apr)
M. Taylor, B.Sc., Ph.D.[9]	Band 5
R. Viola, B.Sc., Ph.D.	Band 5
R.E. Wheatley, B.Sc., Ph.D.	Band 5
I. Young, B.Sc., Ph.D.	Band 5
D.C. Gordon, H.N.C.	Band 6
J. Liu, B.Sc., M.Sc., Ph.D.	Band 6
Heather A. Ross, H.N.C., Ph.D., C.Biol., M.I. Biol.	Band 6
Kathryn M. Wright, M.A., Ph.D.	Band 6
Sandra Caul, H.N.C.	Band 7
R. Neilson, H.N.C., M.Sc.	Band 7
D.A.M. Prior, H.N.C.	Band 7
Susan Verrall, H.N.C.	Band 7 (P/T)
Gladys Wright, H.N.C.	Band 7
D. Crabb	Band 8
G. Dunlop, O.N.C.	Band 8
Margaret Garland	Band 8
Lesley George	Band 8
Diane McRae	Band 8
Julie A. Duncan	Band 10 (P/T)
Evelyn Good	Band 10 (P/T)
A.T. Hall, B.Sc.	Band 10 (P/T)
B. McGill	Band 11 (P/T) (HELM)

Crop Genetics Department (CG)

Head : G.R. Mackay, B.Sc., M.Sc., C.Biol., F.I.Biol.[4,5]	Band 3
J.E. Bradshaw, M.A., M.Sc., Ph.D.[6]	Band 4
M.F.B. Dale, B.Sc., Ph.D.[6]	Band 4
G. Bryan, B.Sc., M.Sc., Ph.D.	Band 5 (Appt. April)
I. Chapman, B.Sc.	Band 5
M.J. De,Maine, B.Sc., M.Phil.	Band 5
S. Millam, B.Sc., Ph.D.	Band 5
G. Ramsay, B.Sc., Ph.D.	Band 5
W. De Jong, B.Sc., Ph.D.	Band 6 (Appt. May)
K. Harding, B.Sc., Ph.D.	Band 6
Alison K. Lees, B.Sc., Ph.D.	Band 6
Ruth M. Solomon-Blackburn, B.A., M.Sc.	Band 6
Helen E. Stewart, C.Biol., M.I.Biol.	Band 6
Jane McNicoll, H.N.C., B.Sc.	Band 7 (Prom. Jan)
G.E.L. Swan	Band 7
D. Todd, B.Sc.	Band 7
R.N. Wilson, N.C.H.	Band 7
Eva Bennett	Band 8
M.P.L. Campbell	Band 8
Norma Dow	Band 8
Marjorie Grant, H.N.D.	Band 9
Moira Myles, O.N.C.	Band 9 (Prom. Oct)
Sharon Neilson	Band 9
A. Margaret McInroy	Band 10
Gail Simpson	Band 10

[1] Visiting Professor in the University of Strathclyde
[2] Visiting Professor in the University of Dundee
[3] Visiting Professor in the University of Edinburgh
[4] Honorary Senior Lecturer in the University of St. Andrews
[5] Honorary Senior Lecturer in the University of Dundee
[6] Honorary Lecturer in the University of Dundee
[7] Honorary Research Fellow in the University of Dundee
[8] Honorary Professor of Botany, Florida International University
[9] Honorary Lecturer in the University of Glasgow
[10] Honorary Professor, Oregon State University
[11] Honorary Fellow in the University of Edinburgh
[12] Honorary Lecturer in the University of Aberdeen

Chemistry Department (Chem)

Head : W.W. Christie, B.Sc., Ph.D., D.Sc., C.Chem., F.R.S.C. Band 3
B.A. Goodman, B.Sc., Ph.D., C.Chem., F.R.S.C. Band 4
D.W. Griffiths, M.A., Ph.D, C. Chem., M.R.S.C. Band 5
G.W. Robertson, B.Sc., C.Chem., M.R.S.C. Band 5
C.M. Scrimgeour, B.Sc., Ph.D.[6] Band 5
H. Bain, H.N.C., L.R.S.C. Band 6
N. Deighton, B.Sc., Ph.D., C.Chem., M.R.S.C. Band 6 (Appt. Feb)
G. Dobson, B.Sc., Ph.D. Band 6 (Appt. Jun)
Sheila Glidewell, M.A., M.Sc., Ph.D. Band 6 (Appt. Feb)
Winifred M. Stein, H.N.C., B.Sc. Band 6
K. Taylor, H.N.C., B.Sc. Band 7
Fiona Falconer, H.N.C. Band 8
Jean Wilkie Band 10
Quality Assurance Officer : T. Shepherd, B.Sc., Ph.D. Band 6

Fungal and Bacterial Plant Pathology Department (FBPP)

Head : J.M. Duncan, B.Sc., Ph.D.[5] Band 3
G.D. Lyon, B.Sc., M.Sc., Ph.D., D.I.C.[6] Band 4
A.C. Newton, B.Sc., Ph.D.[6] Band 4
P. Birch, B.Sc., Ph.D. Band 6
D. Cooke, B. Sc., Ph.D. Band 6
Lizbeth J. Hyman, B.A., M.Sc. Band 6
R. Lowe Band 6
I. Toth, B.Sc., Ph.D. Band 6
Jacqueline Heilbronn, H.N.C. Band 7
Naomi A. Williams, H.N.C. Band 7
D.C. Guy, H.N.D. Band 8

Nematology Department (Nem)

Head : D.L. Trudgill, B.Sc., Ph.D., C.Biol., F.I.Biol., F.S.O.N.[5] Band 3
M.S. Phillips, B.Sc. Band 4
W.M. Robertson, H.N.C., F.L.S. Band 4
A. Kumar, B.Sc., Ph.D. Band 5 (Tr. from CMG Aug)
Vivian Blok, B.Sc., M.Sc., Ph.D. Band 6
J.T. Jones, B.Sc., Ph.D. Band 6
B. Harrower, H.N.D., B.Sc. Band 7
Ailsa Smith, B.Sc. Band 7
Anne M. Holt Band 8 (P/T)
Alison Paterson Band 10 (P/T)

Soft Fruit & Perennial Crops Department (SFPC)

Head : R.J. McNicol, B.Sc.[5] Band 3
A.T. Jones, B.Sc., Ph.D.[5] Band 3 (IMP)
B. Williamson, B.Sc., M.Sc., Ph.D., D.Sc.[6] Band 4
A.N.E. Birch, B.Sc., Ph.D., C.Biol., M.I.Biol. Band 5
R.M. Brennan, B.Sc., Ph.D. Band 5
B. Fenton, B.Sc., Ph.D., C.Biol., M.I.Biol. Band 5 (Prom. Apr)
S.C. Gordon, H.N.C. Band 5
Julie Graham, B.Sc., Ph.D. Band 5 (Prom. Apr)
G. Thow, B.Sc., Ph.D. Band 6 (Tr. from FBPP Jan)
Alison Dolan, H.N.C. Band 7 (P/T) (Appt. Nov)
Wendy J. McGavin, B.Sc. Band 7
Gaynor Malloch, D.C.R., B.Sc. Band 7
Sandra L. Gordon, H.N.C. Band 8
Kay Greig, Dip. H.E. Band 8
Departmental Administrator : Maureen Murray Band 8

Virology Department (Vir)

Head : P.F. Palukaitis, B.Sc., Ph.D. Band 3 (Appt. Jan)
M.A. Mayo, B.Sc., Ph.D., C.Biol., M.I.Biol.[5] Band 3 (IMP)
H. Barker, B.Sc., Ph.D. Band 4
D.J.F. Brown, B.A., Ph.D., C.Biol., M.I. Biol., F.R.S.N., F.S.O.N. Band 4
I.M. Roberts, H.N.C., Dip.R.M.S. Band 4
D.J. Robinson, M.A., Ph.D.[6] Band 4
Lesley Torrance, B.Sc., Ph.D.[6] Band 4
J.A.T. Woodford, M.A., Ph.D.[6] Band 4
G.H. Duncan, H.N.C. Band 5
S.A. MacFarlane, B.Sc., D.Phil. Band 5
B. Reavy, B.Sc., D.Phil. Band 5
S. Santa Cruz, B.Sc., Ph.D. Band 5 (Prom. Apr)
Maud M. Swanson, B.Sc., Ph.D. Band 6
G.H. Cowan, H.N.D. Band 7
Sheila M.S. Dawson, H.C. Band 7
Jill Middlefell-Williams, H.N.C. Band 7 (Tr. from CG May)
Kara D. Webster, H.N.C. Band 7
Fiona Carr Band 8 (P/T)
Gillian L. Fraser Band 8
Sheena S. Lamond Band 8

Scientific Liaison & Information Services Department (SLIS)

Head : W.H. Macfarlane Smith, B.Sc., Ph.D., C.Biol., M.I.Biol., F.I. Mgt. Band 4
T. G. Geoghegan, A.B.I.P.P., A.M.P.A. Band 5
T.D. Heilbronn, B.Sc., M.Sc. Band 6
I.R. Pitkethly, H.N.D. Band 6
P. Smith, B.Sc. Band 6
Sarah E. Stephens, B.Sc., M.A., A.L.A. Band 6
I. Black, H.N.C. Band 7
S. Clark, H.N.C. Band 7
S.F. Malecki, A.B.I.P.P. Band 7
Ursula M. McKean, M.A., Dip. Lib. Band 7
G. Menzies Band 7
Janette Keith Band 11 (P/T)
Safety Coordinator : Kathryn M. Wright, M.A., Ph.D. Band 6

Administration Department (Admin)

Secretary & Financial Manager : R.J. Killick, B.Sc., M.B.A., M.A., Ph.D., C.Biol., M.I.Biol.	Band 4
Financial Controller : R.R. Boath, C.A.	Band 4
Accountant : S.L. Howie, C.A.	Band 5
Assistant Secretary : D.L. Hood, B.Admin., Dip. Ed., L.T.I., A.I.I.M.	Band 6
Personnel Officer : I. Paxton, H.N.C., M.Sc., M.I.P.D.	Band 6
European Liaison Officer : Joan Duffin, B.Sc., P.G.C.E., M.B.A., Dipl. Mngt.	Band 6
Anne Pack	Band 7
Catherine Skelly	Band 7
Theresa Ower, B.A.	Band 8 (Appt. Feb)
Elizabeth L. Stewart	Band 8
Dianne L. Beharrie, Dip. Ed.	Band 8
Rhona G. Davidson	Band 8
Pam Duncan	Band 8
Kristy L. Grant, B.A.	Band 8
Sarah-Jane Simms, H.N.D.	Band 8
Joyce Davidson	Band 8
Sheena Forsyth	Band 8
Barbara V. Gunn	Band 9 (Tr. from SLIS Jul)
Media Kitchen	
Wendy Ridley	Band 7
Evelyn Warden	Band 10
W. Burry	Band 11 (HELM)
J. McMillan	Band 11 (P/T) (HELM)

Engineering & Maintenance Department (EM)

Head: S. Petrie, B.Sc.	Band 4 (Prom. Oct)
D. Gray, H.N.C.	Band 6
A. Low	Band 7
I.C. McNaughton, H.N.C.	Band 7 (Prom. Oct)
K. Henry	Band 8 (Prom. Apr)
G.C. Roberts	Band 8
R. White	Band 8
J. Anderson	Band 9
D. Byrne	Band 9
E. Lawrence	Band 9
R.D. McLean	Band 8
C.G. Milne	Band 9
R. Pugh	Band 9
C. Conejo	Band 10 (Prom. Jun)
J. Flight	Band 10
N. McInroy	Band 10
D.L.K. Robertson	Band 10
J. Rowe	Band 10 (Prom. Jun)
M.J. Soutar	Band 10 (Tr. fro, EGFR Jun)
J. Oldershaw	Band 11
Departmental Administrator :	
Wendy A. Patterson, H.N.D.	Band 8 (P/T)

Estate, Glasshouse & Field Research Department (EGFR)

Head : G. Wood, B.Sc., Ph.D., F.E.T.C.	Band 4
P.A. Gill, H.N.D.	Band 6
J.R.K. Bennett	Band 7
W.D.J. Jack, B.Sc.	Band 7
D.S. Petrie	Band 7
B.D. Robertson, N.E.B.S.M., H.N.C., Dip. Mgt., M.B.A.	Band 7
A. Grant	Band 8
A.W. Mills	Band 8
R. Ogg	Band 8
D.G. Pugh	Band 8
J.T. Bennett	Band 9
L.A. McNicoll	Band 9
G. Dow	Band 10
B. Fleming	Band 10
I. Fleming	Band 10
A.C. Fuller	Band 10
G.S. Lacey	Band 10
J. Mason	Band 10
T.A. Mason, N.E.B.S.M.	Band 10
C. McCreadie	Band 10
R. Murray	Band 10
Gillian Pugh	Band 10
Angela M. Thain	Band 10 (P/T)
J.K. Wilde	Band 10
J. Abernethy	Band 11 (P/T) (HELM)
M. Torrie	Band 11 (P/T) (HELM)
Departmental Administrator :	
Wendy A. Patterson, H.N.D.	Band 8 (P/T)

Biomathematics and Statistics Scotland (BioSS)

King's Buildings, University of Edinburgh	
Director : R.A. Kempton, M.A., B.Phil.[11]	Band 3
G.J. Gibson, B.Sc., Ph.D.	Band 4
C.A. Glasbey, M.A., Dip. Math. Stats., Ph.D., D.Sc., M.I.S.I.[11]	Band 4
E.A. Hunter, B.Sc., M.Phil.[11]	Band 4
Janet M. Dickson, B.Sc.	Band 5
G.W. Horgan, B.A., M.Sc.	Band 5
M. Talbot, F.I.S., M.Phil. [11]	Band 4
F.G. Wright, B.Sc., M.Sc., Ph.D.	Band 5
J.A.N. Filipe, B.Sc., M.Sc., Ph.D.	Band 6 (Appt. Apr)
A.D. Mann, B.Sc.	Band 6
I.M. Nevison, M.A.	Band 6
Muriel A.M. Kirkwood, D.A.	Band 8
Diane Glancy	Band 10 (P/T)
Karyn Linton	Band 9 (P/T)
Amy G. Stewart	Band 10 (P/T)
Secretary : Elizabeth M. Heyburn, M.A.	Band 7
Ayr Unit	
D.A. McNulty, B.Sc., Ph.D.	Band 6
Aberdeen Unit, RRI	
Head : M.F. Franklin, B.Sc., M.Sc., Ph.D. [12]	Band 4
C. J. Harbron, B.Sc.	Band 6
M.J. Metcalf, B.Sc., Ph.D.	Band 6 (Appt. Apr)
Carol A. Reid, B.Sc., Dip. Acc., Ph.D.	Band 6 (Appt. Oct)
Karen A. Robertson, B.Sc.	Band 7
Aberdeen Unit, MLURI	
Head : D.A. Elston, B.A., M.Sc.	Band 4
D.J. Hirst, B.Sc., Ph.D.	Band 5
Elizabeth I. Duff, B.Sc.	Band 6
Dundee Unit	
Head : J.W. McNicol, B.Sc., M.Sc.	Band 4
Christine A. Hackett, B.A., Dip. Math. Stats., Ph.D.	Band 5
T. Connolly, B.Sc., Ph.D.	Band 6

Short-term Contracts

SOAEFD Flexible Funding

BioSS

Nicole H. Augustin, M.Sc.	Band 6 (Appt. Sep)
Elizabeth J. Austin, M.A., D.Phil.	Band 6
I.J. McKendrick, B.Sc., Ph.D.	Band 6 (Appt. Oct)
T.S. Smart, B.A., P.G.C.E., M.Sc.	Band 6 (Appt. Oct)
Verena M. Trenkel, Dipl. Biol., M.Sc.	Band 6
Kerry J. Brown, B.Sc.	Band 7 (Appt. Sep)
Maria L.Durban-Reguera, B.Sc., Dip. Math. Stats.	Band 7
S.A.R. Williams, B.Sc.	Band 7

Cell and Molecular Genetics

F. Commerford, B.Sc., Ph.D.	Band 6
A. Ibrahim, B.Sc., Ph.D.	Band 6
J. Provan, B.Sc., Ph.D.	Band 6
Karen McLean, B.Sc.	Band 7 (Appt. Jan)
C. McQuade	Band 10

Cellular and Environmental Physiology

C. Clegg, B.Sc., Ph.D.	Band 6
E. Grist, B.Sc., M.Sc., Ph.D.	Band 6 (Appt. Jan)
A.M. Cooper, H.N.D.	Band 7
G. Henderson, B.Sc., M.Sc.	Band 7 (Appt. Sep)
D. Kiezebrink, B.Sc., M.Sc.	Band 7 (Appt. Sep)
Sarah Tiller	Band 7
Lisa Vettraino	Band 7(Tr. from FBPP Sep)
Alexandra Holmes, H.N.D., P.G.Dip. Biotech.	Band 10

Crop Genetics

Elise Flipse, Ir., Ph.D.	Band 6
Sharon Anderson, B.Sc., M.Sc.	Band 7

Fungal and Bacterial Plant Pathology

Rachel Toth, B.Sc., Ph.D.	Band 6 (Appt. Nov)
Francis Gourlay, B.Sc.	Band 7

Soft Fruit & Perennial Crops

Phil Irving, B.Sc., P.G.Dip.	Band 7
Kathryn Watt, B.Sc.	Band 7

Nematology

Irene E. Geoghegan	Band 7

Virology

S. Chapman, B.A., Ph.D.	Band 6
D.A.C. Jones, B.Sc., Ph.D.	Band 6
A. Ziegler, B.Sc., Ph.D.	Band 6
S. Main, B.Sc.	Band 8

BBSRC

Cell and Molecular Genetics

Linda Cardle, B.Sc., Ph.D.	Band 6 (Appt. Aug)
M. Macaulay, H.N.C., B.Sc.	Band 7

Chemistry

Samantha Gill, B.Sc., M.Sc.	Band 7

Virology

Lisa Smolenska, B.Sc.	Band 7

CEC

Cell and Molecular Genetics

Angela Collins, B.Sc.	Band 6
L. Ramsay, B.Sc., Ph.D.	Band 6 (Appt. Jul)
H. Dewar, B.Sc.	Band 7

Cellular and Environmental Physiology

M.R. MacLeod, B.Sc., Ph.D.	Band 6
Sigrun Holdhus, Cand. mag.	Band 7
Paula M. Hebden, B.Sc.	Band 8

Chemistry

I.S. Begley, B.Sc., Ph.D.	Band 7
Claire Fernie, B.Sc.	Band 7 (Appt. Feb)

Crop Genetics

Mary McGregor	Band 11 (P/T)

Nematology

M. Armstrong, B.Sc., M.Sc.	Band 7
Lisa Duncan, B.Sc., Ph.D.	Band 7 (Appt. Nov)
Alison Prior, B.Sc.	Band 7 (Appt. Feb)
Jane Wishart, B.Sc.	Band 7 (Tr. from CEP Aug)
Jean Harkins	Band 10 (P/T)
A. Stevenson, B.Sc.	Band 10 (P/T)

Virology

C. Bragard, B.Sc., Ph.D.	Band 6
K. Harper, B.Sc., Ph.D.	Band 6
Sybil M. Macintosh, B.Sc.	Band 7

Gene Shears

Cell and Molecular Genetics

D.J. Leader, B.Sc.	Band 6
Jennifer Watters, H.N.D.	Band 8 (P/T)

Leverhulme Trust

Cellular and Environmental Physiology/ Virology

Petra C. Boevink, B.Sc., Ph.D.	Band 6

MAFF

BioSS

Kerry J. Brown	Band 7

Cellular and Environmental Physiology

Sheena J. Rodger O.N.C.	Band 8

Soft Fruit & Perennial Crops

Emily Cobb, H.N.C.	Band 10

Virology

Michelle Leslie	Band 7

McCains PLC

Crop Genetics

Venetia Mahoney	Band10

ODA

Cell and Molecular Genetics

N. Wilson	Band 8

Soft Fruit & Perennial Crops

Karen B. Howat, H.N.D.	Band 8 (Appt. Jan)

Virology

M. Taliansky, Ph.D., D.Sc.	Band 6

PMB

Cellular and Environmental Physiology

M. Young, H.N.D., M.Sc.	Band 7
Mandy Morris	Band 10 (P/T)

Fungal and Bacterial Plant Pathology

J. Claxton, B.Sc., Ph.D.	Band 7 (Appt. May)
S. McDonald, B.Sc., M.Sc.	Band 7 (Appt. Sep)
Jane Roberts, H.N.C.	Band 10 (Tr. from CG Jun)

Scotia

Chemistry

D.F. Coakley, B.Sc.	Band 6

SmithKline Beecham R&D Fund

SFPC/CEP

Linda Sommerville, B.Sc.	Band 7
Mary Woodhead, B.Sc., Ph.D.	Band 7

Miscellaneous funding

Soft Fruit & Perennial Crops

P. Lanham, B.Sc., Ph.D.	Band 6

Resignations

Name	Dept.	Band	Month
Maureen Campbell	Admin	9	January
S.D. Chasalow	BioSS	6	March
T. Connolly	BioSS	6	December
C.R. Dalrymple	EGFR	9	May
R. Forrest	FBPP	6	October
Anne T. Hall	CEP	10	November
C.J. Harbron	BioSS	6	February
D. Hitchcock	BioSS	6	March
R.A. Jefferies	CEP	5	March
E. Mann	E&M	9	November (Appt. Oct)
R. Milligan	CG	9	November
Linzi Ross	SFPC	10	September
R. Smith	E&M	7	March (Appt. Jan)
Jane Wishart	CEP	7	July
I.J. Young	CG	10	October

Staff Retirements

Name	Dept.	Band	Month
R. Clark	SLIS	5	May
Elizabeth Fyffe	Admin	10	Sept

Voluntary and Flexible Retirements

Name	Dept.	Band	Month
Lorna Doig	EGFR	9	July
Lorraine Galloway	Admin	7	September
G. Goleniewski	CEP	6	January
R. Kidger	SLIS	5	March
Freida Soutar	Admin	6	July

Mylnefield Research Services Ltd

Managing Director : N.W. Kerby, B.Sc., Ph.D., C.Biol., F.I.Biol.
Administrative Executive Officer : Anne Cameron, H.N.C.
Marketing Executive Officer : Kate Bridgens, B.A., M.Sc.
Personal Secretary : Linda Butler

Carole Bachelier, B.Sc.
Leila Blackman, B.Sc., Ph.D. (Appt. Aug - MRS Fellowship)
P. Davie, O.N.C.
Patricia Dobson
Jane E Fairlie, O.N.C.
D. N. Harris, B.Sc., M.Sc.
R.E. Harrison, B.Sc., Ph.D.
P. Hedley, B.Sc., Ph.D.
P.P.M. Iannetta, B.Sc., Ph.D.
Angela Ingram, B.Sc. (Appt. Jun)
S. Nikki Jennings, B.Sc. (Appt. Jul)
C. Jones, B.Sc.
Fiona McMahon, B.Sc.
Sarah Miller, B.Sc. (Appt. Nov)
Susan Mitchell, B.Sc.
Jacqueline Murphy, B.Sc., Ph.D. (Appt. Apr)
Vasantha Ramanathan, B.Sc., M.Phil., Dip. Biotech., Ph.D.
Claire Reid, B.Sc.
Joanne Russell, B.Sc., Ph.D. (Appt. Nov)
Fiona Slack, M.A. (Appt. Jul)

Honorary Research Professors

Professor P. Broda, M.A., M.Sc., Ph.D., D.Sc., Hon.D.Sc.
Professor F. Gunstone, B.Sc., Ph.D., D.Sc., F.R.S.C., F.T.S.E., C.Chem.
Professor B.D. Harrison, C.B.E., B.Sc., Ph.D., D.Ag.For., F.R.S., F.R.S.E.
Professor N. L. Innes, O.B.E., B.Sc., Ph.D., D.Sc., C.Biol., F.I. Biol., F.R.S.E., F.I. Hort.
Professor P.H. Nye, M.A., B.Sc., F.R.S.
Professor B. Sleeman, B.Sc., Ph.D., D.Sc., C.Math., F.I.M.A., F.R.S.E.
Professor Janet Sprent, O.B.E., B.Sc., D.Sc., Ph.D., A.R.C.S., F.R.S.E.
Professor Sir W. Stewart, B.Sc., Ph.D., D.Sc., C.Biol., F.I.Biol., F.R.S., F.R.S.E.
Professor C.E. Taylor, C.B.E., B.Sc., Ph.D., F.R.S.E., C.Biol., F.I.Biol.

Honorary Research Fellows

R.A. Brown, B.Sc., M.Sc., Ph.D.
Professor H. Griffiths, B.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.

Postgraduate Students

Name	Dept.	Subject
M. Armstrong	Nem	Molecular heterogeneity in potato cyst nematodes.
Nicole Augustin	BioSS	Statistical spatio-temporal models with applications in vegetation dynamics.
Suzanne Baker	FBPP	The effect of biotic and abiotic stress on the molecular processes underlying *ml-o* resistance in barley.
O. Brendel	CEP	^{13}C and genetic variation in native Scots pine.
K. Clacher	SFPC	Production of year-round *Rubus* crops in the UK.
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.
Maria Durban-Reguera	BioSS	Modelling spatial trends and local competition effects in field trials, using generalised additive models.
M. Ehwaeti	Nem	Root-knot nematodes, biology & 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 *Solanum phureja*.
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.
C. Jones	CEP	Molecular basis of ripening in *Rubus*.
D. Kiezebrink	CEP	Modelling soil & 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 *Aceria cajani*, the mite vector of the agent of sterility mosaic of pigeonpea in different regions of Asia.
Fevronia Lioliopoulou	Vir	Studies on molecular interactions between PMTV and its vector, *Spongospora subterranea* f.sp. *subterranea*.
Gaynor Malloch*	SFPC	Genetic variation in the family Byturidae.
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.
A.A.F.L.K. Perera	CMG	Molecular diversity in coconut.
Alexandra Popovich	SFPC	Development of a rapid screening system for gene function.
J. Provan	CMG	Development of simple sequence repeat markers in potato.
C. Regalado	CEP	Spatio-temporal dynamics of microorganisms in a heterogeneous environment.
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 *Botrytis* grey mould of chickpea using PGIP genes.
Lisa Smolenska	Vir	The use of potato virus X for high level production of foreign proteins in plants.
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.

* Permanent member of staff

Service on External Committees or Organisations

Name	Position	Committee or Organisation
T.J.W. Alphey	Secretariat	CHABOS & SMAC
H. Barker	Member	Association of Applied Biologists (Virology Group)
	Member	Working group to produce FAO/IPGRI Technical Guidelines for the Safe Movement of Potato Germplasm
A.G. Bengough	Member	Scottish Soils Discussion Group Committee
A.N.E. Birch	Committee Member	IOBC Working Group - Breeding for resistance to insects and mites
V.C. Blok	Member	AAB Nematology Sub-Group
R. Brennan	Adviser	SmithKline Beecham Blackcurrant R&D Committee
D.J.F Brown	Co-chairman	Russian Society of Nematology International Symposium
	Member	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
J.W.S. Brown	Committee Member	BBSRC Genes and Development
J.W. Crawford	Member	Industrial Liaison Committee, University of Abertay, Dundee
	Member	Management Group, Centre for Non-Linear Systems in Biology
H.V. Davies	Member	Organising Committee of International Potato Molecular Biology Symposium
	Consultative Committee	Kluwer Academic Press
	Member	Monsanto Programme Review Board
R.P. Ellis	Member	BSPB Cereal Group
	Member	SAC Recommended List Consultative Committee - BSPB Representative
B.P. Forster	Co-ordinator	International Committee for Barley Chromosome Mapping: Chromosome 4
	Committee Member	European Group on Barley Genetics and Physiology
P.A. Gill	Member	Institute of Horticulture - Scottish Branch
	Member	Institute of Horticulture - Horticultural Affairs Standing Committee
	Member	UK Controlled Environment Group
	Member	Dundee College Horticultural Users Liaison Group
	Member	University of Dundee - Botanic Garden Advisory Group
C.A. Glasbey	Council Member	Royal Statistical Society
B.S. Griffiths	Member	Scientific Committee, Substrate Utilisation in the Characterisation of Microbial Communities Conference, Innsbruck, Austria.
T.D. Heilbronn	Publicity Officer	Association for Crop Protection in Northern Britain
J.R. Hillman	Chairman	Agriculture, Horticulture & Forestry Sector Panel, UK Foresight Programme
	Chairman	SCRI/SASA/SAC Liaison Group
	Chairman	Tayside Biocentre Group
	Deputy Chairman	Board of Directors, Mylnefield Research Services Ltd
	Member	Board of Directors, CHABOS
	Member	SOAEFD Joint Consultative Committee
	Member	ECRR Board of Management
	Member	SNSA Adviser to Committee
	Member	Senate, University of Dundee
	Member	University of Strathclyde Sub-Board for the Degree of B.Sc. in Horticulture
	Member	SSPDC Management Committee
	Member	Tayside Economic Forum
	Member	PSRE Network Steering Committee
	Adviser	International Foundation for Science, Stockholm
	Adviser	University of St Andrews
	Examiner	University of London
	Examiner	University of Glasgow
	Examiner	University of Edinburgh
	Examiner	Oxford Brookes University
D.J. Hirst	Member	ECN Statistical and Technical Advisory Group
D.L. Hood	Secretary & Treasurer	Scottish Society for Crop Research
G.W. Horgan	Member	RSS Local Group Committee
E.A. Hunter	Scientific Coordinator	EU AAIR 2322 Project
R.A. Kempton	President	British Region, International Biometric Society
	Chairman	Internal Award Fund Committee, International Biometric Society
	Member	Scientific Programme Committee, International Biometric Conference, Amsterdam
	Member	Organising Committee, 7th IMA Conference on Mathematics in Medicine and Biology, Oxford
R.J. Killick	Member	SMAC
	Member	BBSRC Pay Advisory Group and Pay Negotiation Team
	Member	Continuing Professional Development Advisory Group, University of Dundee
	Company Secretary	Mylnefield Research Services Ltd
W.H. Macfarlane Smith	Member	BBSRC Joint Committee on Health & Safety
	Member	BSPB Oilseed & Industrial Crops Group
	Member	ECRR PR Officers' Group
	Member	SABRI Safety Officers' Group
	Member	NPTC Plant Variety Development Panel

Name	Position	Committee or Organisation
D.K.L. MacKerron	Chairman	EAPR Physiology Section
	Secretary	Potato-Crop Sub-committee, SSCR
G.R. Mackay	Co-ordinator	Global Initiative on Late Blight (GILB)
B. Marshall	Member	Terrestrial Sciences RG&TA Committee NERC
	External Reviewer	MAFF Nitrate R&D Programme
M.A. Mayo	Member	Executive Committee of ICTV
	Chair	Plant Virus Sub-committee of ICTV
	Member	Virus data Sub-committee of ICTV
	Member	IUBS/IUMS International Commission on Bionomenclature
G.J. McDougall	Member	Organising Committee, First International Conference on Cellular and Molecular Aspects of Plant Cell Differentiation, Lebanon
U.M. McKean	Joint Chair	Scottish Agricultural Librarians' Group
R.J. McNicol	Member	HDC Soft Fruit Trialling Sub-Committee
	Member	Soft Fruit Sub-Committee, SSCR
	Adviser	SNSA Committee
	Adviser	SSFG Ltd Board
	Adviser	Soft Fruit Committee of Horticulture Research International
	Member	CHABOS - Control of Food Intake Committee
I.M. Morrison	Member	Agriculture & Environment Group Committee
	Member	Society of Chemical Industry
	Member	Energy & Industrial Cropping Group
	Member	National Farmers Union of Scotland
	Member	COST 814-II Alternative Fibre Crops Panel
A. Newton	Member	International Congress on Plant Pathology, Local Arrangements Committee
	Committee Member	United Kingdom Cereal Pathogen Virulence Survey
	Web Server Manager	British Society for Plant Pathology
	UK Representantive	EU COST Action 817
K.J. Oparka	Member	SEB Plant Biology Committee
	Member	International Organising Committee, Plant Membrane Transport Conference, Cambridge 1998
	Member	International Organising Committee, Third International Workshop on Plasmodesmata, Israel 1996
	Conference Organiser	Juan March Fundacion, International Workshop on Plasmodesmata and Macromolecular Trafficking, Madrid 1998
W. Powell	Member	External Review Team, ICARDA
	External Examiner	UCW Aberystwyth
G. Ramsay	Member	UK Plant Genetic Resources Group
K. Ritz	Coordinator	BBSRC/SOAEFD Soil:Plant:Microbe Interactions Initiative
I.M. Roberts	Chairman	BBSRC Electron Microscope Advisory Group
W.M. Robertson	Member	CHABOS Committee
	Safety Representative	Royal Microscopical Society
D.J. Robinson	Member	Advisory Committee on Releases to the Environment
G.R. Squire	Chairman	CHABOS Working Group on Environmental Pollution and Biological Radiation
	Project Coordinator	SOAEFD Coordinated Programme in Vegetation Dynamics
	Member	CHABOS Working Group on Vegetation Dynamics
	Member	CHABOS Working Group on Soil Conservation
S.E. Stephens	Joint Chair	Scottish Agricultural Librarians' Group
	Member	Information Services Group - Scottish Library Association
	Member	Tayside Chief Librarians' Group
M. Talbot	Chairman	Statistics Group of UK Plant Varieties and Seeds Committee
	Member	Statistics Committee of International Seed Testing Association
	Member	Management Board of European Network for Information Technology in Agriculture
W.T.B. Thomas	Convenor	AAB Plant Breeding and Genetics Group
Lesley Torrance	Local Organiser	Third Symposium of the IWGPVFV
	National Rep.	COST action 823
I. Toth	Organising Committee	Crop Protection in Northern Britain, 1996
D.L. Trudgill	President	Governing Body of European Society of Nematology
B. Williamson	Secretary	7th International Congress of Plant Pathology (Finance Committee)
	Treasurer	Association for Crop Protection in Northern Britain
	Secretary	Soft Fruit Sub-committee, SSCR
	Member	Department of Agriculture, Aberdeen University Advisory Committee
T.M.A. Wilson	Member	Program/Advisory Committee, Xth International Congress of Plant Virology, Jerusalem
	Member	Dundee Science Centre Consortium Steering Committee
	Member	Programme Committee, VIIth International Congress of Plant Pathology, Edinburgh
	Member	Church of Scotland, Society Religion and Technology Project 'Ethics of Genetic Engineering of Non-Human Life'
J.A.T. Woodford	Regional Hon. Sec.	Royal Entomological Society
A. Young	Section Representative	SABRI IPMS Branch Executive Committee
I.M. Young	Committee Member	British Standards Soil Quality Committee

Short-Term Workers and Visitors

Name	Country of origin	Dept.	Month/yr of arrival	Length of stay
P. Alary	France	SFPC	Apr 96	6 months
R. Alonso-Sanz	Spain	BioSS	Jul 96	2 months
M.S. Alphey	UK	CG	Jul 96	2 months
Shabina Aslam	UK	SFPC	Apr 96	6 months
Hildburg Beier	Germany	Vir	Feb 96	1 month
A. Belakbir	Morocco	CEP	Jun 96	15 months
Olivier Berdeaux	France	Chem	Jul 96	2 months
G. Bishop	Australia	BioSS	Jan 96	6 months
Leila Blackman	Australia	CEP	Aug 96	1 year
Gillian Boag	UK	CG	Jul 96	10 weeks
W.J. Bodles	UK	FBPP	Sep 95	6 months
C. Bragard	Belgium	Vir	Sep 96	16 months
S. Cable	UK	CMG	Jan 96	4 months
T. Canto	Spain	Vir	Aug 96	14 months
Pilar Blanco Camba	Spain	SFPC	June 96	4 months
K. Chueng	UK	Vir	July 96	1 month
O. David	France	BioSS	Nov 96	2 weeks
F. Decraemer	Belgium	Vir	Sep 96	1 week
Veronique Dewasmes	Belgium	FBPP	Feb 96	4 months
Catherine Dodds	UK	CG	Jan 96	1 month
R. Gaunt	New Zealand	FBPP	Jul 96	2 months
R.A. Genet	New Zealand	CG	Jul 96	6 weeks
F.E. Gildow	USA	Vir	Oct 96	3 weeks
G. Gleixner	Germany	CEP	Jul 96	1 month
S. Gunther	Germany	Vir	Nov 96	4 months
Y. Itabashi	Japan	Chem	Jul 96	3 months
L. Jain	France	CEP	Apr 96	6 months
P. Johns	New Zealand	CEP	Aug 96	2 weeks
Monika Joschko	Germany	CEP	Feb 96	1 week
T. Jung	Germany	FBPP	Aug 96	2 weeks
R. Kemp	UK	SFPC	Mar 96	1 month
R. Lister	UK	Vir	Nov 96	3 months
A. Macrae	UK	CEP	Jun 96	1 week
Chisa Maeda	Japan	CEP	Jan 96	3 months
Vanessa Maughan	UK	CEP	Jul 96	5 months
Claire Mills	UK	CG	Apr 96	6 months
Severine Morel	France	SFPC	Apr 96	9 months
F. Nabugoomu	Uganda	BioSS	Jul 96	1 month
R.A. Naidu	India	Vir	Jun 96	6 weeks
E. Necker	Switzerland	CEP	Feb 96	6 weeks
R. Nookala	India	Vir	Mar 96	4 months
Pi Nyvall	Sweden	CEP	Jun 96	2 months
Fina Opio	Uganda	FBPP	Apr 96	2 months
G.W. Otim-Nape	Uganda	Vir	Feb 96	5 months
P-J. Pange	Greece	BioSS	Jan 96	3 weeks
Virpiliena Parviainen	Finland	SFPC	Apr 96	6 months
A. Pesnyakevich	Belarus	FBPP	Feb 96	3 months
Carol Peterson	Canada	CEP	Apr 96	1 month
N. Pike	UK	FBPP	Nov 95	6 weeks
Nicola Robertson	UK	Vir	Jun 96	3 months
T. Rogasik	Germany	CEP	Feb 96	1 week
E. Ryabov	Russia	Vir	May 96	1 year
Katherine Selfe	UK	CMG	Oct 96	3 months
B. Sharga	Ukraine	FBPP	Feb 96	3 months
Jane Shearer	UK	Vir	Mar 96	2 months
Claire Simpson	UK	FBPP	Apr 96	3 months
W. Sledz	Poland	FBPP	Jul 96	2 months
M. Smith	UK	CEP	Aug 95	1 year
Darja Stanic	Slovenia	Vir	Jun 96	3 months
Tracey Sturgeon	UK	CG	Jul 96	6 weeks
P. Susi	Finland	Vir	Jan 96	8 months
R. Taylor	New Zealand	FBPP	Jul 96	2 months
W. Thomas	New Zealand	SFPC	May 96	5 months
R. Turgeon	USA	CEP	Sep 96	2 months
Laura Vicente	Portugal	Chem	Jun 96	3 months
B. Weischer	Germany	Vir	Nov 96	1 week
X. Zhou	China	Vir	Apr 96	15 months

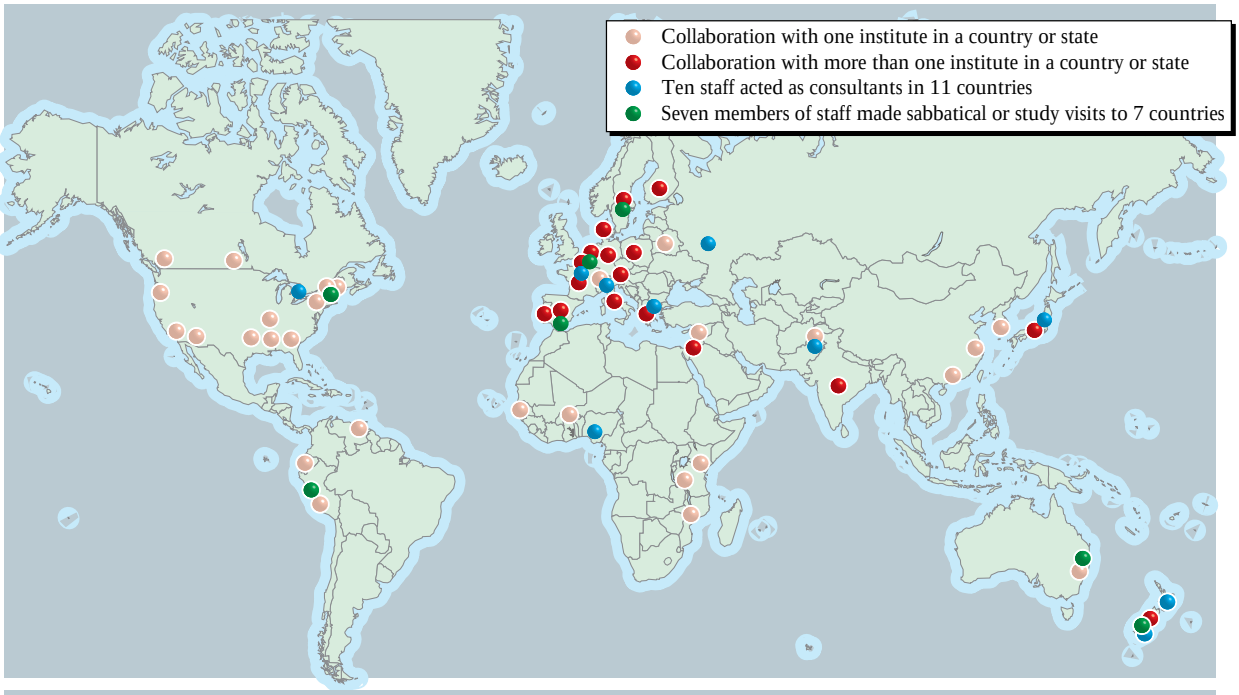
Editorial Duties

Name	Position	Journal Title
H. Barker	Editorial Board	*Annals of Applied Biology*
A.G. Bengough	Joint Editor	*British Society of Soil Science Newsletter*
	Editorial Board	*Annals of Botany*
B. Boag	Editorial Board	*Annals of Applied Biology*
	Editorial Board	*Nematologia Mediterranea*
R.M. Brennan	Associate Editor	*Journal of Horticultural Science*
D.J.F. Brown	Honorary Chief Editor	*Russian Journal of Nematology*
	Editorial Board	*Nematologia Mediterranea*
W.W. Christie	Editorial Board	*Chemistry and Physics of Lipids*
	Editorial Board	*Lipid Technology*
	Managing Editor	The Oily Press Ltd
D. Cooke	Editor	*Molecular Plant Pathology Online*
J.M. Duncan	Associate Editor	*Journal of Horticultural Science*
M.F. Franklin	Editorial Board	*British Journal of Nutrition*
	Editorial Board	*Journal of Agricultural Science*
C.A. Glasbey	Associate Editor	*Biometrics*
J.R. Hillman	Publication Committee	*Journal of Horticultural Science*
	Editorial Board	*Agricultural Systems*
	Editorial Board	*Journal of Agricultural Science*
R.A. Kempton	Editorial Board	*Journal of Agricultural Biological and Environmental Statistics*
	Editorial Board	*Heredity*
D.K.L. MacKerron	Assistant Editor	*Journal of Horticultural Science*
	Member of Editorial Board	*Euphytica*
M.A. Mayo	Editorial Board	*Virology*
	Editorial Board	*Encyclopedia of Virology*
J.W. McNicol	Editorial Panel	*Annals of Applied Biology*
I.M. Morrison	Management Committee and Editorial Board	*Journal of the Science of Food and Agriculture*
A.C. Newton	Senior Editor	*Molecular Plant Pathology On-Line*
K.J. Oparka	Editor	*Plant Physiology*
	International Advisory Board	*Journal of Experimental Botany*
P. Palukaitis	Senior Editor	*Virology*
	Senior Editor	*Molecular Plant-Microbe Interactions*
M. S. Phillips	Associate Editor	*Journal of Nematology*
D. Robinson	Editorial Advisory Board	*New Phytologist*
	Consulting Editor	*Plant and Soil*
D.J. Robinson	Editorial Board	*Journal of Virological Methods*
G.R. Squire	Advisory Board	*New Phytologist*
	Editorial Board	*Experimental Agriculture*
	Advisory Board	*Crop Physiology Abstracts*
D. L. Trudgill	Advisory Board	*European Journal of Plant Pathology*
	Editorial Board	*Nematologica*
	Editorial Board	*Fundamental and Applied Nematology*
	Associate Editor	*Journal of Nematology*
R. Viola	Editorial Board	*ACTA Botanica Neerlandica*
R. Waugh	Editorial Board	*Molecular Biotechnology*
B. Williamson	Editor	*Annals of Applied Biology*
T.M.A. Wilson	Senior Editor	*Journal of General Virology*
I.M. Young	Joint Editor	*British Society of Soil Science Newsletter*

Awards and Distinctions

Name	Dept.	Degree/Award/Distinction/Appointment
D.L. Trudgill	Nem	President of the European Society of Nematologists
D.J.F. Brown	Vir	Fellow of the American Society of Nematologists
C.A. Glasbey	BioSS	Member of the International Statistical Institute
D.J.F. Brown	Vir	Distinguished Service Award from the European Society of Nematologists
D.J.F. Brown	Vir	Skrjabin Memorial Medal awarded by the Russian Society of Parasitologists
C.G. Simpson	CMG	Peter Massalski Prize
D.J.F. Brown	Vir	Honorary Lecturer, University of Dundee
G.J. Gibson	BioSS	Honorary Lecturer, Department of Statistics & Modelling Science, University of Strathclyde
C.A. Glasbey	BioSS	D.Sc., University of Edinburgh
A. Anderson	CEP	Ph.D., University of Dundee
Miray Arli	Vir	Ph.D., University of Dundee
S. Barr	CG	Ph.D., University of Dundee
J. Claxton	FBPP	Ph.D., University of Bath
Sarah Cox	Vir	Ph.D., University of Dundee
Pauline Douglas	FBPP	Ph.D., University of Dundee
Lisa Duncan	Nem	Ph.D., University of Glasgow
Alison Mackie	CEP	Ph.D., University of Dundee
H. Pakniyat	CMG	Ph.D., University of Reading
Sarah Preston	CEP	Ph.D., University of Aberdeen
Gurinderjit Randhawa	CMG	Ph.D., University of Dundee
W. Ribeiro	CMG	Ph.D., University of Dundee
F. Wachira	CMG	Ph.D., University of Dundee
Yvonne Charters	CG	M.Sc., University of Dundee
Samantha Gill	Chem	M.Sc., University of Wales, Aberystwyth
Lizbeth Hyman	FBPP	M.Sc., University of Dundee
P. Read	FBPP	M.Sc., University of Aberdeen
Gillian Clark	CMG	B.Sc., Open University

International Collaboration and Consultancies



Research is executed within an international framework that encourages information transfer. The extent of SCRI's international commitment during 1996 is reflected in the collaborative research that was undertaken with 113 Institutions in 36 countries.

SCRI Research Programme
1996-1997

SOAEFD funded research programme showing: SOAEFD project number; Title (prefixed ROA for ROAMEd core-funded projects; FF for Flexible Fund projects); Scientific Project Leader. In addition to this list, there are research projects undertaken on behalf of various bodies, including other governmental bodies, commerce and levy boards.

SCR/361/92	ROA Genetic control of pathogenicity and host specificity at the molecular level in the fungal pathogens *Phytophthora* and *Rhynchosporium*	Duncan J M
SCR/367/92	ROA Post-transcriptional processes in plant gene expression	Brown J W S
SCR/387/92	ROA Aphid vectors of potato virus Y complex in Scotland in relation to environmental change	Woodford J A T
SCR/397/93	ROA Novel methodology for the determination of lipid structure and its application to plant biochemistry and food lipids	Christie W W
SCR/398/93	ROA Chemical strategies for the study of natural defence compounds present in plant species, including faba beans, potatoes, brassicas and soft fruit crops	Griffiths D W
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/406/93	ROA Fungus-transmitted viral pathogens of potato, cereal and peanut: fundamental model studies and comparative analyses of their genomes, gene expression, transmission by fungi and molecular cytopathology	Torrance L
SCR/409/93	ROA Establish methods for cloning antibody-coding sequences to produce recombinant antibodies from bacterial cultures	Torrance L
SCR/410/93	ROA Host gene-mediated and transgenic resistance: a study of inheritance, expression and molecular mechanisms to improve crop protection against four important potato viruses	Barker H
SCR/412/93	ROA Transformation of *Rubus*, *Ribes*, *Fragaria* and *Vaccinium* and evaluation of the biological value of the resultant transgenic plants	McNicol R J
SCR/413/93	FF Development of improved diagnostic tests for potato virus Y in a post-harvest testing scheme	Barker H
SCR/414/93	FF Carbon partitioning: rôle of rhizosphere carbon-flow in regulating soil microbial diversity and activity	Griffiths B S
SCR/415/93	FF Antibody gene repertoire cloning to produce a diverse array of specific antibodies	Torrance L
SCR/416/93	FF Foodweb analysis of below ground ecosystems using natural abundance of stable isotopes	Handley L L
SCR/418/94	ROA Free radicals, antioxidants and metalloenzymes; their identification and behaviour in plants and plant derived foods	Goodman B A
SCR/419/94	ROA Non-invasive approaches to the study of structure, composition and developmental processes in plants and plant parasites using magnetic resonance technologies	Goodman B A

SCR/420/94	ROA Production, isolation and characterisation of plant fibres for industrial applications	Morrison I M
SCR/421/94	ROA Biosynthetic control of fibre constituents during development and differentiation of fibre cells and genetic modification of these processes	Morrison I M
SCR/422/94	ROA Processing of plant fibres by novel and environmentally acceptable methods	Morrison I M
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/425/94	ROA Influence of the host on gene expression of plant parasitic nematodes including *Globodera rostochiensis* and *G. pallida*	Jones J T
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 *Globodera*, *Heterodera* and *Meloidogyne* 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/431/94	ROA Devise and operate methods for detecting and quantifying genetic resistance to pathogens of the potato causing late blight, early blight, blackleg, stem canker, skinspot, dry rot, silver scurf, gangrene, common scab and powdery scab	Bradshaw J E
SCR/432/94	ROA Integrated approaches for rapid and efficient gene transfer and characterisation in potato	Millam S
SCR/433/94	ROA Development of Polymerase Chain Reaction (PCR)-based sequence tagged site markers for potato and barley	Waugh R
SCR/434/94	ROA Dissection of regulatory mechanisms governing invertase gene expression in potato	Machray G C
SCR/435/94	ROA To clone the *Hero* gene of tomato which confers resistance to potato cyst nematode by transposon tagging	Kumar A
SCR/436/94	ROA Molecular approach to study the functions of polyamines in plant cell proliferation and morphogenesis	Kumar A
SCR/440/94	FF Investigation of *in vitro* 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, *Solanum tuberosum*	Mackay G R
SCR/444/95	ROA Low temperature stress in *Ribes*, *Rubus* and other woody genera	McNicol R J
SCR/445/95	ROA Collection and evaluation and genetic resources of *Rubus*, *Ribes* and *Fragaria*	McNicol R J
SCR/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/447/95	ROA Inheritance of resistance to potato virus diseases and production of resistant enhanced potato germplasm	Solomon Blackburn R
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 *Erysiphe graminis* on barley and oats, *Phytophthora fragaria* on strawberries and raspberries and *Erwinia* 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 *Hordeum vulgare* (barley) and *Arabidopsis thaliana*	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/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 *Phytophthora* 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/460/95	FF Use of natural abundance of stable isotopes to study sources of C, N and S for growth of bloom-forming green macroalgae and fate of these algae in the eutrophic Ythan Estuary	Handley L L
SCR/461/95	FF Native Scots Pine: establishing a scientific basis for its conservation	Powell 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

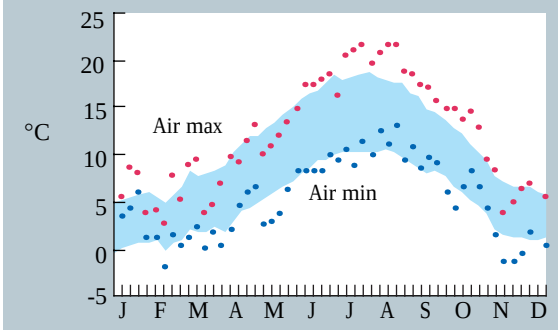
SCR/481/96	ROA Evaluation, improvement, maintenance and exploitation of biodiversity in germplasm collections of brassicas for improved pest resistance (particularly cabbage and turnip root flies) and nutritional value	Birch A N E
SCR/482/96	ROA Detection, identification, genetic variation and ecology of virus and insect, mite and nematode pests and virus vectors, especially of soft fruit crops, and strategies for their effective control	Jones A T
SCR/483/96	ROA Soft rot erwinias and blackleg disease: aetiology, epidemiology and pathogenicity, selection of resistant potato cultivars and their mechanisms of resistance	Lyon G D
SCR/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 *Botrytis cinerea* 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
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/490/96	ROA Produce and maintain pathogen-tested stocks of soft fruit cultivars and index imported materials for infection	Jones A T
SCR/491/96	FF Characterisation of novel gene promoters from maize	Brown J W S
SCR/803/94	FF Fundamental studies to develop plant virus-like particles expressed in *Escherichia coli* 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
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 Vegetation dynamics in heterogeneous species-rich vegetation	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

Meteorological Records

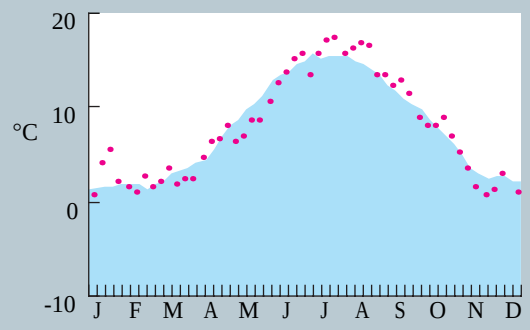
D.K.L. MacKerron

Detailed meteorological records are kept regularly at SCRI. The graphs shown are for weekly values for 1996 and the long term average for 1961-1990( ).

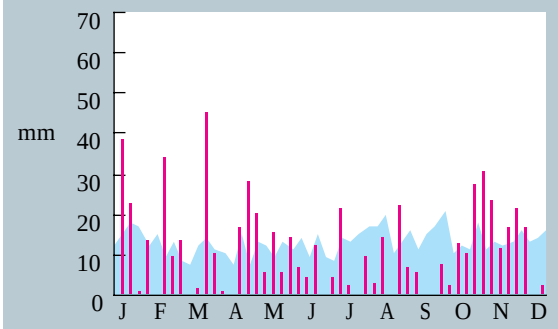
Air temperature



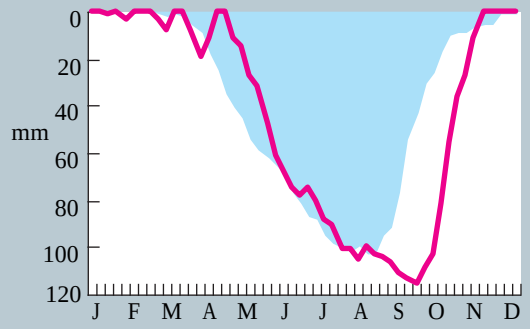
10cm soil temperature



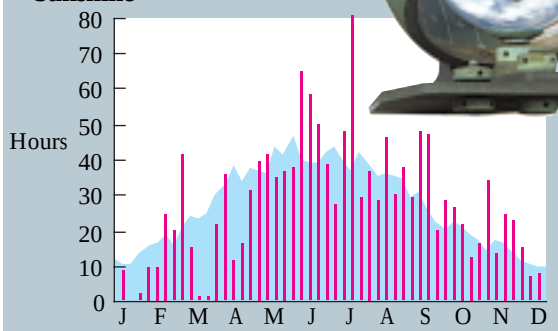
Rainfall



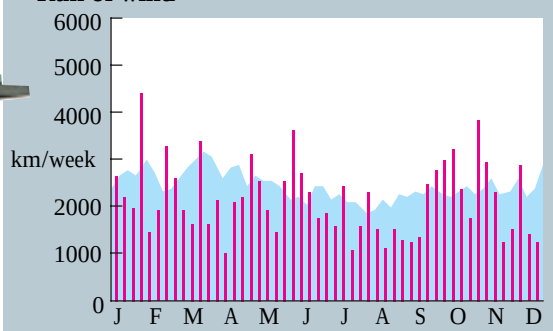
Soil moisture deficit



Sunshine



Run of wind



Cumulative Index 1990 - 1996/7

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

Soft fruit and perennial crops

Molecular biology

Cellular and environmental physiology

Chemistry

Fungal and bacterial diseases

Plant viruses

Zoology

Biomathematics and Statistics Scotland

Other Articles

Institutes supported by the Biotechnology and Biological Sciences Research Council

BBSRC Office	Polaris House, North Star Avenue, Swindon, Wilts SN2 1UH	01793-413200
BBSRC Bioscience IT Services	West Common, Harpenden, Herts AL5 2JE	01582-762271
Babraham Institute	Babraham Hall, Babraham, Cambridge CB2 4AT	01223-832312
Laboratory of Molecular Signalling	Babraham Institute, P.O. Box 158, Cambridge CB2 3ES	01223-336600
Institute for Animal Health	Compton, Near Newbury, Berkshire RG20 7NN	01635-578411
Compton Laboratory	Compton, Near Newbury, Berkshire RG20 7NN	01635-578411
Pirbright Laboratory	Ash Road, Pirbright, Woking, Surrey GU24 0NF	01483-232441
BBSRC & MRC Neuropathogenesis Unit	Ogston Building, West Mains Road, Edinburgh EH9 3JF	0131-667-5204
Institute of Arable Crops Research	Harpenden, Herts AL5 2JQ	01582-763133
IACR - Long Ashton Research Station	Long Ashton, Bristol BS18 9AF	01275-392181
IACR - Rothamsted	Harpenden, Herts AL5 2JQ	01582-763133
IACR - Broom's Barn	Highham, Bury St. Edmunds, Suffolk IP28 6NP	01284-810363
Institute of Food Research	Earley Gate, Whiteknights Rd, Reading RG6 6BZ	01189-357055
Norwich Laboratory	Norwich Research Park, Colney, Norwich NR4 7UA	01603-255000
Reading Laboratory	Earley Gate, Whiteknights Rd, Reading RG6 6BZ	01189-357000
Institute of Grassland and Environmental Research	Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB	01970-828255
Aberystwyth Research Centre	Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB	01970-828255
North Wyke Research Station	Okehampton, Devon EX20 2SB	01837-82558
Bronydd Mawr Research Station	Trecastle, Brecon, Powys LD3 8RD	01874-636480
Trawsgoed Research Farm	Trawsgoed, Aberystwyth, Dyfed SY23 4LL	01974-261615
John Innes Centre	Norwich Research Park, Colney, Norwich NR4 7UH	01603-452571
Roslin Institute	Roslin, Midlothian EH25 9PS	0131-527-4200
Silsoe Research Institute	Wrest Park, Silsoe, Bedford MK45 4HS	01525-860000
Horticultural Research International	Wellesbourne, Warwick CV35 9EF	01789-470382
HRI, East Malling	West Malling, Maidstone, Kent ME19 6BJ	01732-843833
HRI, Wellesbourne	Wellesbourne, Warwick CV35 9EF	01789-470382

Scottish Agricultural and Biological Research Institutes

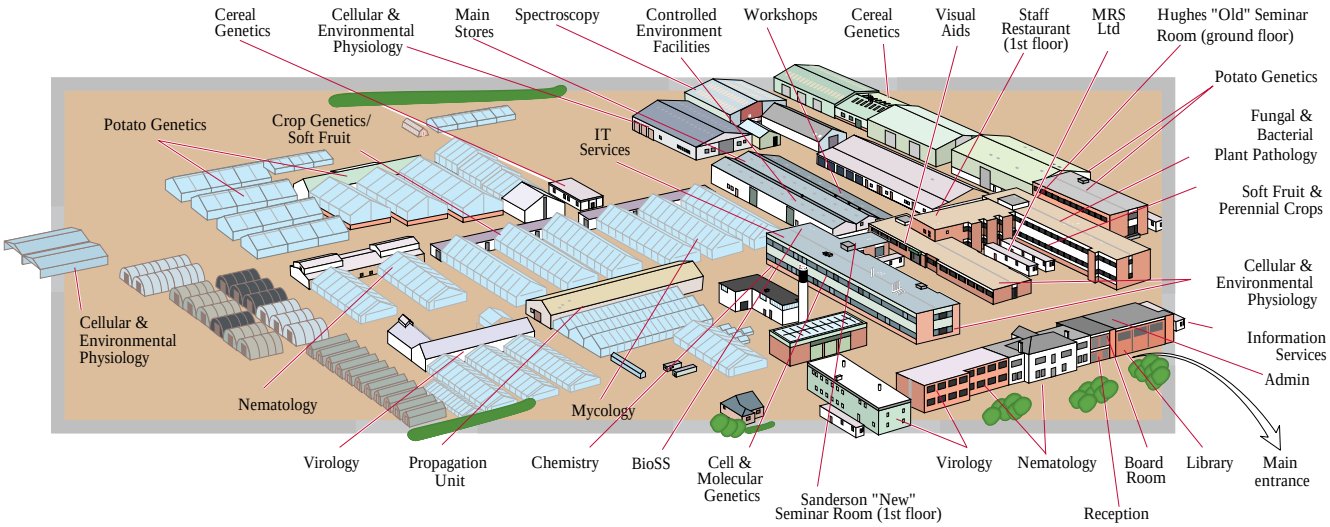
Hannah Research Institute	Ayr, Scotland KA6 5HL	01292-476013
Macaulay Land Use Research Institute	Craigiebuckler, Aberdeen AB9 2QJ	01224-318611
Moredun Research Institute	408 Gilmerton Road, Edinburgh EH17 7JH	0131-664-3262
Rowett Research Institute	Greenburn Road, Bucksburn, Aberdeen AB21 9SB	01224-712751
Scottish Crop Research Institute	Invergowrie, Dundee DD2 5DA	01382-562731
Biomathematics and Statistics Scotland (Administered by SCRI)	University of Edinburgh, James Clerk Maxwell Building, King's Buildings, Mayfield Road, Edinburgh EH9 3JZ	0131-650-4900

List of Abbreviations

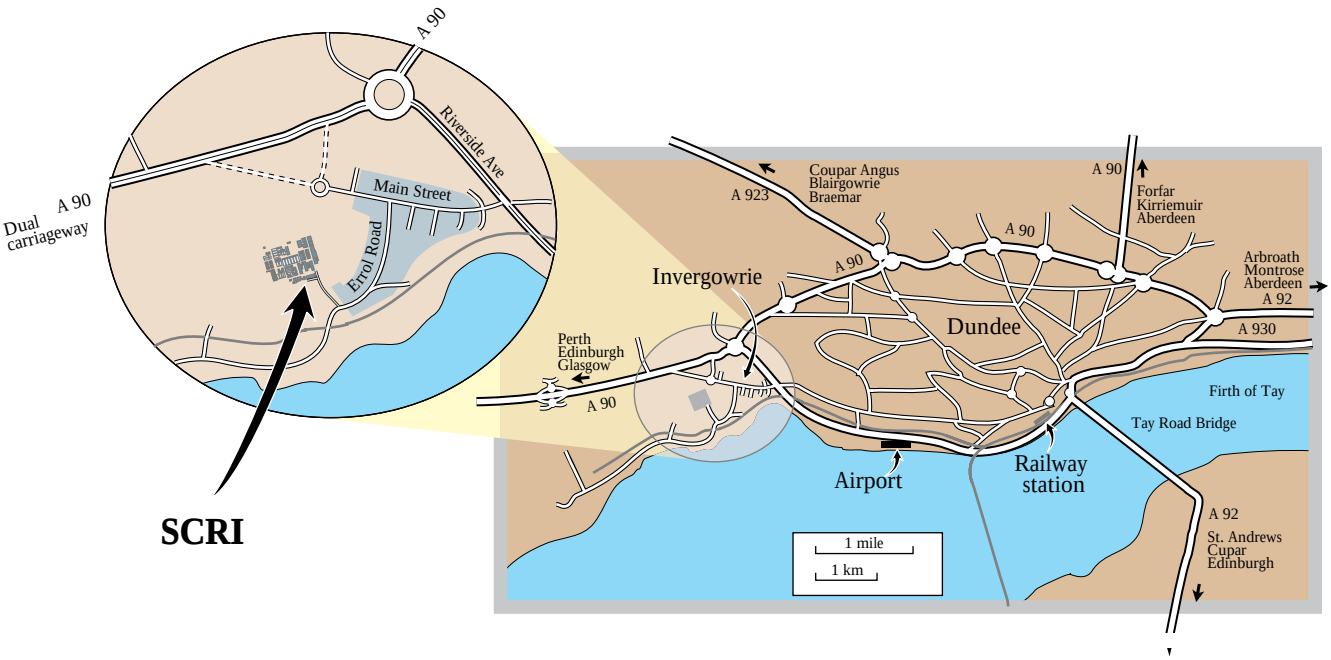
AAB	Association of Applied Biologists
ACRE	Advisory Committee on Releases to the Environment
ADAS	Agricultural Development and Advisory Service
BBSRC	Biotechnology & Biological Sciences Research Council
BCPC	British Crop Protection Council
BioSS	Biomathematics and Statistics Scotland
BSPB	British Society of Plant Breeders
BTG	British Technology Group
CAPS	Cleaved Amplified Polymorphic Sequence
CEC	Commission of the European Communities
CHABOS	Committee of Heads of Agricultural and Biological Organisations in Scotland
CIP	International Potato Centre - Peru
COST	European Co-operation in the field of Scientific and Technical Research
EAPR	European Association for Potato Research
ECRR	Edinburgh Centre for Rural Research
ECSA	European Chips and Snacks Association
EHF	Experimental Husbandry Farm
ELISA	Enzyme linked immunosorbent assay
EPPO	European Plant Protection Organisation
ESTs	Expressed Sequence Tagged Sites
FF	Flexible Funding (SOAEFD)
FLAIR	Food-Linked Agro-Industrial Research
GILB	Global Initiative on Late Blight
GIUS	Glasshouse Investigational Unit for Scotland
H-GCA	Home-Grown Cereals Authority
HDC	Horticultural Development Council
HPLC	High Performance Liquid Chromatography
HRI	Hannah Research Institute
IACR	Institute of Arable Crops Research
ICTV	International Committee for the Taxonomy of Viruses
IOBC	International Organisation for Biological Control
ISHS	International Society for Horticultural Science
ISPP	International Society for Plant Pathology
IVEM	Institute of Virology and Environmental Microbiology
MAFF	Ministry of Agriculture Fisheries and Food
MLURI	Macaulay Land Use Research Institute
MRI	Moredun Research Institute
NERC	National Environmental Research Council
NFT	National Fruit Trials
NFU	National Farmers Union
NIR	Near Infra-Red
NMR	Nuclear Magnetic Resonance
NPTC	National Proficiency Test Council
ODA	Overseas Development Administration
ORSTOM	Organisation for research in science and technology overseas
PCR	Polymerase Chain Reaction
PMB	Potato Marketing Board
PVRO	Plant Variety Rights Office
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RRI	Rowett Research Institute
SABRI	Scottish Agricultural and Biological Research Institutes
SAC	Scottish Agricultural College
SASA	Scottish Agricultural Science Agency
SCRI	Scottish Crop Research Institute
SEB	Society for Experimental Biology
SET	Scottish Enterprise Tayside
SNSA	Scottish Nuclear Stocks Association
SOAEFD	Scottish Office Agriculture, Environment and Fisheries Department
SSCR	Scottish Society for Crop Research
SSFG	Scottish Soft Fruit Growers Ltd
SSPDC	Scottish Seed Potato Development Council
STS	Sequence Tagged Sites
UNDP	United Nations Development Programme
WHO	World Health Organisation

The Scottish Crop Research Institute

Site plan



Access to Scottish Crop Research Institute



SCRI is on the east coast of Scotland, midway between Edinburgh and Aberdeen.

It is located at Invergowrie 6km west of the centre of Dundee. Access is via Riverside Avenue, Main Street and Errol Road.

British Rail has direct InterCity services between Dundee and London, Edinburgh and Glasgow and other UK cities.

Flights are available to Dundee Airport from Edinburgh, Manchester and Aberdeen, and scheduled services operate from many domestic and international destinations to Edinburgh and Glasgow